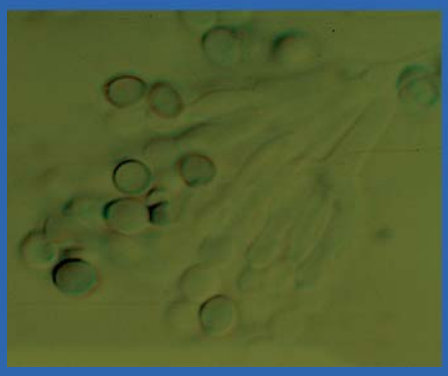
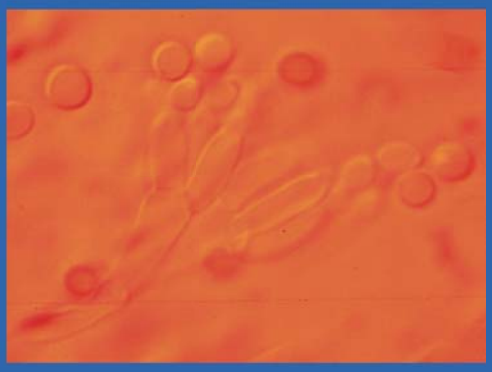


Ulla Lignell

Characterization of Microorganisms in Indoor Environments

Publications of the National Public Health Institute A 3/2008

Department of Environmental Health
National Public Health Institute
Kuopio, Finland
and
Department of Environmental Sciences
University of Kuopio, Finland

Kuopio 2008

Ulla Lignell

CHARACTERIZATION OF MICROORGANISMS IN INDOOR ENVIRONMENTS

A C A D E M I C D I S S E R T A T I O N

To be presented with the permission of the Faculty of Natural and Environmental Sciences, University of Kuopio, for public examination in auditorium L21, Snellmania Building, on February 9th 2008, at 12 o'clock noon.

National Public Health Institute, Department of Environmental Health, Kuopio, Finland

and

Department of Environmental Sciences, University of Kuopio, Finland

Kuopio 2008

Publications of the National Public Health Institute
KTL A3 / 2008

Julkaisija-Utgivare-Publisher

Kansanterveyslaitos (KTL)
Mannerheimintie 166
00300 Helsinki
Puh. vaihde (09) 474 41, telefax (09) 4744 8408

Folkhälsoinstitutet
Mannerheimvägen 166
00300 Helsingfors
Tel. växel (09) 474 41, telefax (09) 4744 8408

National Public Health Institute
Mannerheimintie 166
FIN-00300 Helsinki, Finland
Telephone +358 9 474 41, telefax +358 9 4744 8408

ISBN 978-951-740-770-0
ISSN 0359-3584
ISBN 978-951-740-771-7 (pdf)
ISSN 1458-6290 (pdf)

Kannen kuva - cover graphic:

Yliopistopaino
Helsinki 2008

Supervised by

Research Professor Aino Nevalainen, Ph.D.
Environmental Microbiology Laboratory
Department of Environmental Health
National Public Health Institute
Kuopio, Finland

Professor Pentti Kalliokoski, Ph.D.
Department of Environmental Sciences
University of Kuopio
Kuopio, Finland

Dr. Merja Kontro, D.Sc. (Tech.)
Department of Ecological and Environmental Sciences
University of Helsinki
Lahti, Finland

Reviewed by

Professor Linda Stetzenbach, Ph.D.
Department of Environmental and Occupational Health
University of Nevada
Las Vegas, Nevada, USA

Dr. Regine Szewzyk, Ph.D.
Department of Environmental Hygiene
Federal Environment Agency
Berlin, Germany

Opponent

Professor Tiina Reponen, Ph.D.
Department of Environmental Health
University of Cincinnati
Cincinnati, Ohio, USA

Ulla Lignell, Characterization of microorganisms in indoor environments
Publications of the National Public Health Institute, A3/2008, 116 Pages
ISBN 978-951-740-770-0; 978-951-740-771-7 (pdf-version)
ISSN 0359-3584; 1458-6290 (pdf-version)
http://www.ktl.fi/portal/4043

ABSTRACT

In indoor environments, moisture damage and related microbial growth are associated with adverse health effects. In this thesis, five studies were conducted to characterize how the microbial conditions in indoor environments are affected by the use of the building, by the presence of moisture damage, renovation of that damage, and time. A special focus was placed on the development of detection methods of an interesting bacterial genus, *Streptomyces*.

The effects of nutrients on the growth of streptomycetes were studied on 26 media. In addition, pH effects were examined with seven media. The pH range was 4.0 to 11.5 in intervals of 1.5 units. Glucose and tryptone allowed good growth of streptomycetes. The widely used tryptone yeast extract glucose (TYG) medium and tryptone soy agar (TSA) proved to be suitable media for the growth of streptomycetes isolated from indoor environments. The pH range for growth and sporulation was large, depended on nutrients, and was not a species-specific property.

Microbial concentrations were analyzed in eight school kitchens and compared with other parts of the schools. The airborne microbial concentrations in the kitchens were lower than those encountered in the other parts of the schools.

The effects of moisture damage on microbial flora in indoor environments were investigated in eight schools including school kitchens by air and surface sampling, in two schools two years before renovation via air samples and in 81 homes with house dust samples. It was found that moisture damage elevated microbial concentrations. In the moisture-damaged schools (index schools, n=6), culturable airborne microbial concentrations were higher than in the reference schools (n=2) and this was also true for kitchen facilities. Microbial concentrations were higher on moisture-damaged surfaces, than on undamaged surfaces. In addition, high concentrations of microbes, especially bacteria, were detected from undamaged surfaces in moisture-damaged kitchens. Moisture damage also affected the diversity of mycobiota. In the index school, diversity was larger than in the reference school.

Symptom prevalence of occupants was enquired by questionnaires among kitchen and clerical personnel in eight schools and among pupils in two schools. The expo-

sure occurring in moisture-damaged schools was associated with a high prevalence of symptoms.

The effects of moisture damage renovation were studied by air sampling in two schools, one of which was undergoing renovation procedures and in addition, the pupils' symptoms were asked with a questionnaire. Remediation measures decreased microbial concentrations in the index school. Although the culturable airborne concentrations decreased in the first year after remediation, the mycobiota did not change. Only after thorough cleaning in the second year after remediation, did the microbial profile approach that found in the reference school. When repairs were underway, there were no differences between the schools in the symptom prevalence of children. After the remediation, prevalence of respiratory symptoms decreased in the index school.

The long-term variation in microbial concentrations was followed with air samples in five consecutive years in two schools. In the second study year, concentrations were higher than those measured in the other years. Long range microbial transport is possible also in wintertime and simultaneous outdoor air sampling may be needed to obtain a better perspective.

The usefulness of the newly developed qPCR method in assessing microbial conditions in two schools and 81 residences by means of house dust samples was investigated and also compared with the traditional culture method. QPCR proved to be a feasible method in analyzing microbial concentrations. Concentrations obtained were several orders of magnitude higher than those detected by culturing. Since non-viable spores and microbial fragments can also cause adverse health effects, qPCR may give a more accurate view of the microbial exposure and be more informative than conventional culturing.

Concentrations of several fungi in house dust analyzed by the quantitative polymerase chain reaction (qPCR) method increased when the extent of moisture damage increased in the house.

Keywords: fungi, bacteria, indoor air, school, house dust

Ulla Lignell, Mikrobikarakterisointi sisäympäristöissä
Kansanterveyslaitoksen julkaisuja, A3/2008, 116 sivua
ISBN 978-951-740-770-0; 978-951-740-771-7 (pdf-versio)
ISSN 0359-3584; 1458-6290 (pdf-versio)
http://www.ktl.fi/portal/4043

TIIVISTELMÄ

Kosteusvaurioilla ja mikrobikasvulla on sisätiloissa esiintyessään yhteys terveyshaittoihin. Tässä väitöstutkimuksessa, joka koostuu viidestä osajulkaisusta, tutkittiin, kuinka sisätilojen mikrobistoon vaikuttavat rakennuksen käyttötarkoitus, kosteusvauriot ja niiden korjaus ja vuosien välinen vaihtelu. Painopiste oli bakteereihin kuuluvien streptomykeettien detektiomenetelmien kehittämisessä.

Ravinteiden vaikutusta streptomykeettien kasvuun tutkittiin 26 kasvualustalla. Glukoosi ja tryptoni mahdollistivat streptomykeettien hyvän kasvun. Yleisesti käytössä olevat tryptoni-hiivauute-glukoosialusta (THG) ja tryptoni-soija-agar (TSA) olivat käyttökelpoisia kasvualustoja sisätiloista eristetyille streptomykeeteille. Lisäksi tutkittiin pH:n vaikutusta seitsemällä alustalla. Tutkittu pH-alue oli välillä 4,0 – 11,5 1,5 yksikön välein. Kasvun ja itiöinnin pH-alue oli laaja ja se riippui ravinteista, muttei ollut lajikohtainen ominaisuus.

Mikrobipitoisuuksia mitattiin kahdeksassa koulukeittiössä ja pitoisuuksia verrattiin koulujen muiden tilojen pitoisuuksiin. Mikrobipitoisuudet keittiöiden sisäilmassa olivat pienempiä kuin koulujen muissa tiloissa.

Kosteusvaurioiden vaikutuksia mikrobistoon tutkittiin kahdeksassa koulussa ilma- ja pintanäytteiden avulla mukaan lukien koulujen keittiötilat, kahdessa koulussa kahtena vuonna ennen kosteusvauriokorjauksia ilmanäytteiden avulla ja 81 asunnossa huonepölynäytteiden avulla. Kosteusvauriot kohottivat mikrobipitoisuuksia. Kosteusvaurioituneissa kouluissa ja keittiötiloissa mikrobipitoisuudet sisäilmassa olivat suurempia kuin vertailukouluissa ja -keittiöissä. Mikrobipitoisuudet olivat suurempia vauriopinnoilla kuin vertailupinnoilla. Kosteusvaurioituneissa keittiöissä myös muilla kuin varsinaisilla vauriopinnoilla havaittiin suuria mikrobipitoisuuksia, erityisesti bakteeripitoisuudet olivat korkeita. Kosteusvauriot vaikuttivat myös mikrobidiversiteettiin siten, että sienisukuja havaittiin enemmän kosteusvaurioituneessa kuin vertailukoulussa.

Kyselylomakkeiden avulla selvitettiin sekä keittiö- että kansliahenkilökunnan oireita kahdeksassa koulussa ja oppilaiden oireita kahdessa koulussa. Kosteusvaurioituneissa kouluissa oireilua oli enemmän.

Kosteusvaurioiden korjausten vaikutusta tutkittiin ilmanäytteiden avulla kahdessa koulussa, joista toisessa tehtiin kosteusvauriokorjauksia. Myös oppilaiden oireprofiilit selvitettiin kyselyjen avulla. Korjaukset pienensivät mikrobipitoisuuksia. Mikrobisuvusto normalisoitui vasta perusteellisen siivouksen jälkeen. Korjausten aikana oppilaiden oireprevalensseissa ei ollut eroja tutkittujen koulujen välillä. Korjausten jälkeen hengitystieoireet vähenivät korjatussa koulussa.

Mikrobipitoisuuksien vuosien välistä vaihtelua tutkittiin kahdessa koulussa viitenä peräkkäisenä vuonna otettujen ilmanäytteiden avulla. Toisena tutkittuna vuotena mikrobipitoisuudet olivat muita vuosia korkeammat. Myös talvisaikaan mikrobien kaukokulkeuma on mahdollista, joten ulkoilmanäytteiden ottoa voi tarvita.

Kahdesta koulusta ja 81 asunnosta otettuja huonepölynäytteitä tutkittiin qPCR- ja viljelymenetelmillä. QPCR-pitoisuudet olivat useita kertaluokkia korkeammat kuin viljelyllä saadut pitoisuudet. Koska myös ei-elinkykyiset itiöt ja mikrobifragmentit voivat aiheuttaa terveyshaittoja, qPCR-tekniikan avulla voidaan saada tarkempi kuva mikrobialtistumisesta kuin perinteisellä viljelymenetelmällä.

Asunnon kosteusvaurioituneisuusasteen kasvaessa useiden kvantitatiivisella polymeraasiketjureaktiolla (qPCR) mitattujen sieni-itiöiden pitoisuudet huonepölyssä kasvoivat.

Avainsanat: sienet, bakteerit, sisäilma, koulu, huonepöly

CONTENTS

ABBREVIATIONS

AGI	all glass impinger
ATCC	American Type Culture Collection
a_w	water activity
cfu	colony forming unit
Ct	cycle threshold
d_{50}	cut-off size
DG18	dichloran 18% glycerol agar
DNA	deoxyribonucleic acid
DSMZ	German Collection of Microorganisms and Cell Cultures
ELISA	enzyme-linked immunosorbent assay
EMB	eosin methylene blue agar
EPS	extracellular polysaccharide
FSSST	fungal spore source strength tester
GM	geometric mean
HBSS	Hank's balanced salt solution
HDM	house dust mite
HT	Hickey-Tresner
IAQ	indoor air quality
IFN	interferon
Ig	immunoglobulin
IL	interleukin
I/O	indoor/outdoor
LAL	*Limulus* amoebocyte lysate assay
LPS	lipopolysaccharide
MEA	malt extract agar

MVOC	microbial volatile organic compound
NAL	nasal lavage
NO	nitric oxide
PAN-PCR	particle-associated nucleic acid PCR
PCR	polymerase chain reaction
P-FLEC	particle-field and laboratory emission cell
qPCR	quantitative PCR
RAPD	random amplified polymorphic DNA
RAST	radio allergo-sorbent test
RCS	Reuter centrifugal sampler
rDNA	ribosomal DNA
RFLP	restriction fragment length polymorphism
RH	relative humidity
RODAC	replicate organism detection and counting
rRNA	ribosomal ribonucleic acid
RT-PCR	reverse transcription-PCR
SAS	surface air system
SBS	sick building syndrome
SD	standard deviation
Th	T helper
TNF	tumor necrosis factor
TSA	tryptone soy agar
TYG	tryptone yeast extract glucose agar

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles referred to in the text by their Roman numerals:

I Suutari M, Lignell U, Hyvärinen A, Nevalainen A. Media for cultivation of indoor streptomycetes. Journal of Microbiological Methods 2002, 51: 411-416.

II Kontro M, Lignell U, Hirvonen M-R, Nevalainen A. pH effects on 10 Streptomyces spp. growth and sporulation depend on nutrients. Letters in Applied Microbiology 2005, 41(1):32-38.

III Lignell U, Meklin T, Putus T, Vepsäläinen A, Roponen M, Torvinen E, Reeslev M, Pennanen S, Hirvonen M-R, Kalliokoski P, Nevalainen A. Microbial exposure, symptoms and inflammatory mediators in nasal lavage fluid of kitchen and clerical personnel in schools. International Journal of Occupational Medicine and Environmental Health 2005, 18(2):139-150.

IV Lignell U, Meklin T, Putus T, Rintala H, Vepsäläinen A, Kalliokoski P, Nevalainen A. Effects of moisture damage and renovation on microbial conditions and pupils' health in two schools – a longitudinal analysis of five years. Journal of Environmental Monitoring 2007, 9(3): 225 – 233. Article was selected to be highlighted in "Chemical Science" 2007, 4(3):C20 and was selected for inclusion in "Chemical Biology" virtual journal.

V Lignell U, Meklin T, Rintala H, Hyvärinen A, Vepsäläinen A, Pekkanen J, Nevalainen A. Evaluation of quantitative PCR method for detection of house dust fungi and streptomycetes in relation to moisture damage of the house and comparison with culture. Submitted.

1 INTRODUCTION

Good indoor air quality (IAQ) is important for all of us; most people spend 90 % or more of their time indoors (Schwab et al., 1992). Most of this time consists of the hours spent at home or at work, but for example, school age children spend 20 % of their time in schools (Clench-Aas et al., 1999). Good IAQ consists of many aspects; it is an interaction of a functioning and efficient ventilation and the lowest achievable amounts of chemical, inorganic or organic, and microbial compounds which should not evoke symptoms in the occupants (Spengler et al., 2001).

Moisture damage and microbial growth indoors are associated with adverse health effects among the occupants (Bornehag et al., 2001; Bornehag et al., 2004). Dampness or moisture damage is a common problem in buildings all over the world. In Finland, 70 % of day care centers have signs of moisture problems (Ruotsalainen et al., 1993), as well as 55 % of homes (Nevalainen et al., 1998) and 53 % of schools (Kurnitski et al., 1996). High moisture load in a building can also be found in repeatedly damp facilities, such as institutional kitchens. This may lead to microbial growth on surfaces and structures. There are, however, few studies, which have evaluated microbial conditions in these kinds of facilities.

When building or surface materials become wet due to moisture damage, it is only a matter of time before microbes will start to grow. In fact, moisture is often the growth-limiting factor for microbes, since the other critical factors such as nutrients and suitable temperatures are usually fulfilled (Pasanen et al., 1992a). Microbial growth refers to the growth of fungi, bacteria and other microorganisms. Even though most of the studies in literature have focussed on fungi, bacteria should also be considered in indoor environments. Especially, the presence of actinomycetes has been connected to moisture damage (Nevalainen et al., 1991). Actinomycetes have been extensively studied in different environments (Kutzner, 1986), though seldom in indoor environments.

There are many methods available for detecting microbes in the indoor environment. They can be sampled from air, surfaces, materials, and dust and subsequently detected by culturing, molecular methods or by using chemical markers. The use of several sampling methods enables a more thorough microbial characterization than can be achieved with one method alone. For instance, surface sampling is recommended in addition to air sampling to detect fungi in indoor environments (van Reenen-Hoekstra et al., 1991).

It is well known that indoor microbial concentrations are not stable over time, but they are affected by many factors, such as outdoor air concentrations, season, and

time of day (de Ana et al., 2006; Lin & Li, 1996; Reponen et al., 1992). However, the year-to-year variation in indoor environments is not well understood, although fluctuations in outdoor spore concentrations have been extensively studied (Mullins, 2001). Culturing is the conventional method for microbial characterization, although it underestimates total microbial concentrations (Amann et al., 1995). Molecular methods allow detecting non-culturable and non-viable microbes. This is important since even non-viable microbes may still be able to cause adverse health effects (Hirvonen et al., 1997b).

While the association between moisture and mold damage of the building and adverse health effects of its occupants is well documented, the causal phenomena are still unclear. There is a clear gap in our understanding of how the exposure actually evokes the symptoms (IOM, 2004). Therefore, more studies are needed to improve our knowledge on the microbial ecology of buildings and the qualitative and quantitative aspects of exposure assessment in the occupants.

2 REVIEW OF THE LITERATURE

2.1 Microbes in indoor environments

Environmental microbes, i.e., fungi and bacteria, are ubiquitous being found in both natural and man-made habitats. In indoor environments, the main source for microbes is usually the outdoor air (Shelton et al., 2002; Su et al., 2001). Microbes are able to travel long distances from their sources. For example, atmospheric transport of fungal and bacterial spores can occur in clouds of desert dust spreading the microbes from Africa and Asia to Europe and the Americas. Spores can also survive for the 5 to 7 days needed for transatlantic transport from Africa to the USA. Usually bacteria have been dominating in such dust events, but about 20 fungal genera have also been identified (Shinn et al., 2003).

Outdoor microbial concentrations vary according to the season and time of day, and these variations are also reflected in indoor air. The highest concentrations are usually detected in summer (Bartlett et al., 2004a; Bartlett et al., 2004b; de Ana et al., 2006; Dharmage et al., 2002; Lee & Jo, 2006; Lin & Li, 1996; Medrela-Kuder, 2003; Ren et al., 1999; Reponen et al., 1992). Climate greatly affects airborne fungal concentrations both outdoors and indoors. In cold climates outdoor and indoor concentrations are typically low during the wintertime when there is snow cover on the ground (Reponen et al., 1992).

In addition to outdoor sources, microbes indoors can originate from indoor sources. These can be the occupants themselves and their activities, as well as indoor plants (Lehtonen et al., 1993). Other factors influencing the microbial population include building maintenance, cleanliness, indoor temperature and relative humidity (RH), type of furniture, and carpeting (Dharmage et al., 1999; Levetin, 1995; Reynolds et al., 1990; Smedje & Norbäck, 2001). Microbes may also drift indoors on the clothes of the occupants or in the fur of pets (Lehtonen et al., 1993).

The type of ventilation affects microbial concentrations in indoor environments. Mechanical ventilation is more efficient than natural ventilation in filtering particles from the intake air and in removing pollutants. This difference is also reflected in microbial concentrations, which in naturally ventilated buildings can be 2 – 7 times higher than in buildings with mechanical ventilation (Parat et al., 1997; Wålinder et al., 1997). There are also differences between mechanical ventilation systems: with a mechanical exhaust or fan coil unit system, microbial concentrations were reported to be higher than where there was a mechanical supply and exhaust system or an air-

handling unit (Reponen et al., 1992; Wu et al., 2005). This is probably due to more effective filtering of the intake air.

Fungi may also originate from contamination in special building strucrures, e.g. the crawl space (Airaksinen et al., 2004). Fungi are distributed in the building due to an under-pressure inside the building caused by mechanical ventilation, which is a normal situation in cold climates.

In general, indoor concentrations of fungi are qualitatively similar and quantitatively lower than those in the ambient air, i.e., indoor/outdoor (I/O) fungal concentration ratios are < 1. This is mainly because of ventilation removing particles including fungal spores from the supply air as well as sedimentation processes. Particle removal may take place through filtering in the mechanical ventilation, or through the filtering effect of the building envelope in natural ventilation. However, in cold climates when there is snow cover on the ground and low outdoor concentrations, the indoor concentrations of fungi are often higher than outdoors pointing to the presence of indoor sources (Reponen et al., 1992). The season and climate must be taken into account when interpreting the I/O ratios (Rogers, 2003) or other fungal data. Furthermore, fungi undergo diurnal rhythms to optimize timing of spore release and to ensure spore dispersal (Rogers, 2003). The spore release may be discontinuous and can be affected by the air velocity (Pasanen et al., 1991). There are also differences in the spore release characteristics between different fungi (Kildesø et al., 2003).

In the indoor air, microbes come and go, which is a natural phenomenon. Ventilation and cleaning are the usual removal processes of microbes in indoor environments. However, microbes may also grow indoors in building materials and structures. In such a situation, they may be responsible for different harmful effects. They can damage building structures by discoloring and degrading building materials, as well as causing negative aesthetic effects such as dirty appearance and unpleasant odors (Portnoy et al., 2005). Undoubtedly, the main concern about microbial growth in indoor environments is related to the strong link to the adverse health effects in the occupants (Douwes & Pearce, 2003; Li & Yang, 2004). There is a need to further characterize microbes in different indoor environments and their temporal fluctuation to achieve a more accurate exposure assessment of occupants.

2.2 Moisture damage and microbes

Moisture damage in buildings is closely related to the microbial growth, so-called mold growth. The primary factor controlling microbial growth indoors is the avail-

ability of water, although the characteristics of materials in question play also a role (Grant et al., 1989).

2.2.1 Microbial growth on building materials

Water can penetrate into materials due to many reasons such as water leakage and condensation. In the presence of water, subsequent microbial growth in the materials will occur almost certainly. Growth rate is, however, dependent on, e.g., temperature and the availability of nutrients. Under optimal conditions, a few days may be sufficient for detectable growth (Pasanen et al., 1992a). Especially in cold climates during wintertime, condensation of water on cold surfaces is a problematic phenomenon and can lead to microbial growth even within the wall structures (Pessi et al., 2002). Subsequently, spores from this kind of hidden microbial growth can gain access to the indoor air through cavities in structures (Morey et al., 2003).

Fungi have species-specific demands for moisture conditions to allow them to germinate and grow on materials (Pasanen et al., 2000b). RH of air controls fungal growth only indirectly, for example high humidity, over 85 % RH, can moisten materials and may lead to fungal growth (Burge, 2002; Pasanen et al., 2000a). The moisture content of the material available for microbial growth is expressed as water activity (a_w), which is the ratio of the vapor pressure exerted by water in the material to the vapor pressure of pure water at the same temperature and pressure which corresponds to the RH on the material under equilibration conditions. The lowest value necessary to initiate microbial growth is a_w 0.65 (Górny, 2004). Microbes can be classified as primary, secondary or tertiary colonizers by the a_w values needed for their growth. Most fungi and bacteria relevant in indoor environments require a_w values of $\geq$ 0.8. Temperature and availability of nutrients influence the ability of microbes to grow at given a_w values. Higher a_w values are required for growth at suboptimal temperature and nutrient limitations (Grant et al., 1989).

In moist building materials, a relatively small number of fungal genera grow commonly. Available water and characteristics of the substrate are two of the main regulating factors. In material samples, *Penicillium* spp., *Aspergillus* spp., *Acremonium* spp., *Phoma* spp., *Cladosporium* spp., *Chaetomium* spp., and *Stachybotrys* spp. belong to most frequently found fungal genera (Andersson et al., 1997; Ellringer et al., 2000; Gravesen et al., 1999; Hyvärinen et al., 2002). Bacteria growing on building materials have been studied much less than fungi. A few studies have reported the detection of actinomycetes, mycobacteria, and gram-negative bacteria (Andersson et al., 1997; Hyvärinen et al., 2002; Torvinen et al., 2006).

Microbes may produce secondary metabolites when growing on building materials. These metabolites include non-volatile allergens, mycotoxins and microbial volatile organic compounds (MVOCs). Fungal allergens may cause allergic reactions in sensitized individuals. For some fungi, such as *Alternaria alternata*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Stachybotrys chartarum*, and *Penicillium* spp., main allergens have been characterized (Achatz et al., 1995; Hemmann et al., 1999; Raunio et al., 2001; Shen et al., 1996). Filamentous fungi are able to produce over 300 mycotoxins (Gutarowska & Piotrowska, 2007). The toxin production is stimulated by changes in the environment. Mycotoxins have been detected in air, surface, dust, and material samples from moldy dwellings (Brasel et al., 2005b; Charpin-Kadouch et al., 2006; Engelhart et al., 2002; Tuomi et al., 2000). Toxins can have carcinogenic, immunotoxic, cytotoxic, neurotoxic, mutagenic and teratogenic effects (Gutarowska & Piotrowska, 2007). After becoming airborne, such as when being carried by spores or fungal fragments, toxins can be transported efficiently to tissues via inhalation in the respiratory tract (Gutarowska & Piotrowska, 2007). MVOCs include low molecular weight alcohols, aldehydes, and ketones (Mazur & Kim, 2006). They are associated with fungal as well as streptomycete contamination (Schöller et al., 2002). There are studies supporting (Elke et al., 1999) and refuting (Nilsson et al., 2004) the usefulness of MVOCs as indicators of moisture damage in indoor environments. This is mainly because the specificity of origin of MVOCs to microbes is unclear, as MVOCs may originate from sources other than microbes (Pasanen et al., 1998).

2.2.2 Microbes in the indoor air of moisture-damaged buildings

Microbial growth on moisture-damaged materials may result in the release of microbes into the indoor air (Pasanen et al., 1991). Thus, moisture- or water-damaged, damp buildings may develop microbial indoor air problems. Some of the airborne microbes will eventually become settled on various surfaces with other particles present in the indoor air. Microbes can be resuspended from this so called settled dust and become airborne again (Hunter et al., 1988).

Fungal composition is different if one compares moldy and reference buildings. This has been demonstrated for homes and schools (Meklin et al., 2005; Pasanen, 1992; Strachan et al., 1990; Vesper et al., 2006b). The species diversity can be higher in damp homes/schools than in controls, and some species or genera can be found at elevated concentrations, such as *Aspergillus versicolor*, *Cladosporium* spp., *Fusarium* spp., *Ulocladium* spp., *Bacillus mycoides*, *Mucor* spp., *Exophiala* spp., *Stachybotrys* spp., and *Tritirachium* spp. (Garrett et al., 1998; Hyvärinen, 2002; Meklin et al., 2002a; Meklin et al., 2003; Meklin et al., 2005; Nilsson et al., 2004). Many stud-

ies examining the indoor air and materials have demonstrated that certain microbes are suggested to be indicative of moisture damage. This means that they do not belong to the mycobiota normally found in buildings, but their presence occurs in association with dampness or moisture damage. There is consensus about the indicator status for certain fungi including *Aspergillus versicolor* and *Stachybotrys* spp.. For many fungi, however, there is much more limited evidence for their possible moisture damage indicative status. (Flannigan & Morey, 1996; Hyvärinen, 2002; Lappalainen et al., 2001; Mahooti-Brooks et al., 2004; Meklin, 2002; Samson et al., 1994)

The effect of moisture damage on total or viable microbial concentrations in the indoor air or on settled dust is still controversial. Fungal concentrations have often been higher in moldy homes than in the reference homes (Hunter et al., 1988; O'Connor et al., 2004; Waegemaekers et al., 1989; Vesper et al., 2006b) at least in winter (Reponen et al., 1994). However, differences are not always detected (Meklin et al., 2004; Müller et al., 2002; Nevalainen et al., 1991; Nilsson et al., 2004). This may be due to variations in the definitions of moisture and mold damage, the type of moisture damage and to the spatial and temporal variation in indoor concentrations. Moisture damage can also affect the particle size distribution of indoor fungi. Differences between moldy or moisture-damaged and reference buildings have been detected mostly in the size range of 1.1 – 4.7 µm (Hyvärinen et al., 2001a; Meklin et al., 2002b; Reponen et al., 1994).

In water-damaged schools in Scandinavia, concentrations of airborne fungi have generally been low (Dotterud et al., 1995; Lappalainen et al., 2001; Meklin et al., 2003). However, concentrations can be elevated in the vicinity of damaged areas (Lappalainen et al., 2001). Concentrations of airborne (1→3)-β-D-glucan, a fungal marker, in a school with mold problems were also higher than in a control school (Rylander et al., 1998). In the indoor air of moisture-damaged schools and day care centers (n=9), viable bacterial concentrations, mainly bacilli and mesophilic actinomycetes including *Streptomyces* spp., have been 10^2-10^3 colony forming units (cfu) per cubic meter of air (Andersson et al., 1999). In non-problem schools (n=39), geometric mean (GM) bacterial concentration, mainly micrococci, was 10^2 cfu/m^3 (Bartlett et al., 2004b).

2.2.3 Effects of remediation on microbial indoor environment quality

Dampness and water damage in buildings have been shown to be important risk factors for the adverse health outcomes in epidemiological studies (IOM, 2004). Thus, remediation is needed to eliminate the harmful exposures (IOM, 2004). Remediation measures have been shown to affect the diversity of mycobiota in the indoor air. During remediation of moisture-damaged buildings, more fungal types and slow-

growing mycobacteria have been detected than before the renovation (Hameed et al., 2004; Rautiala et al., 2004). Furthermore, an increase in the concentrations of *Aspergillus* spp., or *Stachybotrys chartarum* (Mahieu et al., 2000; Vesper et al., 2000) and the overall fungal concentration have been observed (Overberger et al., 1995; Rautiala et al., 1996; Rautiala et al., 1998).

The aim of the remediation measures is to reach background microbial concentrations after completion of the remediation process. Airborne fungal concentrations have decreased after remediation in homes, offices, schools and hotel (Barnes et al., 2007; Cooley et al., 1998; Ellringer et al., 2000; Reynolds et al., 1990; Vesper et al., 2000). In successfully remediated buildings, fungal concentrations were lower than in non-complaint portions of the buildings or in reference buildings, but the difference was minor or not found if the remediation was unsuccessful or only partially carried out (Kleinheinz et al., 2006; Kokotti et al., 1999; Meklin et al., 2005). After remediation, fungal concentrations were also lower on undamaged surfaces than before remediation (Ellringer et al., 2000). The effect of cleaning on microbial concentrations has also been shown. Microbial concentrations decreased after thorough remediation and cleaning procedures in a moisture-damaged building (Ebbehøj et al., 2002).

2.3 Sampling of indoor microbes

Sampling methods are needed to quantify and describe the microbial populations in indoor environments. Microbes can be sampled from air, surfaces, dust, and materials. Since there is no technique that would cover all aspects of environmental sampling, the methods need to be selected according to the needs of the investigation.

2.3.1 Air sampling

In general, air is an extreme and oligotrophic environment for microbes, e.g., if compared to soil (Peccia & Hernandez, 2006). This means that special features must be taken into the account in the air sampling. Differences in the aerodynamic diameter of particles can affect the collection efficiencies of air samplers. Furthermore, sampling stress can reduce the viability and/or culturability of many microbes (Stewart et al., 1995). Short sampling times are a common problem in air sampling. The representativeness of sampling decreases and variability between side-by-side samples increases with decreasing sampling time (Godish & Godish, 2006; Verhoeff & Burge, 1997). In one study, however, the sampling duration had no effect on fungal concentrations but a trend of decreasing concentrations was detected for bacteria as the sampling duration increased (Godish & Godish, 2006). Long sampling peri-

ods may lead to low sampling efficiencies due to desiccation effects (Toivola et al., 2004). The limit of detection and the upper limit of range are also important aspects that need to be taken into account by controlling the sampling time (Nevalainen et al., 1992).

Air samplers are based on different physical principles and they can be designed for the detection of culturable or non-culturable microbes. Sampling methods for airborne particles can be subdivided into passive samplers using natural aerosol convection, diffusion or gravity, and active samplers using stationary or personal pumps (An et al., 2004). The stationary sampling is the most widely used method for conducting microbial measurements in indoor environments. In addition, personal sampling has been used especially to characterize exposures during the remediation (Rautiala et al., 1996) or to analyze total individual exposure to microbes (Toivola et al., 2004). Recently, cyclone personal samplers enabling particle size fractioning have been developed which collect bioaerosols into microcentrifuge tubes (Lindsley et al., 2006). Subsequently, these samples can be readily analyzed by, e.g., polymerase chain reaction (PCR) and immunoassays. There are several major reviews on bioaerosol samplers (Eduard & Heederik, 1998; Portnoy et al., 2004; Reponen et al., 2001). There is a large variety in their types and suitability for different needs of field sampling, but none of these samplers can be considered as representing a reference method (Reponen et al., 2001). An important characteristic for the physical sampling efficiency is the cut-off size (d_{50}) above which 50 % or more of the particles are collected. The results obtained with different devices are not easily comparable with each other due to differences in sampling times, volumes and principles.

Table 1 presents some of the commonly used sampler types and their characteristics from various sampling issues point of view.

Table 1. Type of samplers for airborne fungi.

Principle / Type of sampler	Example of device	Advantages	Limitations
Impaction A Impaction on agar media / culturable spore samplers		- determination of culturable organisms - species determination - no sample treatment after sampling - large reference data for cultivation results	- negative result inconclusive - not for non-culturable particles - selection of culture medium - short sampling times - time consuming - slow growing species will be underestimated - clumps of organisms appear as single colonies - counting is difficult in highly contaminated situations
- slit samplers	Mattson-Garvin		
- hole impact onto agar	Andersen 1 stage impactor		
- multistage hole impact onto agar	Andersen 2 or 6 stage impactor, SAS	- particle size distribution	
Impaction B slit impact onto coated slide / total spore traps	Allergenco MK-3, Burkard 24 h sampler	- total spore enumerations - larger sample volume - easy counting - fast	- no species determination - no determination of culturable organisms
Impingers Impaction into liquid	AGI-30, BioSampler	- for high concentrations - culture, total counts, biochemical analyses or immunoassays - extended sampling time - no upper detection limit	- fungi may impinge with low efficiency
Centrifugal acceleration / centrifugal samplers	RCS, aerojet cyclone	- culture, total counts, biochemical analyses or immunoassays	
Filtration / filter cassette permanent or disposable	Sartorius MD8	- culture, total counts biochemical analyses, immunoassays or by PCR after sampling - for high concentrations	- loss of viability - desiccation of bacterial cells
	Button Sampler	- stationary and personal sampling - sampling efficiency 4 l/min like inhalation curve - low sensitivity to wind direction high 99 % collection efficiency - also for large particles - long term sampling	
Natural electrostatics	Electrosampler	-low velocity maintain biological and physical integrity of microbes	

AGI=all glass impinger, RCS=Reuter centrifugal sampler, SAS = surface air system
(An et al., 2004; Blomquist, 1994; Burton et al., 2007; Buttner et al., 1997; Eduard & Heederik, 1998; Engelhart & Exner, 2002; Fabian et al., 2005; Grinshpun et al., 1998; Horner, 2003; Lee et al., 2004b; Moschandreas et al., 1996; Osborne et al., 2006; Pasanen, 2001; Portnoy et al., 2004; Reponen et al., 2001; Stetzenbach et al., 2004; Verhoeff & Burge, 1997; Yao & Mainelis, 2006)

For air sampling, it should be noted that a single sample is usually not sufficient to provide a satisfactory conclusion about microbial conditions in a specific indoor environment since there are spatial and temporal variations in microbial concentrations. Therefore, several samples should be taken. According to a proposal for residences, a sampling campaign should consist of 11 different sampling days in two rooms if one aims to obtain a long time average concentration of the home (Hyvärinen et al., 2001b). Since the outdoor air is an important source of indoor microbes, outdoor air samples should also be collected for comparison (Mazur & Kim, 2006).

2.3.2 Surface sampling

Microbial concentrations and mycobiota on surfaces may differ from those in the indoor air. Therefore, results obtained from surface sampling cannot directly be associated with the exposure to airborne microbes (Niemeier et al., 2006). Surface sampling is, however, one way to determine whether there is a problem and if so, to locate the source of the biocontamination. In fact, surface sampling is recommended as a supplement to air sampling as a way of detecting fungi in indoor environments (van Reenen-Hoekstra et al., 1991).

A common technique for surface sampling is the tape-lift, where a clear cellophane tape (cellotape) is placed onto the suspected area, stained, and placed on a microscope slide (Portnoy et al., 2004; van Reenen-Hoekstra et al., 1991). The presence of spores and mycelia can be estimated semiquantitatively and a few fungal genera, e.g., *Stachybotrys* or groups of genera, e.g., *Aspergillus* and *Penicillium* may be identified by their spore type. The presence of mycelia is an important indication of active growth on the surface. However, the identification is quite limited due to similarities of some spores the uncertainty of collecting characteristic conidiophores or other growth structures that aid in identification.

Surface samples can also be taken from a certain area by a cotton swab or plastic loop into stabilization media with subsequent cultivation on agar media (Beguin & Nolard, 1994; Cooley et al., 1998; Portnoy et al., 2004; van Reenen-Hoekstra et al., 1991). Using this method, concentrations expressed as cfu/cm^2 can be determined. To improve the recovery efficiency, sonication has been used in the rayon swab surface sampling for the small area sampling (10-25 cm^2) with high microbial concentrations (Brown et al., 2007). Results obtained by the sponge swipe method have been comparable with those obtained using the swab kit method (Buttner et al., 2001), although the swab has also been more efficient than the sponge (Buttner et al., 2007). For large sampling areas (1 m^2), a sampling method called the BIsKIT biological sampling kit can be used (Buttner et al., 2004b). Surfaces can also be

sampled by a method called "replicating organism detection and counting" (RODAC). It is a direct agar contact plate, which is commonly used in the hospital infection control (Stetzenbach et al., 2004). The adenosine triphosphate bioluminescence method has been developed to monitor surface cleanliness in, e.g., kitchens (Aycicek et al., 2006; Griffith et al., 2000).

Air turbulence increases the spore release from rough surfaces such as ceiling tiles. As an alternative to the conventional air or surface sampling, the spore release rate can be measured with specifically developed devices (Górny et al., 2001). Portable aerosolization chambers such as the "fungal spore source strength tester" (FSSST) (Sivasubramani et al., 2004) or "particle field and laboratory emission cell" (P-FLEC) (Kildesø et al., 2003) can be used. In these chambers, aerosolized spores from a fungal source are immediately collected into an air sampler. In comparison with the swab method and air sampling, it seemed that FSSST could be a feasible method to detect a mold source (Niemeier et al., 2006).

2.3.3 Dust sampling

House dust is a mixture of many kinds of substances, not simply microorganisms (Macher, 2001b; Portnoy et al., 2004). There are variations in the definitions of dust. In this thesis, house dust refers to the settled dust, not to airborne particles, which can be collected onto filters from the indoor air. It has been hypothesized that microbial measurements of dust reflect the accumulated microbial load over a period of time longer than can be obtained using air samples (Flannigan, 1997; Portnoy et al., 2004). Thus, the evaluation of long-term exposure is possible, although the stability of microbes in dust over time is a matter of debate (Verhoeff et al., 1994). However, only a part of the resuspended dust particles are of respirable size (Flannigan et al., 1994) and thus, they do not exactly reflect inhalation exposure.

House dust samples are usually collected by vacuuming. Samples can be analyzed by culturing for culturable microbes, by chemical methods or by bioassays for ergosterol, endotoxin, glucans or allergens, or by quantitative PCR (qPCR) for microbial DNA. Table 2 presents different vacuuming methods used for detecting culturable microbes in house dust and the obtained microbial concentrations. The literature on this topic is somewhat confusing due to variations in sampling times, areas and sample treatments. Before there can be a valid comparison of results, a harmonization of methods would be needed. In most of the studies, dust samples are collected during site visits by research personnel. A simpler way to obtain samples would be to ask the study participants to take the samples by themselves, e.g., by vacuuming into specifically developed socks using their own vacuum cleaner. The levels of allergens and microbial agents obtained with these two techniques have

Table 2. Summary of studies detecting culturable microbes in vacuumed house dust.

Location	No. of samples per study	Vacuuming method and culture media	Fungal concentration (cfu/g)	Main observation	Study
living room floor, carpet	6-767	- onto different kinds of filters or into clean dust collection bag - 0.70 - 4 m^2, for 1 – 10 min, not mentioned (1 study) - ALK nozzle, not always - sieving (2 studies) - Martin's Rose Bengal Agar, MEA, DG18, cellulose agar, TSA for bacteria	range 1.0×10^3-6.6×10^7 GMs 6×10^4-3×10^5 41 different fungi most frequent genera *Penicillium, Aspergillus, Cladosporium* for mesophilic bacteria overall mean 7.8-8.3×10^6 for thermophilic bacteria overall mean 1.3-1.9×10^4	- highest concentrations in living room - associations of fungal concentrations with irritation, chest illness, moldy odors - background levels - seasonal variation - cultured concentrations 2 to 3 orders of magnitude lower than by qPCR - highest correlation estimate between culture and qPCR 0.437 - toxic sterigmatocystin occasionally in house dust in moldy dwellings	4,5,7,8,9,10, 11,13,14,16, 18,20*,21,32
bedroom floor, carpet	25-419	- canister vacuum cleaner - onto different kinds of filters - 1 - 4 m^2, for 1 - 5 min - ALK nozzle, not always - dilution, not always - MEA, DG18	range 5×10^3-7×10^7 median or GM 9×10^3-7×10^4	- weak association of dustborne fungi with airborne fungi - concentration of *Aspergillus fumigatus* increases after insulated windows and central heating are installed - no association of fungal concentrations with allergic history or respiratory symptoms - increased risk for lower respiratory illnesses with high prevalence of genera	4,6,9,15,17,22,30,31
mattress	25-377	- onto paper or cellulose filters - 1 m^2 ,1- 5 min - dilution, not always - MEA, DG18	median or GM 7×10^3-3×10^4	- peak of fungal concentrations in winter - concentration of *Aspergillus fumigatus* increases after insulated windows and central heating are installed - no association of fungal concentrations with allergic history or respiratory symptoms	3,9,15,17,31
furniture	11-356	- cellulose ester filter or polyethylene filter socks - 0.09- >1 m^2, 3-5 min - homogenizing, not always - dilution, not always - MEA, DG18, cellulose agar	GM 6×10^4-2×10^5 *Mucor, Wallemia sebi* and *Alternaria* prevalent	- genera differ from those in air samples	14,24,27

school, floor	9-72	- HVS-3 sampler, microvacuuming - onto cloth or MCE filters - 2 m^2 - 1 ft^2, 1 - 4 min - dilution or settled dust on horizontal surfaces - DG18	range 2×10^2-8×10^4 for bacteria range 10^4-10^6	- correlation between inflammatory potency and fungal concentrations in floor dust - lowest fungal concentrations in schools - fungal concentrations two-fold increased in damaged areas - no difference between damaged and control schools in fungal concentrations - seasonal variation	1,2,4,19,25, 26
other	12-356	- HVS-3 sampler, microvacuuming - onto different filters, polyethylene filter sock or filterbag (HEPA) - 98 cm^2 – 500 m^2, 5 sec – 5 min - dilution, homogenizing not always - dust from vacuum bags (2 studies) - MEA, DG18, cellulose agar	range 5×10^3-7×10^7 median 15-1×10^4, 85-2×10^4 cfu/100 cm^2 for bacteria (Micrococci, *Bacillus*, gram-neg.) range 3.4×10^6-7×10^3 for actinomycetes mean 4000	- qualitative properties of dust associated with sick building syndrome - no association of fungal concentrations between air and dust samples - increased fungal concentrations when potential for water intrusion increases	4,12,23, 24,28,29

* residential carpets

[1](Allermann et al., 2006), [2](Andersson et al., 1999), [3](Beguin, 1995), [4](Beguin & Nolard, 1996), [5](Chew et al., 2001), [6](Chew et al., 2003), [7](Dales et al., 1999), [8](Dales et al., 1997), [9](Douwes et al., 1998), [10](Engelhart et al., 2002), [11](Gehring et al., 2001), [12](Gyntelberg et al., 1994), [13](Heinrich et al., 2003), [14](Hicks et al., 2005), [15](Hirsch et al., 2000), [16](Horner et al., 2004), [17](Jovanovic et al., 2004), [18](Koch et al., 2000), [19](Lappalainen et al., 2001), [20](Macher, 2001a), [21](Meklin et al., 2004), [22](Müller et al., 2002), [23](Niemeier et al., 2006), [24](Park et al., 2006), [25](Piecková & Wilkins, 2004), [26](Ramachandran et al., 2005), [27](Ren et al., 1999), [28](Rintala et al., 2004), [29](Spurgeon, 2003), [30](Stark et al., 2003), [31](Verhoeff et al., 1994), [32](Vesper et al., 2006b)

been shown to correlate moderately or well (Schram-Bijkerk et al., 2006). The easiest way to obtain dust samples is to collect homeowners' vacuum bags (Vesper et al., 2007). More homogenous subsamples from vacuumed dust can be obtained by discarding coarse material such as hair, small toys, and stones or by sieving the dust sample (Butte & Heinzow, 2002; Vesper et al., 2007). However, analytical results may vary after sieving and are not comparable to dust analysis without sieving. Vortexing with glass beads can be used in order to increase microbial yields from dust samples (Macher, 2001a). Airborne dust can be collected passively by allowing sedimentation for long periods of time. A device called a dustfall collector has been used for estimations of airborne culturable fungi (Würtz et al., 2005).

House dust samples are often stored until analysis. It was reported that storage of house dust at the room temperature or refrigerator for 25 days had no effect on concentrations of culturable fungi or bacteria (Macher, 2001a). Furthermore, the storage at -20 °C for 10 months had no effect on the mite allergen, endotoxin, and β(1→3)-glucan levels, but a loss in cat allergen levels was noted (Fahlbusch et al., 2003).

2.3.4 Material sampling

Bulk samples include pieces of material taken usually from surfaces with suspected microbial growth. Often the results have been expressed as concentrations of culturable microbes per gram of material (cfu/g) (Ellringer et al., 2000; Hyvärinen et al., 2002). Material samples give a good picture of microbial growth occurring at a specific site in question, but it is not known how well the findings reflect the human exposure in the indoor environment.

2.3.5 Sampling for PCR

According to a review article (Peccia & Hernandez, 2006), the most common sampling methods for bioaerosol studies with PCR are the same as for culture methods i.e. the impaction onto non-agar surfaces (20 % of previous studies), impingement (24 %) and filtration (56 %). The best sampling devices for PCR-based measurements appear to be high-volume samplers. *Penicillium roqueforti* has been detected by PCR in samples collected directly to Eppendorf tubes (Williams et al., 2001). The bioaerosol sampler, where air is bubbled through a porous medium submerged in a liquid layer (Agranovski et al., 2002), has been used in conjunction of PCR to identify *Influenza* virus (Pyankov et al., 2007). The sampler is suitable also for detecting bacteria and fungi (Agranovski et al., 2002).

2.3.6 Sampling aspects regarding the exposure assessment

There are no established health-based guidelines for bioaerosols because of the varying nature of the purported exposing agents. Clearly, there is a need for developing consistent methods for quantifying indoor exposures. At its best, the sampling procedure is still conducted over a limited period, like snapshot of the potential overall exposure (Mazur & Kim, 2006). All sampling should be based on an assessment strategy and careful monitoring planning, because no sampling method can collect all the potential pollutants existing in indoor environments (Hui et al., 2006). For the exposure assessment in indoor environments, biomonitoring of exposed individuals with serum samples (fungal specific immunoglobulin (Ig)G, IgE, IgA and IgM antibodies) may also be utilized (Hyvärinen et al., 1999; Johanning et al., 1996; Johanning et al., 1999; Raunio et al., 1999; Vojdani et al., 2003). A comprehensive exposure assessment strategy requires the inspection of the building by trained persons familiar with building science and residential construction and this will entail a thorough inspection of the building and estimation of the contaminated area (Dillon et al., 1999; Nevalainen et al., 1998). It has been stated that a multidisciplinary approach including technical investigations, microbial measurements and health surveys is the best way to approach indoor air problems (Haverinen et al., 1999). Usually, indoor and outdoor exposure assessments are conducted separately. Recently, in a comprehensive study both traffic-derived and biological contaminants in indoor environments were monitored (Miller et al., 2007).

2.4 Detection methods of indoor microbes

Exposure assessment includes a description and quantification of exposing agents. In the case of microbes, this means that microbial communities including various groups of bacteria and fungi should be detected properly. Detection methods are based on culturing, on microscopy after staining, on assessment of total biomass of microbes using chemical markers as well as on molecular methods. It is self evident that different microbes can have different effects on occupants and, therefore, identification of the microbes to the genus or species level is needed.

2.4.1 Culture-based methods

Traditional microbial detection methods are based on culturing. Culturing methods underestimate the total amount of microbes present in the sample. It has been estimated that somewhere between 0.001 – 15 % of bacteria in environmental samples are culturable (Amann et al., 1995). For fungi, the proportion of culturability can be higher – a median value of 87 % was reported (Lee et al., 2006). The culture me-

dium and incubation conditions (Burge, 1995) are two of the factors that further affect the results. There is no single medium that permits the growth of all microbes. Some genera, such as *Stachybotrys*, grow poorly on most media. In addition, interactions between the microbial colonies developing on the agar medium may influence the results. Rapidly growing fungi such as *Mucor* spp. may obscure slowly growing ones such as *Wallemia* spp.. In addition, organisms present at high concentrations may inhibit the growth of less abundant species (MacNeil et al., 1995; Portnoy et al., 2004). The indoor temperatures are usually in the mesophilic range of microbes and therefore, incubation is usually performed at (25 ± 3) °C for a duration of at least 5 days (Samson et al., 1994). Commonly used media for indoor fungi and bacteria are presented in Table 3. The advantages of culture include the possibility to identify the microbial genera or species in samples and to isolate the strains for further characterization. Morphology based identification is usually performed using microscope, but also direct identification of species by image analysis using accurate digital camera is possible (Dörge et al., 2000). However, culturing is time consuming, labor intensive and therefore, a costly technique.

Table 3. Most commonly used or recommended media for different microbial groups in studies of indoor environments.

Microbes	Medium
Hydrophilic fungi	Malt extract agar (MEA)[2,4,5,9,10,12,13,14,15,19,23,27] 2 % MEA[4,7,8,17,18,24,26,29] Media with Rose Bengal[3,25,27]
Xerophilic fungi	Dichloran 18 % glycerol agar (DG18)[4,7,8,13,17,18,19,26,28,29]
Mesophilic bacteria	Tryptone soy agar (TSA)[1,4,8,9,13,16,19,22] Tryptone yeast extract glucose agar (TYG)[7,17,18,20,21,23,25] R2A[4,6,16,19]
Gram-negative bacteria	Eosin methylene blue agar (EMB)[5,16]
Thermophilic actinomycetes	Nutrient agar[11] Half-strength nutrient agar[21] TSA[11] or sample heating before plating[11]

[1](Andersson et al., 1999), [2](Awad & Farag, 1999), [3](Beguin & Nolard, 1994), [4](Burge, 1995), [5](Górny et al., 1999), [6](Hyvärinen et al., 1991), [7](Hyvärinen et al., 2002), [8](Jo & Seo, 2005), [9](Kim & Kim, 2007), [10](Kuo & Li, 1994), [11](Kutzner, 1986), [12](Lee et al., 2004b), [13](Lee & Jo, 2006), [14](Lee et al., 2006), [15](Li & Kuo, 1994), [16](Liu et al., 2000), [17](Meklin et al., 2003), [18](Meklin et al., 2005), [19](Muilenberg, 2003), [20](Nevalainen, 1989), [21](Nevalainen et al., 1991), [22](Obbard & Fang, 2003), [23](Pessi et al., 2002), [24](Ramachandran et al., 2005), [25](Reponen et al., 1992), [26](Samson et al., 1994), [27](Shelton et al., 2002), [28](Stark et al., 2003), [29](van Reenen-Hoekstra et al., 1991)

2.4.2 Non-molecular methods detecting total counts of microbes

In addition to culturable microbes, non-culturable and non-viable microbes are important as exposing agents because they can also cause adverse health effects by evoking allergic or toxic reactions (Hirvonen et al., 1997b; Levetin, 1995). Methods for detecting these microbes include microscopic techniques, bioassays, immunoassays, chemical methods, and molecular methods. Microscopic analyses, usually by epifluorescence microscopy with acridine orange staining (Kepner & Pratt, 1994) or impaction on coated slides with lactophenol staining (see Table 1), enable total cell counts or spore counts, respectively, but identification of fungal species is not possible (Pasanen, 2001). Total cell concentrations of bacterial and fungal bioaerosols can be measured by flow cytometry (Day et al., 2002; Lange et al., 1997).

Microbial communities can also be quantified by chemical methods using markers for the structural or constituent components of microbes. One advantage is that these substances can be used for characterizing and quantifying particular microbial groups. The most commonly used chemical markers are listed in Table 4.

Table 4. Chemical markers used for microbial characterization and quantification.

Microbial group	Constituent	Chemical marker	Reference
Gram-negative bacteria	Endotoxin (lipopolysaccharide=LPS)	3-hydroxy fatty acids (10-14 carbon chains)	1,4,5,7,10,12,13,14
Actinobacteria		3-hydroxy fatty acids (16 or more carbon chains)	11
Total bacteria	Peptidoglycan	Muramic acid	4,7,10,13
Fungal biomass	Ergosterol		3,6,8,10,12,13,14
Fungi (and certain bacteria, some plants, pollen)	(1→3)-β-D-glucan		3,9
Aspergillus and *Penicillium*	extracellular polysaccharides (EPS)		2

[1](Alwis et al., 2006), [2](Douwes et al., 1999), [3](Foto et al., 2005), [4](Fox et al., 2003), [5](Lee et al., 2004a), [6](Miller & Young, 1997), [7](Nilsson et al., 2004), [8](Pasanen et al., 1999), [9](Rylander, 2004), [10](Sebastian & Larsson, 2003), [11](Sebastian et al., 2005), [12](Szponar & Larsson, 2000), [13](Szponar & Larsson, 2001), [14](Szponar et al., 2003b)

In an experiment where schoolroom conditions were monitored, it was found that the concentrations of muramic acid and 3-hydroxy fatty acids in occupied schoolrooms were much higher than in unoccupied rooms, reflecting the fact that children were the main source of the indoor bacteria (Fox et al., 2003). The plant sterol, ergosterol, has been found to be a suitable marker to estimate fungal biomass in samples from damp building material (Hippelein & Rügamer, 2004). Airborne levels of ergosterol and glucan have correlated with the visible mold damage in a house (Foto et al., 2005).

The *Limulus* amoebocyte lysate assay (LAL) is the most commonly used bioassay for endotoxin measurements (Eduard, 1996). Immunoassays for specific antigens and allergens include enzyme-linked immunosorbent assay (ELISA) and the radio allergo-sorbent test (RAST) (Eduard, 1996).

2.4.3 Molecular methods in detection of indoor microbes

The identification of specific bacterial or fungal species has developed greatly as the techniques based on DNA analyses have become available. These methods allow the specific detection of target organisms. With respect to environmental samples, a variety of these techniques has been successfully applied, including the qPCR, restriction fragment length polymorphism (RFLP) analysis, random amplified polymorphic DNA (RAPD) analysis, and multiplex reverse transcription-PCR (RT-PCR)

(Degola et al., 2007; Isik et al., 2003; Vesper et al., 2000; Vesper et al., 1999). These techniques serve different purposes, qPCR is used for quantitative detection, RFLP, RAPD and RT-PCR are used for, e.g., differentiation of species or strains from each other.

The PCR method can be applied to any biological organism containing DNA. In PCR, a sequence of DNA, usually a specific gene or portion of a gene is selected and multiple copies are produced by an enzymatic PCR reaction. The gene sequence selected can also be from a microbial functional gene. The sequence chosen should be specific for the microorganism or a group of microorganisms (Peccia & Hernandez, 2006). These template DNA sequences can be targeted, amplified and quantified by designed DNA primers. The DNA primers used to detect bacteria or fungi are commonly sequences from the 16S or 18S rRNA gene, respectively (Cruz-Perez et al., 2001a).

Extraction of DNA and PCR procedure: Before DNA can be analyzed, it must be extracted from cells. The nucleic acid extraction consists of cell lysis and nucleic acid purification. Enzymatic, chemical or physical methods or their combinations are used to disrupt cell or spore walls which protect the genomic DNA, (Cruz-Perez et al., 2001a). For most environmental bacteria and fungi, an additional step such as bead beating/milling using high energy agitation with micron-sized beads or rapid freeze-thaw cycling is needed for complete cell wall disruption since this will increase the DNA yield (Haugland et al., 2002; Haugland et al., 1999a; Peccia & Hernandez, 2006; Saad et al., 2004).

DNA purification techniques are used for the removal of PCR inhibitory compounds produced by fungal cell lysates, the dust or particulate matter. The purification can be done in many ways, by phenol: chloroform extraction (Dean et al., 2004; Williams et al., 2001) or using microcentrifuge spin columns with cleanup and elution (Haugland et al., 2002). The purification of the DNA extracts is an important step, but depending on the purification method and the type of sample, it may result in a high loss of DNA with recoveries $\leq$ 10 % (Brinkman et al., 2003). This step can, therefore, cause a major limitation particularly in environmental samples with low levels of microbial contamination (Keswani et al., 2005).

The main steps of the PCR procedure are shown in Table 5. Usually, the actual PCR is carried out in three steps, often preceded with one temperature hold at the beginning and one at the end. A typical number of cycles is 20 to 35. Sensitivity of the method can be increased by increasing the number of cycles (Dean et al., 2004).

Table 5. Usual PCR procedure.

Step	Temperature (° C)	Duration	Main action
Initialization	94-96	1-15 min	Ensuring denaturation
Denaturation	94-98	20-30 sec	Cycling begins
Annealing	50-64	20-40 sec	Primers attach to the single-stranded DNA template
Extension/elongation	72	1 min	DNA polymerase synthesizes new DNA strands complementary to the DNA template strands
Final elongation	72	5-15 min	Ensuring full extension
Storage	4-15		Short time storage of the reaction

After the PCR amplicon is obtained, there are several options for the post PCR analysis. Microbial population studies include the DNA fingerprinting, clone library analysis, and DNA microarray hybridization.

Quantitative PCR analysis in indoor studies: QPCR is a faster method than conventional PCR (Zeng et al., 2004). To measure the amplicon concentration, dual-labeled fluorophore-containing DNA probes, such as TaqMan (Heid et al., 1996) or fluorescent dyes, such as Sybr Green are used. Estimations of *Cladosporium* spp. concentrations resulted in similar values with both of these dyes (Zeng et al., 2006). The labeled probe fluoresces when it becomes bound to the target DNA amplicon and if this fluorescence data is reported for each cycle, then the term real-time PCR may also be used. The increase in the amplicon concentration is seen as an increase in the fluorescence intensity, which is monitored with a sequence detector (Stetzenbach et al., 2004). Cycle threshold (Ct) is the cycle number where a statistically relevant increase in the fluorescent signal from the background is first observed. The quantification of the target organism can be based on the co-amplification of an internal control which might be another fungus (e.g., *Geotrichum candidum*) (Haugland et al., 1999b) or on direct comparison with standards of the target organism, a technique also called absolute quantification (Cruz-Perez et al., 2001b; Peccia & Hernandez, 2006).

Most of the techniques used include primers and probes assigned to identify specific fungi or groups of fungi, although universal PCR assay for fungi has also been developed (Zhou et al., 2000). The identification of many fungal species in a single PCR reaction is also possible, for instance by particle-associated nucleic acid PCR (PAN-PCR) (Dean et al., 2006). In indoor studies, qPCR has been most commonly used in analyzing microbes in house dust samples (Meklin et al., 2004; Vesper et al., 2000; Vesper et al., 2007; Vesper et al., 2006a; Vesper et al., 2006b; Vesper et al., 2004; Vesper et al., 2005). In some studies, air samples have been taken for qPCR

analysis (Goebes et al., 2007; Meklin et al., 2007b; Vesper et al., 2000; Zeng et al., 2004; Zeng et al., 2006).

The precision of quantitative PCR is ± 6 – 21 % (Rutledge & Côté, 2003), but there are several possible limiting factors. These include reagent and operator variability and a lack of standardization in data analysis (Burns et al., 2005; Bustin, 2002). In addition, potential cross-reaction of PCR may occur during the analysis (Jensen et al., 1999; Pyankov et al., 2007). Many of the problems associated with differing amplification efficiencies between standards and samples may be removed by using the sigmoidal curve-fitting method (Rutledge, 2004).

Inhibition in qPCR: In environmental samples, inhibitors of qPCR which can give rise to false negative results may be a concern. The inhibition can be due to biological or nonbiological contaminants (Buttner et al., 2001). It is possible to control for this kind of inhibition i.e. by using internal positive controls / standards or internal amplification controls (McDevitt et al., 2004; Paterson, 2007; Stetzenbach et al., 2004). When the inhibition is total, both the sample and internal positive control give negative results. With partial inhibition, the sample results are positive, but Ct for the internal positive control is higher than its self-amplification result (Cruz-Perez et al., 2001a). Inhibitors can also be controlled by the dilution of sample or reference assay in addition to the purification of the DNA samples but this decreases the sensitivity of the assay (Cruz-Perez et al., 2001a; Cruz-Perez et al., 2001b; Haugland et al., 2004; Keswani et al., 2005; Roe et al., 2001). If the inhibitors are known, there are other ways to overcome the inhibition, i.e., the addition of a component to the PCR reaction with a higher affinity for the inhibitor (Keswani et al., 2005) or using thermostable DNA polymerase more resistant to inhibitors (Al-Soud et al., 2000). PCR-negative samples can be subjected to the PCR amplification with universal bacterial primers pA and pH as a control for possible inhibition (Edwards et al., 1989).

Microbial concentrations obtained by qPCR vs. culture: It is not straightforward to compare the results obtained with the culture and qPCR techniques. Quantification of culture results is based on colony forming units, i.e., including the possibility that one colony has originated from an aggregate of several spores. Instead, PCR quantifies all DNA from the particular species or assay group, independent on whether the material originates from culturable or non-culturable spores or their fragments or mycelial material. In addition, qPCR usually is species-specific, whereas the morphological identification connected to culture method is often at the genus-level. Therefore, the qPCR method has been found to be more sensitive than culturing (Brinkman et al., 2003; Buttner et al., 2007; Buttner et al., 2004a; Meklin et al., 2004). A strong correlation was observed between total plate counts and total numbers of *Candida* species determined with qPCR in water samples (Brinkman et al.,

2003). In indoor environments, concentrations obtained by qPCR have been much higher than those obtained by culturing. Airborne concentrations of *Cladosporium* spp. and *Wallemia sebi* by qPCR method were ten-fold higher than those found by cultivation (Zeng et al., 2004; Zeng et al., 2006). Concentrations of *Aspergillus* spp. in house dust by qPCR were 2- to 3-fold higher than those obtained by culturing (Meklin et al., 2004). In an experiment evaluating nine fungal species, the concentrations in house dust correlated weakly but significantly for five species (Vesper et al., 2006b).

2.5 Streptomycetes

Streptomycetes are filamentous gram-positive bacteria belonging to the class *Actinobacteria* and the order *Actinomycetales* (Stackebrandt et al., 1997). They are typical in soil and many other habitats, such as composts, fodders, and aquatic environments (Kutzner, 1986). In fact, they are ecologically important in the degradation of soil litter (Kutzner, 1986). Streptomycetes have a versatile potential for the production of secondary metabolites. Some of these metabolites are useful to man, as they are antibiotics (Lee & Rho, 1993). However, some of the secondary metabolites may be toxic, such as valinomycin (Andersson et al., 1998), potentially irritating, such as amines and sulphur compounds (Claeson & Sunesson, 2005; Wilkins, 1996) and some are volatile and odorous, such as geosmin (Pollak & Berger, 1996; Sunesson et al., 1997).

2.5.1 Growth demands of streptomycetes

The optimum growth temperature for most streptomycetes under laboratory conditions is (25 – 35) °C, while thermophilic streptomycetes are also found (Pridham & Tresner, 1974). The life cycle of streptomycetes involves physiologically distinct stages including spore germination, vegetative hyphal growth, the production of aerial hyphae, and sporulation (Ensign, 1978).

Streptomycetes do not need organic nitrogen and they can utilize many biological polymers, such as cellulose, lignin and chitin as well as sugars, e.g., glucose, xylose, arabinose, and fructose as their carbon source (Kutzner, 1986; Pridham & Tresner, 1974). Glucose has been shown to have a dual effect, in some cases, glucose has been the primary carbon source during growth (Karandikar et al., 1996; Rho & Lee, 1994). However, high levels of either phosphate or glucose have inhibited aerial mycelium formation and sporulation (Ensign, 1978; Ochi, 1986) due to the acidification of the medium following glucose metabolism (Madden et al., 1996; Ochi, 1986). It is possible that acids are consumed when glucose has not inhibited growth

and thus, growth inhibitory pH levels (< 5 for *Streptomyces coelicolor*) are avoided (Viollier et al., 2001). Furthermore, acidification may be prevented by the presence of some nutrients such as adenine, ammonium salts, starch or maltose (Dekleva & Strohl, 1987; Madden et al., 1996; Surowitz & Pfister, 1985). Resistance to the acidification has also increased when nitrate has been used as a nitrogen source (Karandikar et al., 1997), because the consumption of nitrate is associated with a rise in pH. Certain nitrogenous compounds may be associated with streptomycetes differentiation: the depletion of nitrate from the medium coincided with the initiation of aerial mycelium formation and thereafter spore formation and a decrease in medium pH (Karandikar et al., 1997). In addition, sodium nitrate and asparagine, acting as growth limiting factors, were found to promote spore formation (Karandikar et al., 1996).

Under laboratory conditions, good growth and sporulation of microbes are needed for identification. Many compounds can stimulate the sporulation and the growth of *Streptomyces* spp., i.e., yeast extract, cysteines, bacitracin, glutathione, β-nicotinamide adenine dinucleotide, 2 % casamino acids, ammonium, L-asparagine, and carbon sources such as acetate and glycerol (Kutzner, 1986; Novella et al., 1992; Ogata et al., 1992).

The optimal pH range for the growth of streptomycetes is in the neutrophilic range of 6.5 – 8.0 on laboratory media (Pridham & Tresner, 1974). However, basidiophilic and acidophilic streptomycetes also exist (Kutzner, 1986). The acidophilic actinobacteria can be divided into two main groups: strict acidophilic and neutrotolerant acidophilic groups including also streptomycetes, the optimal pH range of which has been found to be 5.5 – 6.5 (Cho et al., 2006). However, the growth of streptomycetes causing potato scab was inhibited at pH values 4.8 – 5.1, but not at 4.0 (Loria et al., 1986). In fact, a high organic load has enabled streptomycetes growth in acidic conditions (Hagedorn, 1976) and in soil with complex substrates like chitin or dead fungal mycelium (Kutzner, 1986). Nutrients can also affect sporulation. In moderate acidic and basic pH values (pH 5.0 – 9.0), sporulation of *Streptomyces antibioticus* was not affected, indicating that sporulation is triggered by a nutritional downshift (Novella et al., 1992). In submerged culture with glucose, optimal sporulation has been detected at pH 7.0, whereas at alkaline pH, the extent of sporulation has been repressed (Rho & Lee, 1994).

Streptomyces coelicor has been shown to possess metabolic pathways supporting anaerobic growth. Its biomass increased in liquid standing cultures under anoxic conditions (van Keulen et al., 2003). Recently, streptomycetes have been shown to germinate at low humidity (a_w 0.67) and even at a_w 0.50 (Doroshenko et al., 2005). This is an important finding, since it has been generally assumed that microbial

growth can only start at a_w 0.7 on laboratory media and at a_w 0.8 on building materials (Grant et al., 1989).

2.5.2 Media for isolation

The isolation of streptomycetes is important for the further characterization of the strains. Sample pre-treatments, such as heating and chemical treatment (soil treated with calcium carbonate), or using selective nutrients in media may improve the sensitivity when isolating streptomycetes from environmental samples (El-Nakeeb & Lechevalier, 1963). The addition of antifungal antibiotics such as pimaricin, nystatin or cycloheximide onto media can prevent fungal growth (Porter et al., 1960).

Various media have been used for the isolation of streptomycetes from environmental samples. Media may contain different carbon and nitrogen sources such as glucose and asparagine, glycerol, potassium nitrate, peptone, casein, and starch (Küster & Williams, 1964). For soil samples, an arginine-glycerol-salt medium was found to be the most suitable for isolation of actinomycetes, the other tested media were chitin medium, modified Benedict's medium, soybean meal-glucose medium, Gauze's agar medium, Czapek's agar medium, egg albumen medium, glucose-asparagine medium, and glycerol-asparaginate agar (El-Nakeeb & Lechevalier, 1963). However, arginine has favored the growth of fungi and therefore, media with glycerol or starch as the carbon source and nitrate or casein as the nitrogen source were preferred, the best being starch/glycerol-casein-KNO_3 agar (Küster & Williams, 1964). Furthermore, actinomycetes are able to grow on extremely meager media, such as on water agar (El-Nakeeb & Lechevalier, 1963).

An experiment was conducted to test 14 different media compositions (Nabais & da Fonseca, 1995). Moderate growth and abundant sporulation occurred on malt and yeast extract or dextrin-yeast extract media. The addition of $CaCO_3$, replacing yeast extract by a combination of yeast and meat extract and peptone as well as the use of higher carbon-nitrogen ratios improved sporulation. Biomass production of *Streptomyces tendae* was supported on American Type Culture Collection (ATCC) sporulation agar, whereas ATCC sporulation agar and Hickey-Tresner (HT) media supported sporulation (Dionigi et al., 1992).

It has been shown for soil bacteria that even minimal changes in the cultivation strategy, such as nutrients and extended incubation time can result in the isolation of previously uncultured, phylogenetically novel bacteria (Sait et al., 2002). The protein fraction extract medium is a new medium for actinobacteria including streptomycetes (Szponar et al., 2003a). It is based on the waste product of beer production,

spent barley grains, and it has been found to be easy to prepare. Furthermore, it promotes rapid spore formation.

Despite the many advantages of molecular methods, innovative selective isolation media are still needed to find rare and uncommon streptomycetes, which can be promising producers of antibiotics and other secondary metabolites. It is well known that the cultivation conditions need to be optimized to achieve the best antibiotic production (Arun & Dharmalingam, 1999; Duangmal et al., 2005; Ishida et al., 2006; Large et al., 1998; Teodoro et al., 2006).

2.5.3 Occurrence of streptomycetes in indoor environments

Actinomycetes including streptomycetes can also be found in indoor environments. They have been isolated from air samples, building materials, and house dust samples from moisture-damaged buildings (Andersson et al., 1997; Hyvärinen et al., 2002; Nevalainen et al., 1991; Rintala et al., 2004). In fact, their presence has been suggested as being indicative of moisture damage (Nevalainen et al., 1991). It is even possible that streptomycetes are among the first pioneer species appearing in moisture-damaged sites in buildings, based on their low needs for nutrients and a_w (Doroshenko et al., 2005; El-Nakeeb & Lechevalier, 1963).

In dust samples, their concentrations have been up to 10^6 cfu/g when this is measured by culturing (Andersson et al., 1999; Awad & Farag, 1999; Rintala et al., 2004). By qPCR, the mean concentrations have ranged from 4×10^4 cells/g to 1.2×10^6 cells/g of dust (Rintala & Nevalainen, 2006). The amount of *Streptomyces*-specific PCR amplification product was significantly higher in moisture-damaged homes than in reference homes (Rintala et al., 2004). In warm climates such as in Brazil and Egypt, actinomycetes have been found in indoor air including homes in concentrations of (11 – 634) cfu/m^3 (Awad & Farag, 1999; Grigorevski-Lima et al., 2006) but in these studies, the presence of moisture damage was not discussed.

Streptomycetes have been found to induce stronger inflammatory responses and cytotoxicity effects than fungi (Hirvonen et al., 1997a; Hirvonen et al., 1997b; Huttunen et al., 2003). These responses have been affected by available nutrients, the building material in question and microbial interactions on the growth site (Hirvonen et al., 2001; Murtoniemi et al., 2001; Penttinen et al., 2007; Roponen et al., 2001b).

2.6 Mycobacteria

Environmental mycobacteria are common in soils, waters, and water distribution systems (Falkinham, 1996; Vaerewijck et al., 2005). They belong to genus *Mycobacterium*, order *Actinomycetales*, and class *Actinobacteria* (Stackebrandt et al., 1997). Some of the environmental mycobacteria are potential pathogens (Falkinham, 1996; Vaerewijck et al., 2005). Mycobacteria are usually not detected by standard microbial culture methods, because their isolation requires selective methods and long incubation periods (Torvinen et al., 2006).

During dismantling of moisture-damaged buildings, mycobacteria have been isolated from air (Rautiala et al., 2004), and these strains have induced inflammatory responses in both human and murine cells (Huttunen et al., 2001; Huttunen et al., 2000). Environmental mycobacteria have been isolated from moisture-damaged material samples (Andersson et al., 1997; Torvinen et al., 2006; Vuorio et al., 1999). In fact, they have been found in 23 % of moisture-damaged material samples (median 10^3 – 10^6 cfu/g) (Torvinen et al., 2006). The percentage of positive samples increased with the increasing concentration of fungi (Torvinen et al., 2006). In addition, a new species of mycobacteria has been isolated from indoor materials (Vuorio et al., 1999).

2.7 Fungi in indoor air

2.7.1 Airborne fungal concentrations

Fungal concentrations in the indoor air have been studied in various indoor environments and climates using different methods. In a review of over 20 studies, the average indoor air concentration in 820 non-complaint residences has been 1252 cfu/m^3, ranging from 17 to 9100 cfu/m^3 (Gots et al., 2003). Microbial conditions in moisture-damaged buildings are presented in Chapter 2.2.2.

In relation to moisture conditions of the buildings, kitchens constitute a special facility due to water usage and cooking. In domestic kitchens, higher GM values for total viable fungi (Drahonovska & Gajdos, 1997) and *Cladosporium* have been detected than are encountered in other rooms (Lee & Jo, 2006). In institutional kitchens, however, fungal concentrations may be low. In a hospital kitchen, fungal concentrations have varied from 13 cfu/m^3 to 106 cfu/m^3 (Marchant et al., 1990).

In cold climates during wintertime with snow cover on the ground, outdoor concentrations of fungi are low and in consequence, also indoor fungal concentrations are typically low, (10^1–10^2) cfu/m^3 (Reponen et al., 1992). In long-term sampling, me-

dian values for culturable fungi have varied between seasons from 37 % to 87 % (Lee et al., 2006) or between 0.7 – 79 % (Niemeier et al., 2006).

It is known that measured airborne fungal concentrations are highly dependent on the sampling and analytical method used (Lee et al., 2004b; Nevalainen et al., 1992). Total airborne concentrations have been 3 – 1000 times higher than concentrations by culturing (Angenent et al., 2005; Fabian et al., 2005; Flannigan, 1997; Toivola et al., 2004; Wu et al., 2002; Wålinder et al., 2001).

2.7.2 Airborne fungi in school environments

In schools, fungal concentrations have been found to be lower than those present in homes (Dotterud et al., 1996), but higher than in bars and internet cafes (Jo & Seo, 2005). One reason for the different levels in these buildings may be differences in ventilation, occupancy and climate. In cold climates such in Finland, fungal concentrations in schools in wintertime seem to be extremely low, at the level of $10^0 – 10^1$ cfu/m^3, and in schools with no moisture damage, it is common to obtain samples with concentrations below the detection limit (Meklin et al., 2003; Meklin et al., 2005). In the school environment, the most common fungi have been *Penicillium* spp., *Aspergillus* spp., yeasts, *Cladosporium* spp. and *Mucor* spp. (Dotterud et al., 1995; Meklin et al., 2005; Ramachandran et al., 2005). A seasonal variation in fungal concentrations was also seen in school environments (Ramachandran et al., 2005). A statistically significant relationship was established between the CO_2 concentration reflecting of air exchange rates and fungal concentrations in schools (Ramachandran et al., 2005). The flooring material can have an effect on airborne fungal concentrations. In non-problem schools, airborne fungal concentrations were three times higher over tiled floors than over carpet (Foarde & Berry, 2004), although the concentrations in dust did not differ between these flooring materials.

2.7.3 Fungal flora in the indoor air

The genera *Cladosporium*, *Penicillium*, and *Aspergillus* are common in the indoor air all over the world in various climates (Awad & Farag, 1999; Beguin & Nolard, 1994; Burge, 2002; Cooley et al., 1998; de Ana et al., 2006; Górny & Dutkiewicz, 2002; Górny et al., 1999; Kim & Kim, 2007; Kuo & Li, 1994; Lee & Jo, 2006; Lee et al., 2006; Li & Kuo, 1994; Li & Kendrick, 1995; Osborne et al., 2006; Pasanen et al., 1992b; Shelton et al., 2002; Stark et al., 2003). In addition, the genus *Alternaria* is common in many countries (Awad & Farag, 1999; Bardana, 2003; Cooley et al., 1998; de Ana et al., 2006; Jo & Seo, 2005; Lee & Jo, 2006; Li & Kendrick, 1995; Osborne et al., 2006). The overall mycobiota is extensive: recent studies have shown

that about 400 fungal species may be found in residences (Gutarowska & Piotrowska, 2007).

Fungi in indoor environments mainly originate from outdoor sources. Thus, common fungal genera found indoors are the same than in outdoor air (Shelton et al., 2002). In case of moisture damage, however, also indoor sources can be significant. Microbes involved in moisture-damage may differ from common microbes in indoor environments as is presented in Chapter 2.2.2.

2.7.4 Particle size of airborne fungi

It is estimated that 70 – 95 % of airborne viable fungi are of respirable size (< 4.7 μm) (Li & Kuo, 1994). The highest fungal concentrations were detected in the size range of 2.1 – 3.3 μm in Finland and in a subtropical climate (Lin & Li, 1996; Reponen, 1995) and between 2.1 – 4.7 μm in Poland (Górny et al., 1999). However, fungal fragments of sub-micron size can also be emitted from the microbial growth surface (Górny et al., 2002; Kildesø et al., 2003). It has also been shown that these fragments may contain mycotoxins (Brasel et al., 2005a).

2.7.5 Guidelines and recommendations for airborne fungal concentrations

There are no health-based guideline values for normal fungal concentrations in the indoor air. Different types of fungi may have different health effects, and at the present, no dose-response data exists for indoor fungi. Therefore, guidelines cannot be based on any observed health effects that could be linked to a certain concentration of fungi (Burge, 2002). In some papers, numerical values have been suggested that might be used to interprete the fungal measurement data. These values range from 50 to 10 000 cfu/m^3 (Burge, 2002; Górny & Dutkiewicz, 2002; Gots et al., 2003; Jo & Seo, 2005; Li & Yang, 2004; Rao et al., 1996; Reponen et al., 1992) with the corresponding value for schools being 1000 spores/m^3 (Santilli, 2002; Santilli & Rockwell, 2003). Ranges are broad due to different climatic conditions and different analyzing methods used in the studies. However, many of the proposed guidelines have been shown to be exceeded in non-air-conditioned and non-complaint homes (Burge, 2002; Gots et al., 2003). No international consensus exists about these interpretations.

Recommendations about how to evaluate IAQ problems in school environments have been published (Haverinen et al., 1999; Santilli, 2002; Wilson et al., 2004). In

Finland, there are recommendations both for schools and homes (Meklin et al., 2007a; Ministry of Social Affairs and Health, 2003).

2.8 Bacteria in the indoor air

GMs of bacterial concentrations in homes and hospitals have varied between 10 cfu/m^3 and 10^4 cfu/m^3 in different studies (Awad & Farag, 1999; Górny & Dutkiewicz, 2002; Lee & Jo, 2006; Nevalainen, 1989; Obbard & Fang, 2003). The main factor affecting the indoor bacterial concentration is the density and activities of occupants (Fox et al., 2005; Liu et al., 2000; Nevalainen, 1989). Occupation may also affect bacterial populations. When a building is occupied, the concentrations of gram-positive bacteria may be elevated and changes in gram-negative bacterial population may occur (Fox et al., 2005). In addition, thermal conditions may affect bacteria in the indoor air. Bacterial concentrations were higher at 20 °C than at 26 °C in a study conducted with six subjects in a simulated office work situation in an environmental chamber (Tham & Zuraimi, 2005). The microbial material is only a minor fraction of total particle concentrations in the air. Viable bacteria of different sizes represented < 1 % of the particle concentrations measured by an optical particle counter (Tham & Zuraimi, 2005).

The diversity of bacterial species present is wide, e.g., gram-positive cocci from *Staphylococcus* spp. and *Micrococcus* spp., which are abundant on human skin, pleomorphic organisms including diphtheroids, rods including *Bacillus* spp., as well as gram-negative *Pseudomonas* spp. and *Moraxella* spp. are common (Gallup et al., 1993; Gandara et al., 2006; Górny et al., 1999; Jaffal et al., 1997; Kim & Kim, 2007; Macher et al., 1991; Nevalainen, 1989; Obbard & Fang, 2003). The concentrations of gram-positive bacteria are generally higher than those of gram-negative bacteria in the indoor air (Fox & Rosario, 1994).

According to a review on the bacteria found in schools and day care centers, viable bacterial concentrations have ranged from 7 cfu/m^3 to 19 500 cfu/m^3 (Daisey et al., 2003). The most commonly observed bacteria were *Micrococcus* spp., *Bacillus* spp., pigmented gram-negative rods, coryneforms and *Staphylococcus* spp.

2.9 Microbes on surfaces

On undamaged surfaces in the indoor environment, the mycobiota can be quite similar to that found in the indoor air. *Cladosporium* is a common fungal genus in outdoor and indoor air, and it has been frequently encountered on undamaged surfaces (Beguin & Nolard, 1994; Ellringer et al., 2000). On visibly damaged surfaces, fungal concentrations between 10^5 cfu/cm^2 and 10^6 cfu/cm^2 have been detected (Jarvis

& Morey, 2001). The main species on moldy surfaces have been *Aspergillus versicolor*, *Cladosporium sphaerospermum*, *Penicillium chrysogenum*, *Ulocladium botrytis*, *Acremonium strictum*, *Phoma herbarum*, *Geomyces pannorum* and *Stachybotrys atra* (*chartarum*) or yeasts (Beguin & Nolard, 1994; Hunter et al., 1988; Macher et al., 1991). On damaged surfaces, the genus *Stachybotrys* has been detected in 30 % of water-damaged houses (N=200) in Texas (Kuhn et al., 2005).

Bacterial concentrations on kitchen surfaces were between 10^4 cfu/cm^2 and 10^7 cfu/cm^2 (Josephson et al., 1997). On damp surfaces, gram-negative rods and gram-positive cocci were found frequently in apartments, especially in kitchens, whereas gram-positive rods were also found on dry surfaces (Macher et al., 1991). In household kitchens, coliform bacteria (Rusin et al., 1998) and other gram-negative rods including pseudomonads (Scott et al., 1982; Speirs et al., 1995) have been found, especially on moist surfaces like sinks and their surrounding surfaces. Furthermore, pseudomonads, *Staphylococcus aureus* and *Bacillus cereus* were found from household kitchen floors.

2.10 Microbes in the house dust

House dust can be considered as being a reservoir for microbes. It is an extremely dry material, and therefore, microbial growth is unlikely to take place on the dust. However, it has nearly optimal pH for microbes and it consists mainly (82 %) of organic material, whereas outdoor dust contains only 18 % of organic matter (Piecková & Wilkins, 2004). However, in case of high RH (84 – 98 %), microbial growth in house dust can occur within a few weeks (Pasanen et al., 1997).

2.10.1 Microbial concentrations in the house dust

The total culturable fungal concentrations are usually in the range 10^3 cfu/g to 10^7 cfu/g in the floor dust (Beguin & Nolard, 1996; Chew et al., 2001; Dales et al., 1997; Gehring et al., 2001; Heinrich et al., 2003; Hicks et al., 2005; Horner et al., 2004; Verhoeff et al., 1994). However, concentrations of yeasts alone may be almost in the same range, 10^3 – 10^6 cfu/g (Glushakova et al., 2004). The microbial concentrations reported in dust samples depend greatly on the analyzing method in use (Verhoeff et al., 1994). If one utilizes qPCR, then fungal concentrations of specific species have been in the range of 10^5 spores/g to 10^8 spores/g (Vesper et al., 2005). Since dust acts as a microbial reservoir, fungal concentrations in the house dust may be related to the damp housing (Dales et al., 1997). However, this association is not clear (Müller et al., 2002). Similarly to airborne microbes in the indoor air, fungal concentrations in house dust exhibit seasonal variations with highest values found in

summer (Heinrich et al., 2003; Koch et al., 2000). In general, there is little debate so far on what kind of concentrations represent low or high levels of dustborne fungi (Rogers, 2003).

Concentrations of culturable bacteria in the house dust have ranged from 10^4 cfu/g to 10^8 cfu/g (Awad & Farag, 1999; Rintala et al., 2004). The dominating genera have been micrococci, *Bacillus*, *Staphylococcus*, *Paenibacillus*, and many actinomycetes including streptomycetes (Andersson et al., 1999; Awad & Farag, 1999).

2.10.2 Fungal flora in the house dust

The airborne concentrations and composition of microbes vary and, therefore, the dust flora representing a longer period of time, is not necessarily comparable to that found in a short period air sample. The most common taxa analyzed by culturing are often as follows: yeasts, *Penicillium*, *Cladosporium*, *Alternaria*, *Aureobasidium*, and *Eurotium* as well as non-sporulating fungi (Hicks et al., 2005; Rogers, 2003; Stark et al., 2003; Wickman et al., 1992). With the qPCR technique, the highest detected concentrations of the analyzed species (33 to 81) have mainly belonged to the same genera as found by culturing, i.e., *Cladosporium cladosporioides*, *C. herbarum*, *Epicoccum nigrum*, *Aureobasidium pullulans*, and *Eurotium* group (Vesper et al., 2006a; Vesper et al., 2006b; Vesper et al., 2005). Housing characteristics may have an influence on fungal concentrations in the house dust, at least at the genus level. The concentrations of *Alternaria* spp. have been high in the presence of dogs or carpeting, and low in apartments compared to other housing types (Chew et al., 2003).

In schools, no difference in the fungal composition or concentrations of house dust was noted between water-damaged and reference schools (Ebbehøj et al., 2005; Piecková & Wilkins, 2004). The most common dustborne taxa in schools have been similar to those found in domestic residences. The predominant fungi have been *Epicoccum* spp., *Alternaria* spp., *Cladosporium* spp. and miscellaneous yeasts (Foarde & Berry, 2004), and common airborne genera *Aspergillus* and *Penicillium* (Piecková & Wilkins, 2004). Carpet dust commonly contains fungi such as *Cladosporium* spp., *Epicoccum nigrum*, yeasts, and *Phoma* spp. (Ramachandran et al., 2005). Bacteria have also been detected from school dust samples. *Pseudomonas* spp. and *Streptomyces* spp. were found in 11 % and 9 % of dust samples taken from classrooms (Smedje & Norbäck, 2001).

2.11 Other aspects of exposure to biological agents

2.11.1 Inflammatory markers in nasal lavage fluid

The exposure of the occupants in damp buildings can be assessed by the determination of inflammatory markers in samples of nasal lavage fluid (NAL). These markers, e.g., lysozyme, eosinophil cationic protein, nitric oxide (NO), and many cytokines such as tumor necrosis factor alpha (TNF-α), interleukins (IL)-1, IL-1β, IL-4, IL-6, and interferon gamma (IFN-γ) are secreted by epithelial and inflammatory cells in the nasal mucosa (Hirvonen et al., 1999; Norbäck et al., 2000; Roponen et al., 2001a; Wieslander et al., 1999). These markers were shown to be elevated during a work period of personnel in a moisture-damaged school and to decrease during vacation (Hirvonen et al., 1999). An association between individual exposure in a moisture-damaged building and increased levels of the proinflammatory cytokines (TNF-α), (IL-1), (IL-1β), (IL-4), and (IL-6) in NAL or cytokine response as indicated by high levels of matrix metalloproteinase 9 has been reported also in other studies (Purokivi et al., 2001; Roponen et al., 2001a; Shoemaker & House, 2006). However, these kinds of association are not invariably found (Ebbehøj et al., 2005). There is some evidence that the increased levels of inflammatory markers seen in moisture-damaged building exposure may be due to mold exposure. An experimentally induced *Aspergillus fumigatus* challenge caused increased production of cytokines in previously exposed individuals (Stark et al., 2006).

2.11.2 Mites

Mites flourish in warm and damp environments where nutrition such as human skin scales, grain and fungi are available. House dust mites (HDM) are ubiquitous and one of the most common sensitizers present in indoor environments all around the world. In addition, storage mites are an important group of mites. They require a higher humidity level than HDM and they may be of importance in damp dwellings and workplaces (Iversen & Dahl, 1990; Pennanen et al., 2007). Storage mites can also cause sensitization (Iversen & Dahl, 1990; Pennanen et al., 2007; Warner et al., 1999; Vidal et al., 2004). Symptomatic employees in a moisture-damaged building displayed a high prevalence of positive skin prick test for storage mites (Roponen et al., 2001a). It has been teported that employees in grocery stores are exposed to storage mites (Koistinen et al., 2006).

2.12 Health effects of moisture damage and microbes in the indoor environment

Dampness, moisture damage and mold problems of buildings are strongly associated with adverse health outcomes. These include asthma and allergy, sick building syndrome (SBS), (chronic) respiratory infections, dry cough, eye irritations, skin symptoms including dermatomycosis, and non-specific symptoms as well as neurological problems, hypersensitivity pneumonitis, and rheumatic diseases. The association between adverse health effects and indoor moisture and molds is shown extensively. There are many reviews summarizing hundreds of studies (IOM, 2004, Bornehag et al., 2001; Bornehag et al., 2004; Douwes & Pearce, 2003; Li & Yang, 2004; Peat et al., 1998; Portnoy et al., 2005). However, no causal relationships between exposing agents and health effects have ever been established, as was also concluded by an expert evaluation report from the Institute of Medicine (IOM, 2004). However, according to that report, there is sufficient evidence to state that there is an association between mold and other agents in damp indoor environments and upper (nasal and throat) and lower respiratory tract symptoms (wheeze and cough), as well as asthma symptoms, and hypersensitivity pneumonitis in sensitized persons. In addition, an international workshop with 15 scientists from eight countries agreed that "exposure to molds may constitute a health threat to children resulting in respiratory symptoms in both the upper and lower airways, an increased incidence of infections and skin symptoms" (Rylander & Etzel, 1999).

Microbial exposure may be one of the causative agents of asthma. In recent studies, increased microbial exposure has been associated with asthma symptoms or atopy in adults (Matheson et al., 2005; Salo et al., 2006). Since 1995, indoor moulds from water-damaged buildings have become the most important causative agent for a diagnosis of occupational asthma in Finland (Piipari & Keskinen, 2005). According to a recent study, about 21 % (4.6 million cases) of asthma in the USA were attributable to exposure to dampness and mold, resulting in approximately $3.5 billion costs annually (Mudarri & Fisk, 2007). In children, it does seem that the presence of moisture damage and visible mould in the main living quarters may increase the risk of asthma in early childhood (Pekkanen et al., 2007).

In intervention studies, symptoms have reduced after moisture damage remediation. When water-damaged schools or homes of asthmatic children have been repaired, a reduction in symptoms and/or in asthma-related acute care visits has been reported (Kercsmar et al., 2006; Meklin et al., 2005; Putus et al., 2004; Savilahti et al., 2000; Åhman et al., 2000). However, when the school renovations were partial, symptoms were reduced only slightly (Meklin et al., 2005).

The relationship between microbial exposure and health is not clear at present. According to the so-called hygiene hypothesis, microbial exposure may also have protective effects against allergy and asthma. It has been observed that microbial exposure early in life, especially endotoxin exposure in farming environments or in homes with a cat or dog (Heinrich et al., 2001), may protect against atopy and asthma by stimulating T helper (Th)1 and inhibiting atopic Th2 immune responses (Braun-Fahrländer & Lauener, 2003; Böttcher et al., 2003). However, asthma can develop either through atopic, i.e., IgE-mediated, or non-atopic, that is non-IgE mediated, mechanisms. It is well known from occupational studies that endotoxin induces asthma through non-allergic mechanisms (Douwes et al., 2002).

2.13 Mechanisms of health effects

Various mechanisms to explain the adverse health effects of the moisture damage environment have been recognized: irritation (IOM, 2004) allergic mechanisms (IgE-mediated hypersensitivity) (Terr, 2004), toxic reactions to mycotoxins (Flannigan et al., 1991), fungal infection (fungus growing in the respiratory tract) (Verhoeff & Burge, 1997), and non-specific inflammation in the airways (Hirvonen et al., 1997a; Huttunen et al., 2003). There is experimental evidence for an association between inflammation and microbial exposure. It is known that the inflammatory and cytotoxic potencies vary significantly between microbial species (Hirvonen et al., 1997b; Huttunen et al., 2000) and also depend on growth conditions (Murtoniemi et al., 2001). Furthermore, interactions between different species have been detected and these interactions may potentiate the ability of the microorganisms to induce apoptosis and cell cycle arrest (Penttinen et al., 2005). Recently, indoor streptomycetes have been shown to be more potent inducers of inflammatory and cytotoxic effects than fungi (Hirvonen et al., 2005). It has been shown that adverse health effects are possible already at low airborne microbial concentrations; synergistic interactions between different exposing agents are one possible explanation for this phenomenon (Huttunen et al., 2004). The causative agents are still obscure and more information is needed about the mechanisms of adverse health effects.

In spite of extensive studies especially on indoor fungi, there is still a need for a better understanding of microbial exposures. A proper exposure assessment requires objective and accurate measurements of exposing agents, and a deeper understanding of the factors determining how, when and why these agents are produced.

3 AIMS OF THE STUDY

Five studies (Studies I-V) were conducted to characterize how the microbial conditions in indoor environments are affected by the use of the building (Study III), by the presence of moisture damage (Studies III, IV, and V), renovation of that damage (Study IV), and time (Study IV). A special focus was placed on the development of detection methods of an interesting bacterial genus, *Streptomyces* (Studies I and II).

The detailed objectives were:

1. to analyze how nutrients and pH affect the growth of streptomycetes and to find a good medium for cultivation of indoor streptomycetes (Studies I and II)

2. to characterize microbial concentrations in school kitchens, comparing them with other facilities in schools (Study III)

3. to investigate how moisture damage can affect the microbial flora in indoor environments (Studies III, IV, and V)

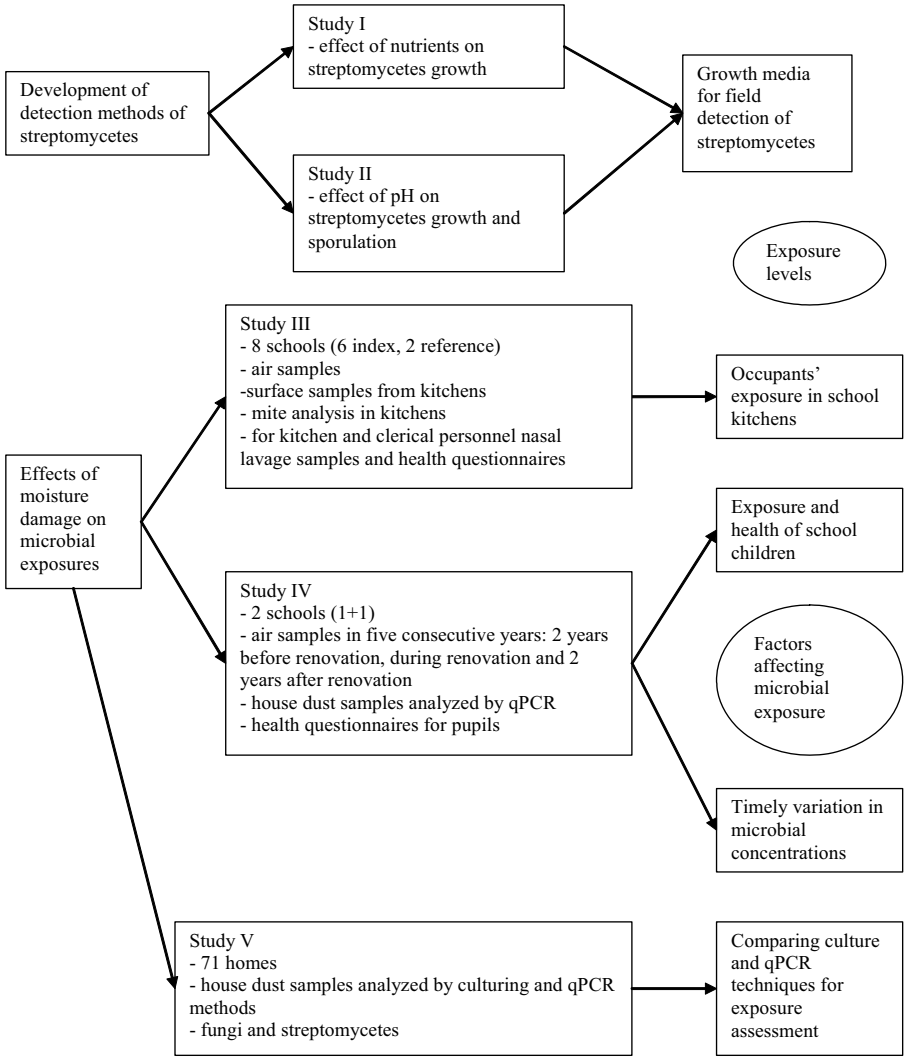
4. to analyze the effect of renovation on indoor microbes and pupils' health (Study IV)

5. to study long-term variation in microbial concentrations in a school environment (IV)

6. to survey the occupants' exposure and symptoms in moisture-damaged schools (Studies III and IV)

7. to investigate the usefulness of the qPCR method in assessing microbial content of house dust in relation to moisture damage (Study V)

Schematic presentation of the studies included in the thesis.



4 MATERIALS AND METHODS

4.1 Laboratory experiments

4.1.1 *Streptomyces* strains

Ten *Streptomyces* strains (VTT E-99-1326 – 1335) used in the Studies I and II were originally obtained from the culture collection of the Environmental Microbiology laboratory, KTL and stored in VTT, Culture Collection of the Technical Research Center, Finland, Biotechnology and Food Research. These strains originated from water-damaged buildings and were isolated using an Andersen six-stage impactor. They were characterized as *S. griseus*-like (7 strains), *S. albidoflavus*-like (2 strains) and *S. coelicolor*-like (1 strain) according to the 16S rDNA sequencing (Suutari et al., 2002). On the basis of biochemical tests, two of the *S. griseus*-like strains (VTT E-99-1326 and VTT E-99-1331) were identified as *S. californicus* and *S. anulatus*, respectively, at German Collection of Microorganisms and Cell Cultures (DSMZ). In order to prepare laboratory experiments, spore suspensions of *Streptomyces* grown on TYG-agar were harvested and inoculated on media at issue.

4.1.2 Growth media

In the growth experiment (Study I), a total of 26 media were used. The medium compositions are presented in Study I, Table 1. All the media were solidified with 1.5 % agar. Five of these, three nutritionally selective media: (i) starch casein KNO_3 –agar, (ii) actinomycete isolation agar with 0.01 g l^{-1} of $FeSO_4 \cdot H_2O$ instead of 0.001 g l^{-1} and (iii) glycerol arginine –agar (Kutzner, 1986) and two nutritionally complex media: TYG-agar and TSA, have been commonly used for the isolation of actinomycetes. The other 21 media were combinations of these media. Seven out of the 26 media were selected for a study examining how pH would affect growth (Study II, Table 1) either on the basis of favoring the growth of streptomycetes in Study I (six media) or on the nutritionally selective basis for the growth of actinomycetes (one medium: starch casein KNO_3).

The pH was adjusted with 1 or 2 M NaOH or HCl before autoclaving at 121 °C for 15 min. In the growth experiment (Study I), the pH was adjusted to 7.0 – 7.2. In the pH effect study (Study II), the pH range of the media was from 4.0 to 11.5 at intervals of 1.5 units. The pH 11.5 was adjusted from pH 10.0 after autoclaving. In Study II, the pH was checked with Ag/AgCl combination pH spear tip electrode (Orion

230A 91-63SC, Orion Research Inc., Boston, MA, USA) after autoclaving from a small amount of solidified agar and readjusted when necessary.

4.1.3 Visual assessment

After the incubation at 20 ± 2 °C, the growth (Study I) or growth and spore formation (Study II) of each strain was assessed visually on each Petri dish and recorded after 5 and 14 days to five groups presented as 20 %, 40 %, 60 %, 80 % or 100 % of maximum amount of mycelium/spores obtained for each strain. When the mycelium/spore formation was barely visible, microscopy (Labophot-2, Nikon, Tokyo, Japan) was used.

4.1.4 Error estimations

Mean of standard deviations (SDs) for growth and sporulation of ten strains at pH 7.0 were calculated based on two independent experiments with incubations of 14 – 16 days on media used in the pH effect study.

4.2 Field studies

4.2.1 The buildings studied

Studies were conducted in Central Finland in schools, including their kitchens, and in domestic residences. All the buildings were technically inspected by a trained civil engineer according a standardized protocol (Nevalainen et al., 1998). Visual signs of moisture were verified with a surface moisture recorder (Doser BD-2, Maxdoser Gmbh & Co.KG, Füssen, Germany).

Schools (Studies III and IV) were considered moisture-damaged when there were frequent signs of moisture and mold damage needing replacement of surface covering or opening, drying or renewing of structures. Reference schools had no moisture damage or only a few minimal signs of moisture. Study III was carried out in eight schools: six moisture-damaged and two reference schools. The classification as moisture-damaged or reference was also applicable to the kitchen facilities of these schools. Study IV was conducted for five consecutive years in two schools, one was moisture-damaged (index) with the other being the reference school. During the study, the moisture-damaged school was thoroughly renovated for all identified faults.

Study V was carried out in 81 residences belonging to a case-control study of childhood asthma. The homes represented a cross-section of Finnish housing. The resi-

dences were categorized into four classes depending on the extent of moisture damage in each home found during technical investigations: no detected moisture damage in class 0 (N=13), moisture damage located in one of the following parts of the home: bathroom, kitchen, living room and/or bedrooms, or in other rooms in class 1 (N=29), moisture damage detected in two of the above-mentioned areas in class 2 (N=13), and in three or all of those areas in class 3 (N=16).

4.2.2 Growth media, incubation and fungal identification

Growth media and incubation conditions used in the field studies are presented in Table 6. The fungi were identified morphologically to the genus level by light microscopy; *Aspergillus fumigatus*, *A. niger*, *A. ochraceus*, *A. penicillioides* and *A. versicolor* were identified to the species level.

Table 6. The growth media and incubation conditions.

Sample type (Study)	Fungi			Bacteria		
	Medium	Temp. (°C)	Time (days)	Medium	Temp. (°C)	Time (days)
Air (III, IV)	2 % MEA	25	7	TYG	20	5, 14[a]
	DG18	25	7			
Surface (III)	2 % MEA	25	7	TYG	20	5, 14[a]
	DG18	25	7	TYG 70 °C[b]	20	5, 14[a]
				EMB	35	1
				Media for mycobacteria[c]	30	90
Material (III)	2 % MEA	25	7	TYG	20	5, 14[a]
	DG18	25	7	TYG 70 °C[b]	20	5, 14[a]
				EMB	35	1
				Media for mycobacteria[c]	30	90
House dust (IV, V)	2 % MEA	25	7	TYG	20	5, 14[a]
	DG18	25	7			

[a)] Total number of colonies was counted after 5 days of incubation, number of actinomycete colonies was counted after 14 days of incubation
[b)] Samples at 70 °C water bath for 10 min before culture
[c)] Six media, contents described in Study III

4.2.3 Air samples

Culturable indoor microbes were sampled with six-stage impactors (Andersen 10-800; Graseby Andersen, Atlanta, Georgia, USA) (Studies III and IV). All the samples were taken during school days in winter. The total number of samples is presented in Table 7. Samples were taken from classrooms, corridors, teachers' rooms, gyms, and washrooms (these were called other facilities of the schools in Study III). In addition, kitchen facilities including kitchen, lunchroom of the school, and coffee

room for the kitchen personnel were sampled in Study III. In Study IV, air samples were taken in five consecutive years. In Study IV, fungal diversity analysis included the samples taken from exactly the same rooms every year. Sampling time was 15 min in Study III and 10 min in Study IV. Concentrations were counted as colony forming units per cubic meter of air (cfu/m^3) using positive hole correction (Andersen, 1958). Samples were taken in wintertime when the outdoor concentrations are extremely low (Reponen et al., 1992). When the temperature is below zero, agar media easily freeze. Therefore, no outdoor air samples were taken.

Table 7. Total numbers of indoor air samples taken from schools.

	Non-kitchen facilities		Kitchen facilities	
	Index	Reference	Index	Reference
Study III	207	90	75	30
Study IV	250	246	n.d.	n.d.
- fungal diversity analysis	195	225	n.d.	n.d.

n.d. = not determined

4.2.4 Surface samples

In Study III, surface samples were taken from kitchen surfaces to characterize further the microbial concentrations and genera in the school kitchen facilities. Samples were taken from damaged surfaces (N=34), their reference surfaces and other undamaged sites (N=71) as described in Study III. Samples (50 – 100 cm^2) were taken with a sterile swab into buffer solution (distilled water with 42.5 mg/l KH_2PO_4 × $7H_2O$, 250 mg/l $MgSO_4$, 8 mg/l NaOH and 0.02% Tween 80 detergent). Series of dilutions were plated on growth media and incubated as presented in Table 6. In addition, TYG at 70 °C and EMB-medium at 35 °C were used for 21 of the samples taken to detect the possible thermophilic and gram-negative bacteria present. From these media, 59 bacterial isolates were collected.

The Mycometer®-test, which is based on the activity of the enzyme β-N-acetylhexosaminidase, was used to detect mold growth on damaged surfaces (N=15) in four kitchens (Study III). Swab samples were collected by rubbing a 9 cm^2 surface area with a moist sterile cotton swab (Macher et al., 1999). Hydrolyzed enzyme was expressed as the fluorescence count, which was categorized into one of three empirically derived categories (Reeslev & Miller, 2000).

4.2.5 House dust samples

In Study IV, dust samples were vacuumed from the sofa in the teachers' room and from the baseboard of the gym onto a glass fiber filter (Munktell filter, Merck euro-

lab, Grycksbo, Sweden) (N=4). In Study V, house dust samples (N=81) were taken from the dust bag of the vacuum cleaners of the occupants. In the laboratory, the dust was sieved and the fine fraction was used for the molecular analysis. Ten samples were excluded from the analyses because they contained a remarkable amount of sand even after sieving.

Samples of Studies IV and V were stored frozen (−20 °C) for molecular analysis. DNA was extracted from 5 mg of dust by a rapid bead-milling method (Haugland et al., 2002). Before the qPCR reactions, DNA-samples were stored at −80 °C. Fungal qPCR analyses for 15 species or groups (Study V, Table 1) using co-extracted DNA from *Geotrichum candidum* as an exogenous reference were performed as described previously (Haugland et al., 2004) with the exception of using a reaction volume of 12 µl instead of 25 µl. All primer and probe sequences, as well as known species comprising the assay groups have been published at the website: www.epa.gov/microbes/moldtech.htm. The qPCR assay for *Streptomyces* spp. was performed as described previously (Rintala & Nevalainen, 2006). All the reactions were monitored with an ABI Prism 7000 real time-PCR instrument (Applied Biosystem). The numbers of spores or conidia were calculated using the standard calibration curves for each target assay groups.

In addition to qPCR analysis, culturing of house dust samples was performed in Study V. Dust (1 – 5 g) was extracted with a sterile buffer solution and the suspensions were held in an ultrasonic bath for 30 min and shaken at 400 – 600 rpm for 60 min. Serial dilutions were plated on the growth media (Table 6).

4.2.6 Other samples

In Study III, six material samples were taken from moisture-damaged sites in the index kitchens. Materials sampled included mainly plate mortar. The samples, 1 – 5 g, were extracted into the buffer solution, held in an ultrasonic bath for 30 min and in a shaker for 60 min. The growth media and incubation conditions are presented in Table 6. Two thermophilic bacteria were isolated. Bacterial isolates from surface and material samples were typed and identified by using colony morphology and routine bacteriological tests.

Mycobacteria were cultured from eight surface and six material samples (Study III). Culturing procedure and media used have been described in Study III and incubation conditions are presented in Table 6.

Dust mites in school kitchens (Study III) were analyzed from dust samples collected on fiberglass filters (MN 640 W) by vacuuming either the floor or an upholstered chair for 20 – 45 sec. Samples (N=18) were stored at −20 °C. From two sub-samples of 25 –

50 mg dust, mites were cleared in lactic acid, picked out under a stereomicroscope, mounted in Heinze PVA medium, counted and identified microscopically. The results were calculated as the number of mites in a gram of dust.

4.2.7 Study populations, nasal lavage and health questionnaires

In Study III, NAL was performed on 28 kitchen workers and 8 clerical personnel (reference group) of the studied schools. The individuals answered a one-page health questionnaire (Appendix 1) about symptoms encountered the preceding week with questions on laryngeal, lower airways, nasal, eye, dermal, and non-specific symptoms. Background factors such as atopic status of the occupants were obtained from a larger questionnaire that will be reported separately. NAL was performed as described earlier (Hirvonen et al., 1999). Nitric oxide (NO) in the NAL supernatant was assayed by the Griess reaction as the stable NO oxidation product nitrite (Green et al., 1982). Cytokines were analyzed by using DuoSet human TNF-α, IL-4, and IL-6 ELISA kits (R&D Systems Europe, Abingdon, Oxon, UK). Cytocentrifuge preparations were made by using 100 µl of resuspended cell suspension, in which the mucus was broken by addition of 0.5% dithiothreitol/0.1% bovine serum albumin. The solution was centrifuged and the slides were stained with May-Grünwald-Giemsa staining (Prat et al., 1993) for the cell differential counts. The Ethical Committee of the Kuopio University Hospital approved the study protocol of Study III.

In Study IV, schoolchildren's health and symptoms were examined over five consecutive years in two schools with questionnaires developed earlier (Meklin et al., 2002a) and consisting of 24 questions on respiratory and general health (Appendix 2). The parents were asked to fill in the questionnaires together with the child, because the pupils were in primary school. In the index school, a total of 2047 questionnaires were filled in as were 1081 questionnaires in the reference school.

4.2.8 Statistical methods

In field experiments, non-parametric tests were used, because normal distributions of the variables could not be found or obtained by logarithmic transformations. The statistical tests used are summarized in Table 8. The SPSS (III, V) and SAS (IV) statistical packages were used for analyses (SAS Institute Inc., 1999; SPSS Inc., 1988).

Table 8. Statistical tests used.

Purpose of use	Test
Correlation of fungal concentrations between - two media - culture and qPCR	Spearman rank correlation Studies III, IV, and V Study V
Difference in fungal and bacterial concentrations	Mann-Whitney test (Study III) Kruskal-Wallis one-way analysis of variance (Studies III and V) Dunn's post hoc test (Study III) (Zar, 1996) Repeated mixed model analysis (Study IV)
Difference in fungal particle size distributions	Repeated mixed model analysis (Study IV)
Inter-rater reliability between culture and qPCR	Cohen's Kappa (Study V)
Difference in NAL data	Mann-Whitney test (Study III)
Differences in symptom prevalence	Logistic regression and χ^2-test (Study IV)

5 RESULTS

5.1 Laboratory experiments

5.1.1 Effects of nutrients and pH on the growth of streptomycetes

Generally, yeast extract supported the best growth of streptomycetes, but did not always hasten the growth rate. Furthermore, glucose and tryptone supported the mycelium biosynthesis (Study I, Table 2). In contrast, when looking at the media in literature, the growth rate was slow on nutritionally selective actinomycete-isolation agar and glycerol-arginine agar. The amount of mycelium had a tendency to increase in the order: starch-casein < glycerol-arginine < glucose-tryptone < Na-caseinate-asparagine. No clear strain-specific differences appeared in the amount of mycelium.

The pH ranges supporting growth depended on which nutrients were provided. Starch-casein-KNO_3 agar enabled the broadest pH ranges for growth but resulted in a small amount of mycelium. The narrowest pH ranges within the neutral and basic area were obtained on media containing Na-propionate, NH_4NO_3, and yeast extract and from the media in literature, on glycerol-arginine agar. The greatest amount of mycelium appeared on TYG and TS agars (Study II, Table 2). The variation in the acid tolerance caused strain-specific differences in growth pH ranges. In terms of sporulation, the pH ranges followed those observed for growth. The sporulation capacity of streptomycetes was good, averagely between 53 % and 92 % of the mycelium was covered with spores, and independent of the pH and the medium composition. Thus, strain-specific differences were greater than those caused by the pH.

5.1.2 Error estimations

The means of standard deviations (SDs) were between 1.0 – 17.0 % for the growth of streptomycetes and between 2.0 – 17 % for the sporulation.

5.2 Field studies

5.2.1 Concentrations, flora and particle size distributions of airborne viable fungi

The concentrations of culturable fungi in indoor air in the index schools were higher than those found in the reference schools (Table 9). This phenomenon was evident both in kitchens ($p<0.05$) and other facilities ($p<0.05$) (Study III) as well as in the two years before ($p=0.0007$, $p=0.0353$) and in the year during the renovation ($p<0.0001$) (Study IV). Similarly, the fungal concentrations in several particle size classes were higher in the index school than in the reference school before and during renovation (Study IV, Fig. 4). The overall fungal concentrations found in the kitchens were lower ($p=0.026$) than in the other facilities of the schools. The concentrations in the index school decreased to the level of the reference school after the renovation and the differences in the particle size distribution also disappeared (Study IV, Fig. 4).

Table 9. The concentrations of airborne culturable fungi in index and reference schools.

Total concentrations	Index		Reference		p-value [b]	Study
	GM[a] (cfu/m^3)	Range (cfu/m^3)	GM[a] (cfu/m^3)	Range (cfu/m^3)		
Kitchen facilities	20	≤ 2 – 650	3	≤ 2 – 29	< 0.05	III
Non-kitchen facilities	30	≤ 2 – 411	7	≤ 2 – 507	< 0.05	III
	18 – 43	4 – 217	4 – 18	≤ 2 – 204	< 0.0001-0.0353	IV [c]
	6 – 10	≤ 2 – 64	6 – 8	≤ 2 – 51	n.s.-0.041	IV [d]

[a)] in the study of five years (Study IV), GM is presented as the range of GMs in different years
[b)] between index and reference schools
[c)] years 1, 2, 3 before and during renovation
[d)] years 4, 5 after renovation
n.s. not significant

The most frequent fungal genera were *Penicillium*, *Cladosporium*, yeasts, non-sporing isolates, and *Aspergillus*. In the index schools, concentrations of the genera *Penicillium*, *Cladosporium*, and yeasts were higher ($p<0.05$, study III) than in the reference schools.

In the index school (Study IV), the diversity of mycobiota was larger than in the reference school, altogether 37 fungal genera were found in the index school compared to 25 genera in the reference school. Furthermore, renovation had an effect on the mycobiota. Occurrence of several fungi, such as *Stachybotrys* spp., *Acremonium* spp. and *Aspergillus penicillioides*, as well as actinomycetes (belonging to bacteria)

increased during the renovation. After the renovation, these microbes were still present although their total concentrations had decreased significantly. Only after the thorough cleaning, did the microbial profile approach that of the reference school.

5.2.2 Concentrations of airborne bacteria

The concentrations of culturable bacteria in indoor air in the index school were higher ($p=0.0232$) than in the reference school before renovations (Study IV). This difference was, however, not evident when the facilities were divided into kitchens and other facilities of the schools (Study III). In the kitchens as a whole, the concentrations were lower ($p<0.001$) than in the other facilities of the schools and this difference remained ($p<0.05$) after classifying the schools into index and reference schools. Bacterial concentrations are summarized in Table 10. After the renovation and the thorough cleaning of the index school, the concentrations decreased. Actinomycetes were mainly detected during the renovation in the index school (GM = 12 cfu/m^3, Study IV) and they were also more frequent ($p = 0.004$, Study III) in index schools than in reference schools.

Table 10. The concentrations of culturable airborne bacteria in index and reference schools.

Total concentrations	Index		Reference		p-value [b]	Study
	GM[a] (cfu/m^3)	Range (cfu/m^3)	GM[a] (cfu/m^3)	Range (cfu/m^3)		
Kitchen facilities	213	18 – 2961	79	11 – 232	n.s.	III
Non-kitchen facilities	581	39 – 6004	503	50 – 6650	n.s.	III
	888 – 1673	54 – 35107	239 – 1336	≤ 2 – 6650	n.s.-0.0232	IV[c]
	185 – 440	0 – 4714	266 – 432	3 – 1439	n.s.	IV[d]

[a)] in the study of five years (Study IV), GM is presented as the range of GMs in different years
[b)] between index and reference schools
[c)] years 1, 2, 3 before and during renovation
[d)] years 4, 5 after renovation
n.s. not significant

5.2.3 Microbes on school kitchen surfaces

In kitchens, both fungal and bacterial concentrations on moisture-damaged surfaces were higher than on undamaged surfaces ($p<0.001$, $p=0.005$, respectively) (Table 11). Furthermore, the concentration of yeasts on damaged surfaces was higher ($p<0.05$) than on undamaged surfaces of the damaged kitchens. Yeasts were also the most frequent fungi found on all surfaces. Bacterial concentrations on undamaged

surfaces in the index kitchens were one or two orders of magnitude higher than in the reference kitchens in half of the samples.

Table 11. Microbial concentrations on kitchen surfaces.

Sample sites	Microbes	GM (cfu/cm^2)	Range (cfu/cm^2)
Damaged surfaces	Fungi	51	<0.25 – 1 500 000
	Mesophilic bacteria	1715	<0.25 – 4 400 000
Undamaged surfaces			
- Index kitchens	Fungi	3	<0.25 – 28 000
	Mesophilic bacteria	95	<0.25 – 3 600 000
- Reference kitchens	Fungi	2	<0.25 – 120
	Mesophilic bacteria	7	<0.25 – 2 600
Specific analyses	Thermophilic bacteria	19	<0.25 – 4 400
	Gram−-bacteria	499	<0.25 – 650 000
	Mycobacteria		1 – 25

Gram-negative rods were the dominant group of bacteria isolated from kitchen surfaces followed by gram-positive cocci, including *Micrococcus* spp. and *Bacillus* spp.

Results of the Mycometer-test were comparable with the cultivation results for 13 out of 15 samples (Study III, Table 2).

5.2.4 Microbes in the house dust

When assessed by culturing,the median concentrations of total fungi were 1.73×10^5 cfu/g (2 % MEA) and 1.28×10^5 cfu/g (DG18) (Study V). Results for genera/species of fungi varied between samples. Many fungal genera were detected in less than half of the samples. In fact, the median concentration was calculable only for *Penicillium* spp., *Cladosporium* spp., and the sum of *Aspergillus* spp., *Penicillium* spp., and *Paecilomyces* spp. Several fungal concentrations between 2 % MEA and DG18 correlated significantly, although the correlation values were not high (Table 2, Study V).

When evaluated by the qPCR method, microbial concentrations were mostly several orders of magnitude higher than the concentrations obtained by the culture method meaning the ratio between culturable and total microbial material was 1:100 or even less (Study V). The highest concentrations were detected for the group assay of *Aspergillus* spp., *Penicillium* spp., and *Paecilomyces variotii*; as well as for *Aureobasidium pullulans*, *Aspergillus penicillioides*, *Cladosporium cladosporioides*, *Peni-*

cillium brevicompactum/stoloniferum, and *Streptomyces* spp. both in schools (Study IV) and homes (Study V).

In the school environment (Study IV), the highest concentrations were found in the teachers' room of the renovated school. In homes (Study V), concentrations of *Penicillium brevicompactum/stoloniferum*, *Wallemia sebi*, *Trichoderma viride/atroviride/koningii*, *Cladosporium sphaerospermum*, *Eurotium amstelodami/chevalieri/herbariorum/rubrum/repens*, as well as the assay group for *Penicillium* spp., *Aspergillus* spp., and *Paecilomyces variotii* were significantly increased in parallel with increasing extent of moisture damage in the home. The trend was similar for *Streptomyces* spp., although the difference in the concentrations was not significant.

5.2.5 Other parameters measured

The concentrations of culturable fungi and bacteria in the material samples at moisture-damaged sites are presented in Table 12. In one material sample with the highest concentrations of actinomycetes and thermophilic bacteria, a relatively high number of mycobacteria was also found (4.0×10^6 cfu/g).

Table 12. Microbial concentrations in material samples.

Microbes	GM (cfu/g)	Range (cfu/g)
Fungi	224	< 45 – 540 000
Actinomycetes	232	< 45 – 130 000
Other mesophilic bacteria	13 284	1 200 – 4 300 000
Thermophilic bacteria	68	< 45 – 14 000

Storage mites were present in 33 % of the samples, with the average of 20 mites in a gram of dust (range 0 – 33.3 mites/g).

Concentrations of inflammatory markers NO and cytokines were slightly increased in the nasal lavage fluid among the kitchen personnel compared with the clerical personnel (Table 13). In addition, the highest TNF-α values were detected in kitchen workers. In the cell differential count, there were no differences between the kitchen and clerical personnel; neutrophilic cells dominated the NAL cell profiles.

Table 13. Concentrations of NO, IL-4 and IL-6 in the NAL samples of kitchen and clerical personnel.

Cytokine	Kitchen personnel		Clerical personnel	
	Median (pg/ml)	Range (pg/ml)	Median (pg/ml)	Range (pg/ml)
NO	0.8 (μM)	0.2 – 17 (μM)	0.5 (μM)	0.2 – 1.1 (μM)
IL-4	25	0 – 200	0	0 – 37
IL-6	4.8	0 – 240	3.2	0 – 68

5.2.6 Symptom prevalence in the school environment

The prevalence of self-reported respiratory and general symptoms was high among both kitchen and clerical personnel (Study III). Among pupils, prevalence of parent- and self-reported stuffy nose, rhinitis, and sore throat were higher in the index school than in the reference school in both years before the renovations (Study IV). During the renovation, differences disappeared and after the renovation, the symptom prevalence decreased in the index school. In the reference school, the prevalence of some symptoms was higher than in the renovated index school.

6 DISCUSSION

6.1 Laboratory experiments

6.1.1 Effects of nutrients and pH on the growth of streptomycetes

The amount of streptomycete mycelium depended on the medium composition. Yeast extract supported mycelium biosynthesis, but the growth rate was not always accelerated. In earlier studies, yeast extract has been reported to promote rapid growth (Kutzner, 1986). In our study, media containing glucose and tryptone supported growth better than media containing glycerol and arginine, or starch and casein. In submerged cultures, however, starch has supported biomass yield better than glucose or glycerol (Glazebrook et al., 1990). In our study, the glucose repression reported in literature (Madden et al., 1996; Ochi, 1986) apparently did not inhibit the growth of streptomycetes on TYG-agar, because the amount of mycelium was good and equal to that obtained with TSA-agar.

The amount of produced mycelium was rather similar for each strain. All the strains belonged to two or three common environmental branches of *Streptomyces* spp., meaning that the diversity of species isolated from indoor environment on TYG was low. This may also be a possible reason for minor differences detected in the mycelium production between strains.

The pH ranges supporting growth depended on the nutrients. Depending on the medium composition, streptomycetes behaved as acidophiles, neutrophiles, and alkalophiles due to pH adaptation. In pH adaptation, the cytoplasmic pH is maintained close to the neutrality by cell membrane proteins functioning as primary proton pumps over the whole growth pH range. In the absence of pH regulation, the intracellular pH is equivalent to that outside cell, when the protons equilibrate across the membrane (Padan et al., 1981). In the absence of nutrients needed for the effective pH adaptation regulation, the growth does not occur at low pH values below 7.0. In our study, the growth below pH 7.0 was restricted on the medium containing Na-propionate, NH_4NO_3, and yeast extract. In earlier studies, poor growth of actinomycetes has been detected on agars containing chitin-mineral salts or glucose-asparagine-salts below pH 6.5 or at 6.2 (Hsu & Lockwood, 1975; Taber, 1960). Nutrient compositions like starch and casein, glucose, tryptone and yeast extract, tryptone and soy peptone and glycerol, arginine and yeast extract enabled pH adaptation and optimal growth over the broadest pH ranges. The high organic load has permitted streptomycetes growth under acidic conditions (Hagedorn, 1976) and in soil with

complex substrates like chitin or dead fungal mycelium (Kutzner, 1986). Presumably, a high organic load may have enabled the functional intracellular pH regulation, and thus, activated the growth of streptomycetes at low pH values.

All the streptomycete strains exhibited excellent sporulation capacities independent of pH and nutrients available. However, the strain specific differences were greater than those caused by the pH. In the submerged culture with glucose, optimal sporulation has been detected at pH 7.0, and at alkaline pH the sporulation has been repressed (Rho & Lee, 1994). In our study on media with glucose, sporulation was the best within the acidic pH range.

Thus, streptomycetes are able to grow at large range of pH values in indoor environments when appropriate nutrients are present. These substrates can be, e.g., fungal mycelium and moisture-damaged plasterboards including starch.

6.1.2 Error estimations

The error estimations also depended on the medium composition. The highest variation in SDs was seen on starch-casein-KNO_3 agar with the weakest growth and the smallest variation on TYG-agar where the growth of streptomycetes was one of the best recorded.

6.2 Field studies

6.2.1 Concentrations, flora and particle size distributions of airborne viable fungi

The concentrations of culturable fungi in indoor air in the six index schools were higher than in the two reference schools. This phenomenon was evident both in the kitchens ($p<0.05$) and other facilities ($p<0.05$) (Study III). Furthermore, the culturable concentrations were higher before ($p=0.0007$, $p=0.0353$) and during renovations ($p<0.0001$) (Study IV). This applied for concentrations in several particle size classes (Study IV). After the repairs were completed (Study IV), the airborne fungal levels were equally low in both schools. These results confirmed the previous findings that microbial concentrations of indoor air can be a sensitive measure of the technical condition of a school building (Cooley et al., 1998; Meklin et al., 2005).

In the five year study (Study IV), the highest fungal concentrations were detected in both schools in the second year, although there was still a concentration difference between the index and the reference school. In this second year, a significant con-

centration increase could be seen even in the reference school. In other years, the fungal concentrations in the reference school remained at the same low level. The year-to-year variation was probably due to the variation in the weather conditions. In the mid-winter, temperatures in the area are usually well below freezing and there is generous snow cover on the ground resulting in very low fungal concentrations in the outdoor air (Reponen et al., 1992). At the time of the measurements, however, the weather was unusually warm during this particular year. The temperatures were above 0 °C for several days, and mild winds from the south may have transported fungal spores along with other particles. The routine aerobiological measurements conducted in Southern Finland showed unusually high wintertime counts of *Cladosporium* spp., one of the most prevalent fungi in the outdoor air (Shelton et al., 2002), up to 160 spores/m^3. The proportion of *Cladosporium* spp. was over 70 % in our indoor measurements for this year, in contrast to the 30 % proportion in other years. It can be concluded that a significant fraction of the indoor spores had actually originated from outdoors. The potential long-range transport added to possible local outdoor sources increased the fungal load in both schools.

In school kitchens, the airborne fungal concentrations were lower than in the other facilities presumably because of more effective ventilation in the kitchens compared with other parts of the school buildings (Ministry of the Environment, 2003). In addition, kitchens are less crowded than classrooms and corridors, where resuspension of spores from floors and their dispersal with clothes is possible (Lehtonen et al., 1993). Kitchens are also humid spaces due to considerable moisture load during cooking and washing. Mean values for RH detected in continuous measurements were between 47 and 61 % in eight kitchens. Temporarily the RH in the air was as high as 80 % (Lignell et al., 2000). In such conditions of high RH, fewer fungal spores may be released into the air (Pasanen et al., 1991). There are only few previous studies on airborne microbial concentrations in kitchen type facilities. In a hospital kitchen, fungal concentrations were noted to be around the same order of magnitude as in our results (Marchant et al., 1990), but in a cafeteria, the range of airborne molds was wider than in our study (Wójcik-Stopczyńska et al., 2003). The highest concentrations were found during the vegetable washing and peeling, which has been shown to be a significant source of indoor microbes (Lehtonen et al., 1993).

In general, the most frequent fungi were *Penicillium* spp., *Cladosporium* spp., yeasts, non-sporulating isolates, and *Aspergillus* spp. both in kitchen and other facilities of the schools (Studies III and IV), in agreement with earlier reports (Dotterud et al., 1995; Pasanen, 1992; Shelton et al., 2002). The effect of moisture damage was seen in elevated levels of *Penicillium* spp. (Studies III and IV), *Cladosporium* spp. (Studies III and IV) and yeasts (Study III) in non-kitchen facilities of the

schools. A similar effect on the concentrations of *Cladosporium* spp. has been reported from residences (Pasanen et al., 1992b). Furthermore, the diversity of mycobiota was highest in the index school (Study IV), as reported also earlier (Meklin et al., 2005). During the renovation, opening the structures and other mechanical disturbances probably released spores of several microbes considered as indicators of moisture damage (Samson et al., 1994). In order to normalize the microbial conditions, both the remediation and thorough cleaning of moisture-damaged buildings play a role (Ebbehøj et al., 2002). This was also seen in our study. Only after the thorough cleaning did the microbial profile approach that of the reference school.

The moisture damage and associated microbial growth evidently acted as a source of all of the size classes of the common fungal spores, as has also been shown in earlier studies (Hyvärinen et al., 2001a; Meklin et al., 2002b; Reponen et al., 1994). This was seen for all but the smallest particle size (<1.1 μm). Viable spores are rare in the particle size fraction below 1.1 μm, but as experimentally shown (Górny et al., 2002), microbial fragments can also be found in the sub-micron size fractions.

6.2.2 Concentrations of airborne bacteria

The concentrations of culturable bacteria in indoor air in the index school before renovations were higher than in the reference school (Study IV). However, this difference disappeared when the facilities were divided into kitchens and other facilities of the schools (Study III). The concentrations in the kitchens were lower than in the other facilities of the schools both in index and reference schools. The low occupancy and effective ventilation in kitchens are clear factors affecting also the bacterial concentrations. Actinomycetes were mainly detected during the renovation in index school. After the renovation and the thorough cleaning of the index school, the bacterial concentrations decreased, which indicated that the moisture damage may have represented a source of indoor air bacteria. In addition, some year-to-year fluctuation in bacterial concentrations was seen in the reference school. In the kitchens, daily activities such as handling of vegetables and washing also contribute to the bacterial concentrations (Wójcik-Stopczyńska et al., 2003).

6.2.3 Microbes on school kitchen surfaces

The concentrations of both fungi and bacteria on moisture-damaged surfaces were significantly higher ($p<0.001$, $p=0.005$, respectively) than on undamaged surfaces in the kitchens. In the moisture-damaged kitchens, even undamaged surfaces were contaminated. On the damaged surfaces, fungal concentrations were typical of the concentrations detected on visibly damaged sites (Jarvis & Morey, 2001) and bacterial

concentrations were at a similar level as those that have been recorded in domestic kitchens (Josephson et al., 1997). Bacterial concentrations in institutional kitchens may usually be lower than in domestic kitchens, due to high water temperature, the use of industrial detergents, and the training of staff about food hygiene (Mattick et al., 2003). However, walls are cleaned less frequently than table surfaces in contact with produce and food, which are cleaned several times every day (Hygieniaopas, 2007). Thus, the results of this study represented the background concentrations of bacteria present in school kitchens.

Gram-negative rods, as found in Study III, and coliform bacteria have usually been detected on moist surfaces of domestic kitchens (Macher et al., 1991; Rusin et al., 1998; Speirs et al., 1995). In addition, gram-positive cocci were frequent; many of them are part of the normal flora of humans (Ruoff, 1999).

6.2.4 Microbes in the house dust

The median concentrations for total fungi analyzed by culture (Study V), were 10^5 cfu/g which is a similar concentration as has been previously detected in house dust samples (Beguin & Nolard, 1996; Chew et al., 2003; Engelhart et al., 2002; Heinrich et al., 2003; Hicks et al., 2005; Horner et al., 2004). When measured by the qPCR method, microbial concentrations were usually several orders of magnitude higher than the concentrations obtained by the culture method, as has been earlier shown for some *Aspergillus* species (Meklin et al., 2004). This means that the ratio between culturable and total microbial material was 1:100 or even less. However, the comparison between the culture and qPCR results is not straightforward. The quantification of culture results is based on colony forming units, i.e., including the possibility that a colony originates from an aggregate of several spores and that not all organisms are capable of producing a colony on artificial laboratory media (non-culturable or stressed cells). Instead, PCR quantifies DNA from the particular species or assay group irrespective of whether the material originates from viable or non-viable spores or their fragments or mycelial material. In earlier studies, estimations of this ratio have varied extensively, from 0.7 % to 87 % for indoor air samples (Lee et al., 2006; Niemeier et al., 2006). Nonetheless, a relatively high proportion of fungal material is non-culturable in the house dust and this should be considered when assessing microbial exposures in indoor environments.

Fungal concentrations on 2 % MEA and DG18 correlated significantly although the values were not high (r_s 0.343 – 0.767). This correlation has been previously shown in air and material samples (Hyvärinen et al., 2002; Hyvärinen et al., 2001b; Meklin et al., 2002a). The correlation between genus-specific culture results and species-specific qPCR results was significant although low (r_s 0.275 – 0.510), as was also

reported earlier (Vesper et al., 2006b). This indicates that the species detected with the qPCR assay were only representing a fraction of the genus present.

According to qPCR, the highest concentrations were detected for the group assay of *Aspergillus* spp. / *Penicillium* spp. / *Paecilomyces variotii*, the genus *Streptomyces*, and for the fungal species *Aureobasidium pullulans*, *Aspergillus penicillioides*, *Cladosporium cladosporioides*, *Penicillium brevicompactum/stoloniferum*, both in schools (Study IV) and in homes (Study V). *A. pullulans* has also earlier been reported to be a prevalent fungus in house dust analyzed by qPCR (Vesper et al., 2006a; Vesper et al., 2006b; Vesper et al., 2005) and the genus *Aureobasidium* has been reported to be prevalent in house dust also using culturing techniques (Hicks et al., 2005; Rogers, 2003; Stark et al., 2003; Wickman et al., 1992). In our study, however, *Aureobasidium* spp. remained almost undetectable with the culture method used, and thus, its presence in the house dust was underestimated.

There was a significant association (p 0.003 – 0.049) between concentrations of several fungi determined with qPCR and the extent of moisture damage in house (Study V), suggesting that these species proliferate whenever moisture damage in a house provides suitable conditions for microbial growth. Furthermore, the high concentration of these species could be "indicative of the moisture damage", which may facilitate in the revelation of moisture damage when the mold is not visible. This association was detected for *Penicillium brevicompactum/stoloniferum*, *Wallemia sebi*, *Trichoderma viride/atroviride/koningii*, *Cladosporium sphaerospermum*, *Eurotium amstelodami/chevalieri/herbariorum/rubrum/repens*, and the assay group for *Penicillium* spp., *Aspergillus* spp. and *Paecilomyces variotii* from which all except the assay group *Penicillium* spp. / *Aspergillus* spp. / *Paecilomyces variotii* have earlier been linked with abnormal mold conditions in visibly damaged homes (Meklin et al., 2004; Vesper et al., 2004). Furthermore, the *Eurotium* group has been linked to homes with asthmatic children (Vesper et al., 2007). In the school environment (study IV), the highest concentrations were found in the sofa in the teachers' room of the renovated school. This sofa was purchased immediately after the renovation but before the thorough cleaning, and thus, it is possible that the detected fungi were residues from the dust liberated during the renovation period. This emphasizes the importance of thorough cleaning after remediation.

6.2.5 Other parameters measured

The concentration range of culturable fungi in material samples was large, < 45 – 540 000 cfu/g, as was also reported earlier (Hyvärinen et al., 2002). The bacterial diversity was large, ranging from mesophilic bacteria including actinomycetes to thermophilic bacteria and mycobacteria.

Low densities of mites were found in 33 % of the vacuumed samples. The mites found belong to storage mite species, for which no threshold values have been suggested, although they may cause allergic symptoms and sensitization (Iversen & Dahl, 1990; Pennanen et al., 2007; Warner et al., 1999; Vidal et al., 2004). The study confirmed that storage mites can also be found in damp dwellings and non-agricultural workplaces, in addition to agricultural storage facilities (Iversen & Dahl, 1990; Pennanen et al., 2007; Solarz, 1998).

The concentrations of measured cytokines in NAL samples among kitchen workers were slightly higher than those found in clerical personnel although the difference did not reach statistical significance. The production of IL-4 was associated with self-reported allergies and repeated respiratory infections, which suggests that work-related exposure was capable of inducing inflammatory responses in the upper airways. Previously, an association between increased cytokine levels in NAL and exposure in moisture-damaged schools has been shown (Hirvonen et al., 1999; Purokivi et al., 2001) and an increased IL-4 concentration appeared to be associated particularly with bacterial exposure (Roponen et al., 2003).

6.2.6 Symptom prevalence in school environment

In the kitchen study (Study III), the majority of respondents (86 %) worked in the moisture-damaged schools. High symptom prevalence was observed especially of respiratory and general symptoms among kitchen and clerical personnel, and the symptoms were associated with the workplace exposure in the moisture-damaged environments. This association was also detected among children (Study IV). Their symptom prevalence was highest in the two years before the renovation. At the end of the process, the symptom profiles were very similar in both schools, pointing to a normalized situation among the pupils. A reduction of pupils' symptoms after remediation has also been reported earlier (Meklin et al., 2005; Putus et al., 2004; Savilahti et al., 2000; Åhman et al., 2000). Reporting bias as over-reporting may have affected the symptom prevalence in the first year studied (Haverinen-Shaughnessy et al., 2004).

7 CONCLUSIONS

Based on the results of this thesis, the following conclusions can be drawn.

1. Glucose and tryptone allowed good growth of streptomycetes. TYG and TSA media enabled good growth. The pH range for growth and sporulation was wide and depended on which nutrients were present. Thus, streptomycetes can grow in indoor environments at a great variety of pH values in the presence of appropriate nutrients.

2. Airborne fungal and bacterial concentrations in school kitchens were lower than in the other facilities of the schools. A large range of different bacteria including actinomycetes, other mesophilic bacteria, mycobacteria, gram-negative bacteria, and thermophilic bacteria were present in school kitchens which is a specific aspect from the point of view of indoor air quality.

3. The moisture damage affected microbial concentrations. Airborne concentrations of viable microbes in the moisture-damaged schools were higher than in the reference schools. Moisture-damaged kitchens differed from the reference kitchens in their higher concentrations of airborne microbes and higher bacterial concentrations on undamaged surfaces.

The moisture damage also affected diversity of mycobiota. In the moisture-damaged school, the microbial diversity was larger than in the reference school, even more extensive during remediation. Concentrations of several fungi in house dust measured with qPCR increased according to the extent of moisture damage in the house.

4. The remediation of the moisture damage decreased microbial concentrations in the school. The microbial profile also approached that of the reference school after the remediation and a thorough cleaning. After the remediation, prevalence of some symptoms in the index school decreased to a level lower than in the reference school.

5. The measurements of airborne microbial concentrations during five consecutive years showed the variation of microbial levels due to climatic conditions. Simultaneous outdoor air sampling may be relevant also in wintertime. However, the concentration difference between a moisture-damaged and reference school remained. This emphasizes the importance of the reference building.

6. The microbial exposure occurring in moisture-damaged schools was associated with a high symptom prevalence among kitchen and clerical personnel and schoolchildren.

7. QPCR method proved to be a feasible method to show the unusual microbial concentrations in the house dust from moisture-damaged buildings. Concentrations as

measured by qPCR were several orders of magnitude higher than the concentrations of culturable fungi, which indicate a more accurate estimate of microbial exposure than conventional culture method.

8 ACKNOWLEDGEMENTS

This work was carried out in the Environmental Microbiology Laboratory, Department of Environmental Health, National Public Health Institute. I would like to thank Professor Jouko Tuomisto, the former director and Professor Terttu Vartiainen, the director of the Department of Environmental Health, for providing the facilities to conduct this study.

I wish to express my gratitude to my principal supervisor Research professor Aino Nevalainen for her support and guidance during this work. I especially admire her talent for the clear expression of complex matters. I wish to express my warmest thanks to my supervisor Professor Pentti Kalliokoski for his advice and always so prompt response and valuable comments. My sincere appreciation and gratitude belong also to my supervisor Merja Kontro, Ph.D., for friendly support and valuable comments.

I am grateful to the official referees of my thesis, Professor Linda Stetzenbach and Dr. Regine Szewzyk, for their constructive criticism and beneficial comments. I also thank Ewen McDonald, Pharm.D., for revising language of this thesis.

I wish to thank all my co-workers, the mold group for excellent co-operation. I express my special thanks to Docent Teija Meklin for very relaxing and pleasure moments with many good laughs. I wish to thank Tuula Putus, M.D., Asko Vepsäläinen, M.Sc., Helena Rintala, Ph.D., and Docent Anne Hyvärinen for many joyful discussions and their contribution into this work.

I express my warm thanks to my other co-authors Research professor Maija-Riitta Hirvonen, Marjut Roponen, Pharm.D., Eila Torvinen, Ph.D., Morten Reeslev, Ph.D., Sirpa Pennanen, Ph.D., and Research professor Juha Pekkanen for the co-operation and valuable comments.

I want to thank my former roommates Riitta Patovirta, M.D., Marja Hänninen, M.Sc., Docent Ulla Haverinen-Shaughnessy, Juho Halla-aho, civ.eng., Jari Koivisto, civ.eng., and current roommates Eeva Kauhanen, M.Sc., and Pasi Kaarakainen, M.Sc (Tech.) for standing me and my chatting.

The late Tuula Wallenius, Arja Rönkkö, Heli Martikainen and Ulla Kukkonen are acknowledged for their technical assistance and Kirsi Korhonen for help with many practical things. I also thank Raija-Leena Pajula from the library of the Neulanen Research Center for very competent service. I extend my thanks to the entire personnel of the Environmental Microbiology Laboratory and the Department of Environmental Health for creating a friendly and inspiring atmosphere.

Most of all, I wish to thank my family and relatives for their support throughout my life as well as my friends for many happy moments we have shared together.

This study was financially supported by the Finnish Work Environment Fund, the Graduate School in Environmental Health (SYTYKE), the Finnish Konkordia Fund, the Northern Savo Regional Fund of the Finnish Cultural Foundation, and University of Kuopio.

Kuopio, January 2008

Ulla Lignell

9 REFERENCES

Achatz, G., Oberkofler, H., Lechenauer, E., Simon, B., Unger, A., Kandler, D., Ebner, C., Prillinger, H., Kraft, D. & Breitenbach, M. (1995). Molecular cloning of major and minor allergens of *Alternaria alternata* and *Cladosporium herbarum*. *Molecular Immunology*, **32**(3), 213-227.

Agranovski, I. E., Agranovski, V., Reponen, T., Willeke, K. & Grinshpun, S. A. (2002). Development and evaluation of a new personal sampler for culturable airborne microorganisms. *Atmospheric Environment*, **36**(5), 889-898.

Airaksinen, M., Pasanen, P., Kurnitski, J. & Seppänen, O. (2004). Microbial contamination of indoor air due to leakages from crawl space: a field study. *Indoor Air*, **14**(1), 55-64.

Al-Soud, W. A., Jönsson, L. J. & Rådström, P. (2000). Identification and characterization of immunoglobulin G in blood as a major inhibitor of diagnostic PCR. *Journal of Clinical Microbiology*, **38**(1), 345-350.

Allermann, L., Wilkins, C. K. & Madsen, A. M. (2006). Inflammatory potency of dust from the indoor environment and correlation to content of NAGase and fungi. *Toxicology in Vitro*, **20**(8), 1522-1531.

Alwis, K. U., Larsson, L. & Milton, D. K. (2006). Suppression of ionization and optimization of assay for 3-hydroxy fatty acids in house dust using ion-trap mass spectrometry. *American Journal of Industrial Medicine*, **49**(4), 286-295.

Amann, R. I., Ludwig, W. & Schleifer, K.-H. (1995). Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiological Reviews*, **59**(1), 143-169.

An, H. R., Mainelis, G. & Yao, M. (2004). Evaluation of a high-volume portable bioaerosol sampler in laboratory and field environments. *Indoor Air*, **14**(6), 385-393.

Andersen, A. A. (1958). New sampler for the collection, sizing, and enumeration of viable airborne particles. *Journal of Bacteriology*, **76**, 471-484.

Andersson, A. M., Weiss, N., Rainey, F. & Salkinoja-Salonen, M. S. (1999). Dust-borne bacteria in animal sheds, schools and children's day care centres. *Journal of Applied Microbiology*, **86**(4), 622-634.

Andersson, M. A., Mikkola, R., Kroppenstedt, M., Rainey, F. A., Peltola, J., Helin, J., Sivonen, K. & Salkinoja-Salonen, M. S. (1998). The mitochondrial toxin produced by *Streptomyces griseus* strains isolated from an indoor environment is valinomycin. *Applied and Environmental Microbiology*, **64**(12), 4767-4773.

Andersson, M. A., Nikulin, M., Köljalg, U., Andersson, M. C., Rainey, F., Reijula, K., Hintikka, E.-L. & Salkinoja-Salonen, M. (1997). Bacteria, molds, and toxins in water-damaged building materials. *Applied and Environmental Microbiology*, **63**(2), 387-393.

Angenent, L. T., Kelley, S. T., St Amand, A., Pace, N. R. & Hernandez, M. T. (2005). Molecular identification of potential pathogens in water and air of a hospital therapy pool. *Proceedings of the National Academy of Sciences of the United States of America*, **102**(13), 4860-4865.

Arun, D. & Dharmalingam, K. (1999). Short Note: *Streptomyces peucetius* converts anthracycline intermediates efficiently in culture media containing oil cake as carbon source. *World Journal of Microbiology & Biotechnology*, **15**(2), 333-334.

Awad, A. H. A. & Farag, S. A. (1999). An indoor bio-contaminants air quality. *International Journal of Environmental Health Research*, **9**(4), 313-319.

Aycicek, H., Oguz, U. & Karci, K. (2006). Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. *International Journal of Hygiene and Environmental Health*, **209**(2), 203-206.

Bardana, E. J., Jr. (2003). Indoor air quality and health Does fungal contamination play a significant role? *Immunology and Allergy Clinics of North America*, **23**(2), 291-309.

Barnes, C. S., Dowling, P., Van Osdol, T. & Portnoy, J. (2007). Comparison of indoor fungal spore levels before and after professional home remediation. *Annals of Allergy Asthma & Immunology*, **98**(3), 262-268.

Bartlett, K. H., Kennedy, S. M., Brauer, M., van Netten, C. & Dill, B. (2004a). Evaluation and a predictive model of airborne fungal concentrations in school classrooms. *Annals of Occupational Hygiene*, **48**(6), 547-554.

Bartlett, K. H., Kennedy, S. M., Brauer, M., van Netten, C. & Dill, B. (2004b). Evaluation and determinants of airborne bacterial concentrations in school classrooms. *Journal of Occupational and Environmental Hygiene*, **1**, 639-647.

Beguin, H. (1995). Mould biodiversity in homes II. Analysis of mattress dust. *Aerobiologia*, **11**, 3-10.

Beguin, H. & Nolard, N. (1994). Mould diversity in homes I. Air and surface analysis of 130 dwellings. *Aerobiologia*, **10**, 157-166.

Beguin, H. & Nolard, N. (1996). Prevalence of fungi in carpeted floor environment: analysis of dust samples from living-rooms, bedrooms, offices and school classrooms. *Aerobiologia*, **12**, 113-120.

Blomquist, G. (1994). Sampling of biological particles. *Analyst*, **119**(1), 53-56.

Bornehag, C.-G., Blomquist, G., Gyntelberg, F., Järvholm, B., Malmberg, P., Nordvall, L., Nielsen, A., Pershagen, G. & Sundell, J. (2001). Dampness in buildings and health, Nordic interdisciplinary review of the scientific evidence on associations between exposure to "dampness" in buildings and health effects (NORDDAMP). *Indoor Air*, **11**, 72-86.

Bornehag, C.-G., Sundell, J., Bonini, S., Custovic, A., Malmberg, P., Skerfving, S., Sigsgaard, T. & Verhoeff, A. (2004). Dampness in buildings as a risk factor for health effects, EUROEXPO: a multidisciplinary review of the literature (1998-2000) on dampness and mite exposure in buildings and health effects. *Indoor Air*, **14**, 243-257.

Brasel, T. L., Douglas, D. R., Wilson, S. C. & Straus, D. C. (2005a). Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins on particulates smaller than conidia. *Applied and Environmental Microbiology*, **71**(1), 114-122.

Brasel, T. L., Martin, J. M., Carriker, C. G., Wilson, S. C. & Straus, D. C. (2005b). Detection of airborne *Stachybotrys chartarum* macrocyclic trichothecene mycotoxins in the indoor environment. *Applied and Environmental Microbiology*, **71**(11), 7376-7388.

Braun-Fahrländer, C. & Lauener, R. (2003). Farming and protective agents against allergy and asthma. *Clinical and Experimental Allergy*, **33**(4), 409-411.

Brinkman, N. E., Haugland, R. A., Wymer, L. J., Byappanahalli, M., Whitman, R. L. & Vesper, S. J. (2003). Evaluation of a rapid, quantitative real-time PCR method for enumeration of pathogenic *Candida* cells in water. *Applied and Environmental Microbiology*, **69**(3), 1775-1782.

Brown, G. S., Betty, R. G., Brockmann, J. E., Lucero, D. A., Souza, C. A., Walsh, K. S., Boucher, R. M., Tezak, M. S., Wilson, M. C., Rudolph, T., Lindquist, H. D. A. & Martinez, K. F. (2007). Evaluation of rayon swab surface sample collection method for *Bacillus* spores from nonporous surfaces. *Journal of Applied Microbiology*, **103**(4), 1074-1080.

Burge, H. A. (1995). Bioaerosol investigations. In *Bioaerosols*, ed. H. A. Burge, CRC Press, Inc. Boca Raton, Florida, pp. 1-23.

Burge, H. A. (2002). An update on pollen and fungal spore aerobiology. *Journal of Allergy and Clinical Immunology*, **110**(4), 544-552.

Burns, M. J., Nixon, G. J., Foy, C. A. & Harris, N. (2005). Standardisation of data from real-time quantitative PCR methods - evaluation of outliers and comparison of calibration curves. *BMC Biotechnology*, **5**(Art. No. 31).

Burton, N. C., Grinshpun, S. A. & Reponen, T. (2007). Physical collection efficiency of filter materials for bacteria and viruses. *Annals of Occupational Hygiene*, **51**(2), 143-151.

Bustin, S. A. (2002). Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *Journal of Molecular Endocrinology*, **29**(1), 23-39.

Butte, W. & Heinzow, B. (2002). Pollutants in house dust as indicators of indoor contamination. *Reviews of Environmental Contamination and Toxicology*, **175**, 1-46.

Buttner, M. P., Cruz-Perez, P. & Stetzenbach, L. D. (2001). Enhanced detection of surface-associated bacteria in indoor environments by quantitative PCR. *Applied and Environmental Microbiology*, **67**(6), 2564-2570.

Buttner, M. P., Cruz, P., Stetzenbach, L. D. & Cronin, T. (2007). Evaluation of two surface sampling methods for detection of *Erwinia herbicola* on a variety of materials by culture and quantitative PCR. *Applied and Environmental Microbiology*, **73**(11), 3505-3510.

Buttner, M. P., Cruz, P., Stetzenbach, L. D., Klima-Comba, A. K., Stevens, V. L. & Cronin, T. D. (2004a). Determination of the efficacy of two building decontamination strategies by surface sampling with culture and quantitative PCR analysis. *Applied and Environmental Microbiology*, **70**(8), 4740-4747.

Buttner, M. P., Cruz, P., Stetzenbach, L. D., Klima-Comba, A. K., Stevens, V. L. & Emanuel, P. A. (2004b). Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. *Applied and Environmental Microbiology*, **70**(12), 7040-7045.

Buttner, M. P., Willeke, K. & Grinshpun, S. (1997). Sampling and analysis of airborne microorganisms. In *Manual of Environmental Microbiology*, eds. C. J. Hurst, G. R. Knudsen, M. J. McInerney, L. D. Stetzenbach & M. V. Walter, ASM Press. Washington, D.C., pp. 629-640.

Böttcher, M. F., Björkstén, B., Gustafson, S., Voor, T. & Jenmalm, M. C. (2003). Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clinical and Experimental Allergy*, **33**, 295-300.

Charpin-Kadouch, C., Maurel, G., Felipo, R., Queralt, J., Ramadour, M., Dumon, H., Garans, M., Botta, A. & Charpin, D. (2006). Mycotoxin identification in moldy dwellings. *Journal of Applied Toxicology*, **26**(6), 475-479.

Chew, G. L., Douwes, J., Doekes, G., Higgins, K. M., van Strien, R., Spithoven, J. & Brunekreef, B. (2001). Fungal extracellular polysaccharides, β (1->3)-glucans and culturable fungi in repeated sampling of house dust. *Indoor Air*, **11**(3), 171-178.

Chew, G. L., Rogers, C., Burge, H. A., Muilenberg, M. L. & Gold, D. R. (2003). Dustborne and airborne fungal propagules represent a different spectrum of fungi with differing relations to home characteristics. *Allergy*, **58**, 13-20.

Cho, S.-H., Han, J.-H., Seong, C. N. & Kim, S. B. (2006). Phylogenetic diversity of acidophilic sporoactinobacteria isolated from various soils. *the Journal of Microbiology*, **44**(6), 600-606.

Claeson, A.-S. & Sunesson, A. L. (2005). Identification using versatile sampling and analytical methods of volatile compounds from *Streptomyces albidoflavus* grown on four humid building materials and one synthetic medium. *Indoor Air*, **15**, 41-47.

Clench-Aas, J., Bartonova, A., Gronskei, K. E. & Walker, S.-E. (1999). Air pollution exposure monitoring and estimation. *Journal of Environmental Monitoring*, **1**, 333-336.

Cooley, J. D., Wong, W. C., Jumper, C. A. & Straus, D. C. (1998). Correlation between the prevalence of certain fungi and sick building syndrome. *Occupational and Environmental Medicine*, **55**, 579-584.

Cruz-Perez, P., Buttner, M. P. & Stetzenbach, L. D. (2001a). Detection and quantitation of *Aspergillus fumigatus* in pure culture using polymerase chain reaction. *Molecular and Cellular Probes*, **15**, 81-88.

Cruz-Perez, P., Buttner, M. P. & Stetzenbach, L. D. (2001b). Specific detection of *Stachybotrys chartarum* in pure culture using quantitative polymerase chain reaction. *Molecular and Cellular Probes*, **15**, 129-138.

Daisey, J. M., Angell, W. J. & Apte, M. G. (2003). Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information. *Indoor Air*, **13**, 53-64.

Dales, R. E., Miller, D. & McMullen, E. (1997). Indoor air quality and health: validity and determinants of reported home dampness and moulds. *International Journal of Epidemiology*, **26**(1), 120-125.

Dales, R. E., Miller, D. & White, J. (1999). Testing the association between residential fungus and health using ergosterol measures and cough recordings. *Mycopathologia*, **147**(1), 21-27.

Day, J. P., Kell, D. B. & Griffith, G. W. (2002). Differentiation of *Phytophthora infestans* sporangia from other airborne biological particles by flow cytometry. *Applied and Environmental Microbiology*, **68**(1), 37-45.

de Ana, S. G., Torres-Rodríguez, J. M., Ramírez, E. A., García, S. M. & Belmonte-Soler, J. (2006). Seasonal distribution of *Alternaria*, *Aspergillus*, *Cladosporium* and *Penicillium* species isolated in homes of fungal allergic patients. *Journal of Investigational Allergology & Clinical Immunology*, **16**(6), 357-363.

Dean, T. R., Betancourt, D. & Menetrez, M. Y. (2004). A rapid DNA extraction method for PCR identification of fungal indoor air contaminants. *Journal of Microbiological Methods*, **56**, 431-434.

Dean, T. R., Kohan, M., Betancourt, D. & Menetrez, M. Y. (2006). A simple polymerase chain reaction-sequencing analysis capable of identifying multiple medically relevant filamentous fungal species. *Mycopathologia*, **162**(4), 265-271.

Degola, F., Berni, E., Dall'Asta, C., Spotti, E., Marchelli, R., Ferrero, I. & Restivo, F. M. (2007). A multiplex RT-PCR approach to detect aflatoxigenic strains of *Aspergillus flavus*. *Journal of Applied Microbiology*, **103**(2), 409-417.

Dekleva, M. L. & Strohl, W. R. (1987). Glucose-stimulated acidogenesis by *Streptomyces peucetius*. *Canadian Journal of Microbiology*, **33**(12), 1129-1132.

Dharmage, S., Bailey, M., Raven, J., Abeyawickrama, K., Cao, D., Guest, D., Rolland, J., Forbes, A., Thien, F., Abramson, M. & Walters, E. H. (2002). Mouldy houses influence symptoms of asthma among atopic individuals. *Clinical and Experimental Allergy*, **32**, 714-720.

Dharmage, S., Bailey, M., Raven, J., Mitakakis, T., Thien, F., Forbes, A., Guest, D., Abramson, M. & Walters, E. H. (1999). Prevalence and residential determinants of fungi within homes in Melbourne, Australia. *Clinical and Experimental Allergy*, **29**, 1481-1489.

Dillon, H. K., Miller, J. D., Sorenson, W. G., Douwes, J. & Jacobs, R. R. (1999). Review of methods applicable to the assessment of mold exposure to children. *Environmental Health Perspectives*, **107** (Suppl. 3), 473-480.

Dionigi, C. P., Millie, D. F., Spanier, A. M. & Johnsen, P. B. (1992). Spore and geosmin production by *Streptomyces tendae* on several media. *Journal of Agricultural and Food Chemistry*, **40**(1), 122-125.

Doroshenko, E. A., Zenova, G. M., Zvyagintsev, D. G. & Sudnitsyn, II. (2005). Spore germination and mycelial growth of streptomycetes at different humidity levels. *Microbiology*, **74**(6), 690-694.

Dotterud, L. K., Vorland, L. H. & Falk, E. S. (1996). Mould allergy in schoolchildren in relation to airborne fungi and residential charasteristics in homes and schools in Northern Norway. *Indoor Air*, **6**, 71-76.

Dotterud, L. K., Vorland, L. H. & Falk, E. S. (1995). Viable fungi in indoor air in homes and schools in the Sør-Varanger community during winter. *Pediatric Allergy and Immunology*, **6**, 181-186.

Douwes, J., Doekes, G., Heinrich, J., Koch, A., Bischof, W. & Brunekreef, B. (1998). Endotoxin and β(1->3)-glucan in house dust and the relation with home characteristics: a pilot study in 25 German houses. *Indoor Air*, **8**, 255-263.

Douwes, J. & Pearce, N. (2003). Invited commentary: Is indoor mold exposure a risk factor for asthma? *American Journal of Epidemiology*, **158**(3), 203-206.

Douwes, J., Pearce, N. & Heederik, D. (2002). Does environmental endotoxin exposure prevent asthma? *Thorax*, **57**(1), 86-90.

Douwes, J., van der Sluis, B., Doekes, G., van Leusden, F., Wijnands, L., van Strien, R., Verhoeff, A. & Brunekreef, B. (1999). Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: Relations with culturable fungi, reported home dampness, and respiratory symptoms. *Journal of Allergy and Clinical Immunology*, **103**(3), 494-500.

Drahonovska, H. & Gajdos, P. (1997). Monitoring indoor air quality in the Czech Republic. *Indoor + Built Environment*, **6**, 198-203.

Duangmal, K., Ward, A. C. & Goodfellow, M. (2005). Selective isolation of members of the *Streptomyces violaceoruber* clade from soil. *FEMS Microbiology Letters*, **245**(2), 321-327.

Dörge, T., Carstensen, J. M. & Frisvad, J. C. (2000). Direct identification of pure *Penicillium* species using image analysis. *Journal of Microbiological Methods*, **41**(2), 121-133.

Ebbehøj, N. E., Hansen, M. O., Sigsgaard, T. & Larsen, L. (2002). Building-related symptoms and molds: a two-step intervention study. *Indoor Air*, **12**(4), 273-277.

Ebbehøj, N. E., Meyer, H. W., Würtz, H., Suadicani, P., Valbjørn, O., Sigsgaard, T., Gyntelberg, F. & Members of a working group under the Danish mold in buildings program (DAMIB). (2005). Molds in floor dust, building-related symptoms, and lung function among male and female schoolteachers. *Indoor Air*, **15**(Suppl. 10), 7-16.

Eduard, W. (1996). Measurement methods and strategies for non-infectious microbial components in bioaerosols at the workplace. *Analyst*, **121**(9), 1197-1201.

Eduard, W. & Heederik, D. (1998). Methods for quantitative assessment of airborne levels of non-infectious microorganisms in highly contaminated work environments. *American Industrial Hygiene Association Journal*, **59**(2), 113-127.

Edwards, U., Rogall, T., Blöcker, H., Emde, M. & Böttger, E. C. (1989). Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Research*, **17**(19), 7843-7853.

El-Nakeeb, M. & Lechevalier, H. A. (1963). Selective isolation of aerobic actinomycetes. *Applied Microbiology*, **11**, 75-77.

Elke, K., Begerow, J., Oppermann, H., Krämer, U., Jermann, E. & Dunemann, L. (1999). Determination of selected microbial volatile organic compounds by diffusive sampling and dual-column capillary GC-FID - a new feasible approach for the detection of an exposure to indoor mould fungi? *Journal of Environmental Monitoring*, **1**(5), 445-452.

Ellringer, P. J., Boone, K. & Hendrickson, S. (2000). Building materials used in construction can affect indoor fungal levels greatly. *American Industrial Hygiene Association Journal*, **61**, 895-899.

Engelhart, S. & Exner, M. (2002). Short-term versus long-term filter cassette sampling for viable fungi in indoor air: comparative performance of the Sartorius MD8 and the GSP sampler. *International Journal of Hygiene and Environmental Health*, **205**(6), 443-451.

Engelhart, S., Loock, A., Skutlarek, D., Sagunski, H., Lommel, A., Färber, H. & Exner, M. (2002). Occurrence of toxigenic *Aspergillus versicolor* isolates and sterigmatocystin in carpet dust from damp indoor environments. *Applied and Environmental Microbiology*, **68**(8), 3886-3890.

Ensign, J. C. (1978). Formation, properties, and germination of actinomycete spores. *Annual Review of Microbiology*, **32**, 185-219.

Fabian, M. P., Miller, S. L., Reponen, T. & Hernandez, M. T. (2005). Ambient bioaerosol indices for indoor air quality assessments of flood reclamation. *Journal of Aerosol Science*, **36**(5-6), 763-783.

Fahlbusch, B., Koch, A., Douwes, J., Bischof, W., Gehring, U., Richter, K., Wichmann, H.-E. & Heinrich, J. (2003). The effect of storage on allergen and microbial agent levels in frozen house dust. *Allergy*, **58**, 150-153.

Falkinham, J. O. (1996). Epidemiology of infection by nontuberculous mycobacteria. *Clinical Microbiology Reviews*, **9**(2), 177-215.

Flannigan, B. (1997). Air sampling for fungi in indoor environments. *Journal of Aerosol Science*, **28**(3), 381-392.

Flannigan, B., McCabe, E. M. & McGarry, F. (1991). Allergenic and toxigenic micro-organisms in houses. *Society for Applied Bacteriology symposium series*, **20**, 61S-73S.

Flannigan, B. & Morey, P. R. (1996). Control of moisture problems affecting biological indoor air quality. *ISIAQ, Guideline: TFI-1996*, Ottawa, Canada.

Flannigan, B., Vicars, S., Pasanen, A. & Pasanen, P. (1994). Bioaerosols from house dust. In *Health implications of fungi in indoor environments*, eds. R. A. Samson, B. Flannigan, M. E. Flannigan, A. P. Verhoeff, O. C. G. Adan & E. S. Hoekstra, Elsevier Science B.V. Amsterdam, pp. 65-74.

Foarde, K. & Berry, M. (2004). Comparison of biocontaminant levels associated with hard vs. carpet floors in nonproblem schools: Results of a year long study. *Journal of Exposure Analysis and Environmental Epidemiology*, **14** (Suppl. 1), S41-S48.

Foto, M., Vrijmoed, L. L., Miller, J. D., Ruest, K., Lawton, M. & Dales, R. E. (2005). A comparison of airborne ergosterol, glucan and Air-O-Cell data in relation to physical assessments of mold damage and some other parameters. *Indoor Air*, **15**(4), 257-266.

Fox, A., Harley, W., Feigley, C., Salzberg, D., Sebastian, A. & Larsson, L. (2003). Increased levels of bacterial markers and CO_2 in occupied school rooms. *Journal of Environmental Monitoring*, **5**(2), 246-252.

Fox, A., Harley, W., Feigley, C., Salzberg, D., Toole, C., Sebastian, A. & Larsson, L. (2005). Large particles are responsible for elevated bacterial marker levels in school air upon occupation. *Journal of Environmental Monitoring*, **7**(5), 450-456.

Fox, A. & Rosario, R. M. T. (1994). Quantification of muramic acid, a marker for bacterial peptidoglycan, in dust collected from hospital and home air-conditioning filters using gas-chromatography mass-spectrometry. *Indoor Air*, **4**(4), 239-247.

Gallup, J. M., Zanolli, J. & Olson, L. (1993). Airborne bacterial exposure: preliminary results of volumetric studies performed in office buildings, schools, and homes in California. *Indoor Air '93*, July 4-8,1993, Helsinki, Finland. 167-170.

Gandara, A., Mota, L. C., Flores, C., Perez, H. R., Green, C. F. & Gibbs, S. G. (2006). Isolation of *Staphylococcus aureus* and antibiotic-resistant *Staphylococcus aureus* from residential indoor bioaerosols. *Environmental Health Perspectives*, **114**(12), 1859-1864.

Garrett, M. H., Rayment, P. R., Hooper, M. A., Abramson, M. J. & Hooper, B. M. (1998). Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. *Clinical and Experimental Allergy*, **28**, 459-467.

Gehring, U., Douwes, J., Doekes, G., Koch, A., Bischof, W., Fahlbusch, B., Richter, K., Wichmann, H. E. & Heinrich, J. (2001). β(1 -> 3)-glucan in house dust of German homes: Housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds. *Environmental Health Perspectives*, **109**(2), 139-144.

Glazebrook, M. A., Doull, J. L., Stuttard, C. & Vining, L. C. (1990). Sporulation of *Streptomyces venezuelae* in submerged cultures. *Journal of General Microbiology*, **136**, 581-588.

Glushakova, A. M., Zheltikova, T. M. & Chernov, I. (2004). Groups and sources of yeasts in house dust. *Microbiology*, **73**(1), 94-98.

Godish, D. R. & Godish, T. J. (2006). Relationship between sampling duration and concentration of culturable airborne mould and bacteria on selected culture media. *Journal of Applied Microbiology*, **102**(6), 1479-1484.

Goebes, M. D., Hildemann, L. M., Kujundzic, E. & Hernandez, M. (2007). Real-time PCR for detection of the *Aspergillus* genus. *Journal of Environmental Monitoring*, **9**(6), 599-609.

Górny, R. L. (2004). Filamentous microorganisms and their fragments in indoor air - a review. *Annals of Agricultural and Environmental Medicine*, **11**, 185-197.

Górny, R. L. & Dutkiewicz, J. (2002). Bacterial and fungal aerosols in indoor environment in Central and Eastern European countries. *Annals of Agricultural and Environmental Medicine*, **9**, 17-23.

Górny, R. L., Dutkiewicz, J. & Krysinska-Traczyk, E. (1999). Size distribution of bacterial and fungal bioaerosols in indoor air. *Annals of Agricultural and Environmental Medicine*, **6**, 105-113.

Górny, R. L., Reponen, T., Grinshpun, S. A. & Willeke, K. (2001). Source strength of fungal spore aerosolization from moldy building material. *Atmospheric Environment*, **35**(28), 4853-4862.

Górny, R. L., Reponen, T., Willeke, K., Schmechel, D., Robine, E., Boissier, M. & Grinshpun, S. A. (2002). Fungal fragments as indoor air biocontaminants. *Applied and Environmental Microbiology*, **68**(7), 3522-3531.

Gots, R. E., Layton, N. & Pirages, S. W. (2003). Indoor health: background levels of fungi. *American Industrial Hygiene Association Journal*, **64**, 427-438.

Grant, C., Hunter, C. A., Flannigan, B. & Bravery, A. F. (1989). The moisture requirements of moulds isolated from domestic dwellings. *International Biodeterioration*, **25**, 259-284.

Gravesen, S., Nielsen, P. A., Iversen, R. & Nielsen, K. F. (1999). Microfungal contamination of damp buildings - examples of risk constructions and risk materials. *Environmental Health Perspectives*, **107**(Suppl. 3), 505-508.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and (^{15}N) nitrate in biological fluids. *Analytical Biochemistry*, **126**, 131-138.

Griffith, C. J., Cooper, R. A., Gilmore, J., Davies, C. & Lewis, M. (2000). An evaluation of hospital cleaning regimes and standards. *Journal of Hospital Infection*, **45**(1), 19-28.

Grigorevski-Lima, A. L., Silva, R. G., Linhares, L. F. & Coelho, R. R. R. (2006). Occurrence of actinomycetes in indoor air in Rio de Janeiro, Brazil. *Building and Environment*, **41**(11), 1540-1543.

Grinshpun, S. A., Aizenberg, V. & Willeke, K. (1998). Personal aerosol sampler with curved porous inlet: wind tunnel evaluation using a full-size manikin. *Journal of Aerosol Science*, **29**(Suppl. 1), S1137-S1138.

Gutarowska, B. & Piotrowska, M. (2007). Methods of mycological analysis in buildings. *Building and Environment*, **42**(4), 1843-1850.

Gyntelberg, F., Suadicani, P., Nielsen, J. W., Skov, P., Valbjørn, O., Nielsen, P. A., Schneider, T., Jørgensen, O., Wolkoff, P., Wilkins, C. K., Gravesen, S. & Norn, S. (1994). Dust and the sick building syndrome. *Indoor Air*, **4**(4), 223-238.

Hagedorn, C. (1976). Influences of soil acidity on *Streptomyces* populations inhabiting forest soils. *Applied and Environmental Microbiology*, **32**(3), 368-375.

Hameed, A. A. A., Yasser, I. H. & Khoder, I. M. (2004). Indoor air quality during renovation actions: a case study. *Journal of Environmental Monitoring*, **6**(9), 740-744.

Haugland, R. A., Brinkmann, N. & Vesper, S. J. (2002). Evaluation of rapid DNA extraction methods for the quantitative detection of fungi using real-time PCR analysis. *Journal of Microbiological Methods*, **50**, 319-323.

Haugland, R. A., Heckman, J. L. & Wymer, L. J. (1999a). Evaluation of different methods for the extraction of DNA from fungal conidia by quantitative competitive PCR analysis. *Journal of Microbiological Methods*, **37**, 165-176.

Haugland, R. A., Varma, M., Wymer, L. J. & Vesper, S. J. (2004). Quantitative PCR analysis of selected *Aspergillus, Penicillium* and *Paecilomyces* species. *Systematic and Applied Microbiology*, **27**, 198-210.

Haugland, R. A., Vesper, S. J. & Wymer, L. J. (1999b). Quantitative measurement of *Stachybotrys chartarum* conidia using real time detection of PCR products with the TaqMan™ fluorogenic probe system. *Molecular and Cellular Probes*, **13**, 329-340.

Haverinen-Shaughnessy, U., Pekkanen, J., Nevalainen, A., Moschandreas, D. & Husman, T. (2004). Estimating effects of moisture damage repairs on students' health - a longterm intervention study. *Journal of Exposure Analysis and Environmental Epidemiology*, **14**, S58-S64.

Haverinen, U., Husman, T., Toivola, M., Suonketo, J., Pentti, M., Lindberg, R., Leinonen, J., Hyvärinen, A., Meklin, T. & Nevalainen, A. (1999). An approach to management of critical indoor air problems in school buildings. *Environmental Health Perspectives*, **107**(Suppl. 3), 509-514.

Heid, C. A., Stevens, J., Livak, K. J. & Williams, P. M. (1996). Real time quantitative PCR. *Genome Research*, **6**, 986-994.

Heinrich, J., Gehring, U., Douwes, J., Koch, A., Fahlbusch, B., Bischof, W. & Wichmann, H. E. (2001). Pets and vermin are associated with high endotoxin levels in house dust. *Clinical and Experimental Allergy*, **31**, 1839-1845.

Heinrich, J., Hölscher, B., Douwes, J., Richter, K., Koch, A., Bischof, W., Fahlbusch, B., Kinne, R. W. & Wichmann, H.-E. (2003). Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment. *Journal of Exposure Analysis and Environmental Epidemiology*, **13**, 152-160.

Hemmann, S., Menz, G., Ismail, C., Blaser, K. & Crameri, R. (1999). Skin test reactivity to 2 recombinant *Aspergillus fumigatus* allergens in *A fumigatus*-sensitized asthmatic subjects allows diagnostic separation of allergic bronchopulmonary aspergillosis from fungal sensitization. *Journal of Allergy and Clinical Immunology*, **104**(3), 601-607.

Hicks, J. B., Lu, E. T., De Guzman, R. & Weingart, M. (2005). Fungal types and concentrations from settled dust in normal residences. *Journal of Occupational and Environmental Hygiene*, **2**, 481-492.

Hippelein, M. & Rügamer, M. (2004). Ergosterol as an indicator of mould growth on building materials. *International Journal of Hygiene and Environmental Health*, **207**(4), 379-385.

Hirsch, T., Hering, M., Bürkner, K., Hirsch, D., Leupold, W., Kerkmann, M. L., Kuhlisch, E. & Jatzwauk, L. (2000). House-dust-mite allergen concentrations (Der f 1) and mold spores in apartment bedrooms before and after installation of insulated windows and central heating systems. *Allergy*, **55**(1), 79-83.

Hirvonen, M.-R., Huttunen, K. & Roponen, M. (2005). Bacterial strains from moldy buildings are highly potent inducers of inflammatory and cytotoxic effects. *Indoor Air*, **15**(Suppl. 9), 65-70.

Hirvonen, M.-R., Nevalainen, A., Makkonen, N., Mönkkönen, J. & Savolainen, K. (1997a). Streptomyces spores from mouldy houses induce nitric oxide, TNFa and IL-6 secretion from RAW264.7 macrophage cell line without causing subsequent cell death. *Environmental Toxicology and Pharmacology*, **3**, 57-63.

Hirvonen, M.-R., Ruotsalainen, M., Roponen, M., Hyvärinen, A., Husman, T., Kosma, V.-M., Komulainen, H., Savolainen, K. & Nevalainen, A. (1999). Nitric oxide and proinflammatory cytokines in nasal lavage fluid associated with symptoms and exposure to moldy building microbes. *American Journal of Respiratory and Critical Care Medicine*, **160**, 1943-1946.

Hirvonen, M.-R., Ruotsalainen, M., Savolainen, K. & Nevalainen, A. (1997b). Effect of viability of actinomycete spores on their ability to stimulate production of nitric oxide and reactive oxygen species in RAW264.7 macrophages. *Toxicology*, **124**(2), 105-114.

Hirvonen, M.-R., Suutari, M., Ruotsalainen, M., Lignell, U. & Nevalainen, A. (2001). Effect of growth medium on potential of *Streptomyces anulatus* spores to induce inflammatory responses and cytotoxicity in RAW264.7 macrophages. *Inhalation Toxicology*, **13**(1), 55-68.

Horner, W. E. (2003). Assessment of the indoor environment: evaluation of mold growth indoors. *Immunology and Allergy Clinics of North America*, **23**(3), 519-531.

Horner, W. E., Worthan, A. G. & Morey, P. R. (2004). Air- and dustborne mycoflora in houses free of water damage and fungal growth. *Applied and Environmental Microbiology*, **70**(11), 6394-6400.

Hsu, S. C. & Lockwood, J. L. (1975). Powdered chitin agar as selective medium for enumeration of actinomycetes in water and soil. *Applied Microbiology*, **29**(3), 422-426.

Hui, P. S., Wong, L. T. & Mui, K. W. (2006). Feasibility study of an express assessment protocol for the indoor air quality of air-conditioned offices. *Indoor and Built Environment*, **15**(4), 373-378.

Hunter, C. A., Grant, C., Flannigan, B. & Bravery, A. F. (1988). Mould in buildings: the air spora of domestic dwellings. *International Biodeterioration*, **24**, 81-101.

Huttunen, K., Hyvärinen, A., Nevalainen, A., Komulainen, H. & Hirvonen, M.-R. (2003). Production of proinflammatory mediators by indoor air bacteria and fungal spores in mouse and human cell lines. *Environmental Health Perspectives*, **111**(1), 85-92.

Huttunen, K., Iivanainen, E., Jussila, J., Katila, M.-L. & Hirvonen, M.-R. (2001). Comparison of mycobacteria-induced cytotoxicity and inflammatory responses in human and mouse cell lines. *Inhalation Toxicology*, **13**, 977-991.

Huttunen, K., Pelkonen, J., Nielsen, K. F., Nuutinen, U., Jussila, J. & Hirvonen, M.-R. (2004). Synergistic interaction in simultaneous exposure to *Streptomyces californicus* and *Stachybotrys chartarum*. *Environmental Health Perspectives*, **112**(6), 659-665.

Huttunen, K., Ruotsalainen, M., Iivanainen, E., Torkko, P., Katila, M.-L. & Hirvonen, M.-R. (2000). Inflammatory responses in RAW264.7 macrophages caused by mycobacteria isolated from moldy houses. *Environmental Toxicology and Pharmacology*, **8**, 237-244.

Hygieniaopas. (2007). *Elintarvikehygienian perusteet. 9th ed.* Elintarvike ja Terveys -lehti. (in Finnish)

Hyvärinen, A. (2002). Characterizing moisture damaged buildings - environmental and biological monitoring. In *Publications of the National Public Health Institute A8*, Kuopio University Printing Office, Finland. Kuopio, pp. 121.

Hyvärinen, A., Meklin, T., Vepsäläinen, A. & Nevalainen, A. (2002). Fungi and actinobacteria in moisture-damaged building materials - concentrations and diversity. *International Biodeterioration & Biodegradation*, **49**(1), 27-37.

Hyvärinen, A., Reiman, M., Meklin, T., Husman, T., Vahteristo, M. & Nevalainen, A. (1999). Fungal exposure and IgG-levels of occupants in houses with and without mold problems. *Third International Conference on Bioaerosols, Fungi and Mycotoxins*, Saratoga Springs, New York. 166-168.

Hyvärinen, A., Reponen, T., Husman, T. & Nevalainen, A. (2001a). Comparison of the indoor air quality in mould damaged and reference buildings in a subarctic climate. *Central European Journal of Public Health*, **9**(3), 133-139.

Hyvärinen, A., Vahteristo, M., Meklin, T., Jantunen, M., Nevalainen, A. & Moschandreas, D. (2001b). Temporal and spatial variation of fungal concentrations in indoor air. *Aerosol Science and Technology*, **35**(2), 688-695.

Hyvärinen, A. M., Martikainen, P. J. & Nevalainen, A. I. (1991). Suitability of poor medium in counting total viable airborne bacteria. *Grana*, **30**(2), 414-417.

IOM (Institute of Medicine; National Academics of Science). (2004). *Damp Indoor Spaces and Health. Committee Report.* The National Academies Press, Washington, D.C., USA.

Ishida, K., Hung, T. V., Lee, H. C., Liou, K., Shin, C. H., Yoon, V. J. & Sohng, J. K. (2006). Degradation of clavulanic acid during the cultivation of *Streptomyces clavuligerus*; instability of clavulanic acid by metabolites and proteins from the strain. *Journal of Microbiology and Biotechnology*, **16**(4), 590-596.

Isik, N., White, L., Barnes, R., Poynton, C. J. & Mills, K. I. (2003). A simple PCR/RFLP analysis can differentiate between *Candida albicans*, *Aspergillus niger*, and *Aspergillus fumigatus*. *Molecular Biotechnology*, **24**(3), 229-231.

Iversen, M. & Dahl, R. (1990). Allergy to storage mites in asthmatic patients and its relation to damp housing conditions. *Allergy*, **45**(2), 81-85.

Jaffal, A. A., Banat, I. M., ElMogheth, A. A., Nsanze, H., Bener, A. & Ameen, A. S. (1997). Residential indoor airborne microbial populations in the United Arab Emirates. *Environment International*, **23**(4), 529-533.

Jarvis, J. Q. & Morey, P. R. (2001). Allergic respiratory disease and fungal remediation in a building in a subtropical climate *Applied Occupational and Environmental Hygiene*, **16**(3), 380-388.

Jensen, J. S., Bruun, B. & Gahrn-Hansen, B. (1999). Unexpected cross-reaction with *Fusobacterium necrophorum* in a PCR for detection of mycoplasmas. *Journal of Clinical Microbiology*, **37**(3), 828-829.

Jo, W.-K. & Seo, Y.-J. (2005). Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes. *Chemosphere*, **61**, 1570-1579.

Johanning, E., Biagini, R., Hull, D., Morey, P., Jarvis, B. & Landsbergis, P. (1996). Health and immunology study following exposure to toxigenic fungi (*Stachybotrys chartarum*) in a water-damaged office environment. *International Archives of Occupational and Environmental Health*, **68**, 207-218.

Johanning, E., Landsbergis, P., Gareis, M., Yang, C. S. & Olmsted, E. (1999). Clinical experience and results of a sentinel health investigation related to indoor fungal exposure. *Environmental Health Perspectives*, **107**(Suppl. 3), 489-494.

Josephson, K. L., Rubino, J. R. & Pepper, I. L. (1997). Characterization and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner. *Journal of Applied Microbiology*, **83**, 737-750.

Jovanovic, S., Felder-Kennel, A., Gabrio, T., Kouros, B., Link, B., Maisner, V., Piechotowski, I., Schick, K. H., Schrimpf, M., Weidner, U., Zöllner, I. & Schwenk, M. (2004). Indoor fungi levels in homes of children with and without allergy history. *International Journal of Hygiene and Environmental Health*, **207**(4), 369-378.

Karandikar, A., Sharples, G. P. & Hobbs, G. (1997). Differentiation of *Streptomyces coelicolor* A3(2) under nitrate-limited conditions. *Microbiology*, **143**, 3581-3590.

Karandikar, A., Sharples, G. P. & Hobbs, G. (1996). Influence of medium composition on sporulation by *Streptomyces coelicolor* A3(2) grown on defined solid media. *Biotechnology Techniques*, **10**(2), 79-82.

Kepner, R. L., Jr. & Pratt, J. R. (1994). Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. *Microbiological Reviews*, **58**(4), 603-615.

Kercsmar, C. M., Dearborn, D. G., Schluchter, M., Xue, L., Kirchner, H. L., Sobolewski, J., Greenberg, S. J., Vesper, S. J. & Allan, T. (2006). Reduction in asthma morbidity in children as a result of home remediation aimed at moisture sources. *Environmental Health Perspectives*, **114**(8), 1574-1580.

Keswani, J., Kashon, M. L. & Chen, B. T. (2005). Evaluation of interference to conventional and real-time PCR for detection and quantification of fungi in dust. *Journal of Environmental Monitoring*, **7**, 311-318.

Kildesø, J., Würtz, H., Nielsen, K. F., Kruse, P., Wilkins, K., Thrane, U., Gravesen, S., Nielsen, P. A. & Schneider, T. (2003). Determination of fungal spore release from wet building materials. *Indoor Air*, **13**(2), 148-155.

Kim, K. Y. & Kim, C. N. (2007). Airborne microbiological characteristics in public buildings of Korea. *Building and Environment*, **42**(5), 2188-2196.

Kleinheinz, G. T., Langolf, B. M. & Englebert, E. (2006). Characterization of airborne fungal levels after mold remediation. *Microbiological Research*, **161**, 367-376.

Koch, A., Heilemann, K.-J., Bischof, W., Heinrich, J. & Wichmann, H. E. (2000). Indoor viable mold spores - a comparison between two cities, Erfurt (eastern Germany) and Hamburg (western Germany). *Allergy*, **55**, 176-180.

Koistinen, T., Ruoppi, P., Putus, T., Pennanen, S., Harju, A. & Nuutinen, J. (2006). Occupational sensitization to storage mites in the personnel of a water-damaged grocery store. *International Archives of Occupational and Environmental Health*, **79**(7), 602-606.

Kokotti, H., Halonen, R., Reiman, M., Seuri, M., Keskikuru, T. & Kalliokoski, P. (1999). Partial repairs of the causes of a moisture problem did not improve the quality of indoor air. *Indoor Air '99*, August 8-13, Edinburgh, Scotland. 203-208.

Kuhn, R. C., Trimble, M. W., Hofer, V., Lee, M. & Nassof, R. S. (2005). Prevalence and airborne spore levels of *Stachybotrys* spp. in 200 houses with water incursions in Houston, Texas. *Canadian Journal of Microbiology*, **51**, 25-28.

Kuo, Y. M. & Li, C. S. (1994). Seasonal fungus prevalence inside and outside of domestic environments in the subtropical climate. *Atmospheric Environment*, **28**(19), 3125-3130.

Kurnitski, J., Palonen, J., Engberg, S. & Ruotsalainen, R. (1996). Koulujen sisäilmasto - rehtorikysely ja sisäilmastomittaukset. Helsinki University of Technology. (in Finnish)

Kutzner, H. J. (1986). The family Streptomycetaceae. In *The Prokaryotes, A Handbook on Habitats, Isolation, and Identification of Bacteria*, eds. M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel, Vol. 2, Springer-Verlag. New York, USA, pp. 2028-2090.

Küster, E. & Williams, S. T. (1964). Selection of media for isolation of streptomycetes. *Nature*, **202**(4935), 928-929.

Lange, J. L., Thorne, P. S. & Lynch, N. (1997). Application of flow cytometry and fluorescent in situ hybridization for assessment of exposures to airborne bacteria. *Applied and Environmental Microbiology*, **63**(4), 1557-1563.

Lappalainen, S., Kähkönen, E., Loikkanen, P., Palomäki, E., Lindroos, O. & Reijula, K. (2001). Evaluation of priorities for repairing in moisture-damaged school buildings in Finland. *Building and Environment*, **36**(8), 981-986.

Large, K. P., Ison, A. P. & Williams, D. J. (1998). The effect of agitation rate on lipid utilisation and clavulanic acid production in *Streptomyces clavuligerus*. *Journal of Biotechnology*, **63**(2), 111-119.

Lee, A. K. Y., Chan, C. K., Fang, M. & Lau, A. P. S. (2004a). The 3-hydroxy fatty acids as biomarkers for quantification and characterization of endotoxins and Gram-negative bacteria in atmospheric aerosols in Hong Kong. *Atmospheric Environment*, **38**(37), 6307-6317.

Lee, J. H. & Jo, W. K. (2006). Characteristics of indoor and outdoor bioaerosols at Korean high-rise apartment buildings. *Environmental Research*, **101**(1), 11-17.

Lee, K. J. & Rho, Y. T. (1993). Characteristics of spores formed by surface and submerged cultures of *Streptomyces albidoflavus* SMF301. *Journal of General Microbiology*, **139**, 3131-3137.

Lee, K. S., Bartlett, K. H., Brauer, M., Stephens, G. M., Black, W. A. & Teschke, K. (2004b). A field comparison of four samplers for enumerating fungal aerosols I. Sampling characteristics. *Indoor Air*, **14**(5), 360-366.

Lee, T., Grinshpun, S. A., Martuzevicius, D., Adhikari, A., Crawford, C. M. & Reponen, T. (2006). Culturability and concentration of indoor and outdoor airborne fungi in six single-family homes. *Atmospheric Environment*, **40**(16), 2902-2910.

Lehtonen, M., Reponen, T. & Nevalainen, A. (1993). Everyday activities and variation of fungal spore concentrations in indoor air. *International Biodeterioration & Biodegradation*, **31**, 25-39.

Levetin, E. (1995). Fungi. In *Bioaerosols*, ed. H. A. Burge, CRC Press, inc. Boca Raton, Florida, pp. 87-120.

Li, C. S. & Kuo, Y. M. (1994). Characteristics of airborne microfungi in subtropical homes. *The Science of the Total Environment*, **155**(3), 267-271.

Li, D.-W. & Kendrick, B. (1995). A year-round study on functional relationships of airborne fungi with meteorological factors. *International Journal of Biometeorology*, **39**, 74-80.

Li, D.-W. & Yang, C. S. (2004). Fungal contamination as a major contributor to sick building syndrome. *Advances in Applied Microbiology*, **55**, 31-112.

Lignell, U., Husman, T., Meklin, T., Koivisto, J., Vahteristo, M., Vepsäläinen, A., Kettunen, A.-V., Nevalainen, A. & Kalliokoski, P. (2000). Moisture in school kitchens and symptoms of the kitchen personnel. *Healthy Buildings 2000*, Espoo, Finland. Gummerus Kirjapaino Oy, Jyväskylä, Finland. 299-303.

Lin, W.-H. & Li, C.-S. (1996). Size characteristics of fungus allergens in the subtropical climate. *Aerosol Science and Technology*, **25**(2), 93-100.

Lindsley, W. G., Schmechel, D. & Chen, B. T. (2006). A two-stage cyclone using microcentrifuge tubes for personal bioaerosol sampling. *Journal of Environmental Monitoring*, **8**(11), 1136-1142.

Liu, L.-J. S., Krahmer, M., Fox, A., Feigley, C. E., Featherstone, A., Saraf, A. & Larsson, L. (2000). Investigation of the concentration of bacteria and their cell envelope components in indoor air in two elementary schools. *Journal of the Air & Waste Management Association*, **50**, 1957-1967.

Loria, R. A., Kempter, A. & Jamieson, A. A. (1986). Characterization of *Streptomyces* spp. causing potato scab in the Northeast. *American Potato Journal*, **63**, 440.

Macher, J., Ammann, H. A., Burge, H. A., Milton, D. K., Morey, P. R. & eds. (1999). *Bioaerosols: Assessment and Control*. American Conference of Governmental Industrial Hygienist, Cincinnati, OH.

Macher, J. M. (2001a). Evaluation of a procedure to isolate culturable microorganisms from carpet dust. *Indoor Air*, **11**, 134-140.

Macher, J. M. (2001b). Review of methods to collect settled dust and isolate culturable microorganisms. *Indoor Air*, **11**, 99-110.

Macher, J. M., Huang, F.-Y. & Flores, M. (1991). A two-year study of microbiological indoor air quality in a new apartment. *Archives of Environmental Health*, **46**(1), 25-29.

MacNeil, L., Kauri, T. & Robertson, W. (1995). Molecular techniques and their potential application in monitoring the microbiological quality of indoor air. *Canadian Journal of Microbiology*, **41**, 657-665.

Madden, T., Ward, J. M. & Ison, A. P. (1996). Organic acid excretion by *Streptomyces lividans* TK24 during growth on defined carbon and nitrogen sources. *Microbiology*, **142**, 3181-3185.

Mahieu, L. M., De Dooy, J. J., Van Laer, F. A., Jansens, H. & Ieven, M. M. (2000). A prospective study on factors influencing aspergillus spore load in the air during renovations works in a neonatal intensive care unit. *Journal of Hospital Infection* **45**, 191-197.

Mahooti-Brooks, N., Storey, E., Yang, C., Simcox, N. J., Turner, W. & Hodgson, M. (2004). Characterization of mold and moisture indicators in the home. *Journal of Occupational and Environmental Hygiene*, **1**(12), 826-839.

Marchant, R. E., Yoshida, K., Walkinshaw, D., Ross, J. B., Gallant, C. & Shires, D. B. (1990). Skin irritation and dyspnea in kitchen workers: sodium hydroxide. *Indoor Air '90*, July 29 - August 3, 1990, Toronto, Ottawa. 653-657.

Matheson, M. C., Abramson, M. J., Dharmage, S. C., Forbes, A. B., Raven, J. M., Thien, F. C. K. & Walters, E. H. (2005). Changes in indoor allergen and fungal levels predict changes in asthma activity among young adults. *Clinical and Experimental Allergy*, **35**(7), 907-913.

Mattick, K., Durham, K., Hendrix, M., Slader, J., Griffith, C., Sen, M. & Humphrey, T. (2003). The microbiological quality of washing-up water and the environment in domestic and commercial kitchens. *Journal of Applied Microbiology*, **94**, 842-848.

Mazur, L. J. & Kim, J. (2006). Spectrum of noninfectious health effects from molds. *Pediatrics*, **118**(6), E1909-E1926.

McDevitt, J. J., Lees, P. S. J., Merz, W. G. & Schwab, K. J. (2004). Development of a method to detect and quantify *Aspergillus fumigatus* conidia by quantitative PCR for environmental air samples. *Mycopathologia*, **158**(3), 325-335.

Medrela-Kuder, E. (2003). Seasonal variations in the occurrence of culturable airborne fungi in outdoor and indoor air in Cracow. *International Biodeterioration & Biodegradation*, **52**(4), 203-205.

Meklin, T. (2002). Microbial exposure and health in schools - effects of moisture damage and renovation. In *Publications of the National Public Health Institute A13*, Kuopio University Printing Office, Finland. Kuopio, pp. 83.

Meklin, T., Haugland, R. A., Reponen, T., Varma, M., Lummus, Z., Bernstein, D., Wymer, L. J. & Vesper, S. J. (2004). Quantitative PCR analysis of house dust can reveal abnormal mold conditions. *Journal of Environmental Monitoring*, **6**, 615-620.

Meklin, T., Husman, T., Vepsäläinen, A., Vahteristo, M., Koivisto, J., Halla-aho, J., Hyvärinen, A., Moschandreas, D. & Nevalainen, A. (2002a). Indoor air microbes and respiratory symptoms of children in moisture damaged and reference schools. *Indoor Air*, **12**(3), 175-183.

Meklin, T., Hyvärinen, A., Toivola, M., Reponen, T., Koponen, V., Husman, T., Taskinen, T., Korppi, M. & Nevalainen, A. (2003). Effect of building frame and moisture damage on microbiological indoor air quality in school buildings. *American Industrial Hygiene Association Journal*, **64**(1), 108-116.

Meklin, T., Putus, T., Hyvärinen, A., Haverinen-Shaughnessy, U., Lignell, U. & Nevalainen, A. (2007a). *Koulurakennusten kosteus- ja homevauriot Opas ongelmien selvittämiseen*. Edita Publishing Oy, Helsinki. (in Finnish)

Meklin, T., Putus, T., Pekkanen, J., Hyvärinen, A., Hirvonen, M.-R. & Nevalainen, A. (2005). Effects of moisture-damage repairs on microbial exposure and symptoms in schoolchildren. *Indoor Air*, **15**(Suppl. 10), 40-47.

Meklin, T., Reponen, T., McKinstry, C., Cho, S.-H., Grinshpun, S. A., Nevalainen, A., Vepsäläinen, A., Haugland, R. A., LeMasters, G. & Vesper, S. J. (2007b). Comparison of mold concentrations quantified by MSQPCR in indoor and outdoor air sampled simultaneously. *Science of The Total Environment*, **382**(1), 130-134.

Meklin, T., Reponen, T., Toivola, M., Koponen, V., Husman, T., Hyvärinen, A. & Nevalainen, A. (2002b). Size distributions of airborne microbes in moisture-damaged and reference school buildings of two construction types. *Atmospheric Environment*, **36**(39-40), 6031-6039.

Miller, J. D., Dugandzic, R., Frescura, A. M. & Salares, V. (2007). Indoor- and outdoor-derived contaminants in urban and rural homes in Ottawa, Ontario, Canada. *Journal of the Air & Waste Management Association*, **57**(3), 297-302.

Miller, J. D. & Young, J. C. (1997). The use of ergosterol to measure exposure to fungal propagules in indoor air. *American Industrial Hygiene Association Journal*, **58**(1), 39-43.

Ministry of Social Affairs and Health. (2003). *Asumisterveysohje*. (in Finnish)

Ministry of the Environment. (2003). D2 National Building Code of Finland, Indoor Climate and Ventilation of Buildings.

Morey, P. R., Hull, M. C. & Andrew, M. (2003). El Niño water leaks identify rooms with concealed mould growth and degraded indoor air quality. *International Biodeterioration & Biodegradation*, **52**(3), 197-202.

Moschandreas, D. J., Cha, D. K. & Qian, J. (1996). Measurement of indoor bioaerosol levels by a direct counting method. *Journal of Environmental Engineering-ASCE*, **122**(5), 374-378.

Mudarri, D. & Fisk, W. J. (2007). Public health and economic impact of dampness and mold. *Indoor Air*, **17**, 226-235.

Muilenberg, M. L. (2003). Sampling devices. *Immunology and Allergy Clinics of North America*, **23**(3), 337-355.

Mullins, J. (2001). Microorganisms in outdoor air. In *Microorganisms in home and indoor work environments*, eds. B. Flannigan, R. A. Samson & D. J. Miller, Taylor & Francis. London, pp. 3-16.

Murtoniemi, T., Nevalainen, A., Suutari, M., Toivola, M., Komulainen, H. & Hirvonen, M.-R. (2001). Induction of cytotoxicity and production of inflammatory mediators in RAW264.7 macrophages by spores grown on six different plasterboards. *Inhalation Toxicology*, **13**, 233-247.

Müller, A., Lehmann, I., Seiffart, A., Diez, U., Wetzig, H., Borte, M. & Herbarth, O. (2002). Increased incidence of allergic sensitisation and respiratory diseases due to mould exposure: Results of the Leipzig Allergy Risk children Study (LARS). *International Journal of Hygiene and Environmental Health*, **204**(5-6), 363-365.

Nabais, A. M. A. & da Fonseca, M. M. R. (1995). The effect of solid medium composition on growth and sporulation of *Streptomyces clavuligerus*; spore viability during storage at +4°C. *Biotechnology Techniques*, **9**(5), 361-364.

Nevalainen, A. (1989). Bacterial aerosols in indoor air. In *Department of Environmental Hygiene and Toxicology*, Vol. PhD, National Public Health Institute. Kuopio, pp. 85.

Nevalainen, A., Partanen, P., Jääskeläinen, E., Hyvärinen, A., Koskinen, O., Meklin, T., Vahteristo, M., Koivisto, J. & Husman, T. (1998). Prevalence of moisture problems in Finnish houses. *Indoor Air*, **4**, 45-49.

Nevalainen, A., Pasanen, A. L., Niininen, M., Reponen, T., Kalliokoski, P. & Jantunen, M. J. (1991). The indoor air quality in Finnish homes with mold problems. *Environment International*, **17**(4), 299-302.

Nevalainen, A., Pastuszka, J., Liebhaber, F. & Willeke, K. (1992). Performance of bioaerosol samplers - collection characteristics and sampler design considerations. *Atmospheric Environment*, **26A**(4), 531-540.

Niemeier, R. T., Sivasubramani, S. K., Reponen, T. & Grinshpun, S. A. (2006). Assessment of fungal contamination in moldy homes: Comparison of different methods. *Journal of Occupational and Environmental Hygiene*, **3**(5), 262-273.

Nilsson, A., Kihlström, E., Lagesson, V., Wessén, B., Szponar, B., Larsson, L. & Tagesson, C. (2004). Microorganisms and volatile organic compounds in airborne dust from damp residences. *Indoor Air*, **14**(2), 74-82.

Norbäck, D., Wålinder, R., Wieslander, G., Smedje, G., Erwall, C. & Venge, P. (2000). Indoor air pollutants in schools: nasal patency and biomarkers in nasal lavage. *Allergy*, **55**, 163-170.

Novella, I. S., Barbés, C. & Sánchez, J. (1992). Sporulation of *Streptomyces antibioticus* ETHZ 7451 in submerged culture. *Canadian Journal of Microbiology*, **38**(8), 769-773.

O'Connor, G. T., Walter, M., Mitchell, H., Kattan, M., Morgan, W. J., Gruchalla, R. S., Pongracic, J. A., Smartt, E., Stout, J. W., Evans, R., Crain, E. F. & Burge, H. A. (2004). Airborne fungi in the homes of children with asthma in low-income urban communities: The Inner-City Asthma Study. *Journal of Allergy and Clinical Immunology*, **114**(3), 599-606.

Obbard, J. P. & Fang, L. S. (2003). Airborne concentrations of bacteria in a hospital environment in Singapore. *Water, Air, and Soil Pollution*, **144**(1), 333-341.

Ochi, K. (1986). A decrease in GTP content is associated with aerial mycelium formation in *Streptomyces* MA406-A-1. *Journal of General Microbiology*, **132**, 299-305.

Ogata, S., Matsubara, H., Tawara, Y., Tokunaga, S. & Hara, T. (1992). Screening of compounds stimulating spore formation and mycelial growth of pock-forming plasmid-carrying strains in *Streptomyces azureus*. *Applied Microbiology and Biotechnology*, **37**(5), 652-654.

Osborne, M., Reponen, T., Adhikari, A., Cho, S. H., Grinshpun, S. A., Levin, L., Bernstein, D. I. & LeMasters, G. (2006). Specific fungal exposures, allergic sensitization, and rhinitis in infants. *Pediatric Allergy and Immunology*, **17**(6), 450-457.

Overberger, P. A., Wadowsky, R. M. & Schaper, M. M. (1995). Evaluation of airborne particulates and fungi during hospital renovation. *American Industrial Hygiene Association Journal*, **56**, 706-712.

Padan, E., Zilberstein, D. & Schuldinger, S. (1981). pH homeostasis in bacteria. *Biochimica et Biophysica Acta*, **650**, 151-166.

Parat, S., Perdrix, A., Fricker-Hidalgo, H., Saude, I., Grillot, R. & Baconnier, P. (1997). Multivariate analysis comparing microbial air content of an air-conditioned building and a naturally ventilated building over one year. *Atmospheric Environment*, **31**(3), 441-449.

Park, J.-H., Cox-Ganser, J., Rao, C. & Kreiss, K. (2006). Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building. *Indoor Air*, **16**, 192-203.

Pasanen, A.-L. (1992). Airborne mesophilic fungal spores in various residential environments. *Atmospheric Environment*, **26A**(16), 2861-2868.

Pasanen, A.-L. (2001). A review: fungal exposure assessment in indoor environments. *Indoor Air*, **11**(2), 87-98.

Pasanen, A.-L., Heinonen-Tanski, H., Kalliokoski, P. & Jantunen, M. J. (1992a). Fungal microcolonies on indoor surfaces - an explanation for the base-level fungal spore counts in indoor air. *Atmospheric Environment*, **26B**(1), 117-120.

Pasanen, A.-L., Kasanen, J. P., Rautiala, S., Ikäheimo, M., Rantamäki, J., Kääriäinen, H. & Kalliokoski, P. (2000a). Fungal growth and survival in building materials under fluctuating moisture and temperature conditions. *International Biodeterioration & Biodegradation*, **46**(2), 117-127.

Pasanen, A.-L., Korpi, A., Kasanen, J.-P. & Pasanen, P. (1998). Critical aspects on the significance of microbial volatile metabolites as indoor air pollutants. *Environment International*, **24**(7), 703-712.

Pasanen, A.-L., Niininen, M., Kalliokoski, P., Nevalainen, A. & Jantunen, M. J. (1992b). Airborne *Cladosporium* and other fungi in damp versus reference residences. *Atmospheric Environment*, **26B**(1), 121-124.

Pasanen, A.-L., Pasanen, P., Jantunen, M. J. & Kalliokoski, P. (1991). Significance of air humidity and air velocity for fungal spore release into the air. *Atmospheric Environment*, **25A**(2), 459-462.

Pasanen, A.-L., Rautiala, S., Kasanen, J. P., Raunio, P., Rantamäki, J. & Kalliokoski, P. (2000b). The relationship between measured moisture conditions and fungal concentrations in water-damaged building materials. *Indoor Air*, **10**(2), 111-120.

Pasanen, A.-L., Yli-Pietilä, K., Pasanen, P., Kalliokoski, P. & Tarhanen, J. (1999). Ergosterol content in various fungal species and biocontaminated building materials. *Applied and Environmental Microbiology*, **65**(1), 138-142.

Pasanen, P., Korpi, A., Kalliokoski, P. & Pasanen, A.-L. (1997). Growth and volatile metabolite production of *Aspergillus versicolor* in house dust. *Environment International*, **23**(4), 425-432.

Paterson, R. R. M. (2007). Internal amplification controls have not been employed in fungal PCR hence potential false negative results. *Journal of Applied Microbiology*, **102**(1), 1-10.

Peat, J. K., Dickerson, J. & Li, J. (1998). Effects of damp and mould in the home on respiratory health: a review of the literature. *Allergy*, **53**, 120-128.

Peccia, J. & Hernandez, M. (2006). Incorporating polymerase chain reaction-based identification, population characterization, and quantification of microorganisms into aerosol science: A review. *Atmospheric Environment*, **40**(21), 3941-3961.

Pekkanen, J., Hyvärinen, A., Haverinen-Shaughnessy, U., Korppi, M., Putus, T. & Nevalainen, A. (2007). Moisture damage and childhood asthma: a population-based incident case-control study. *European Respiratory Journal*, **29**, 1-7.

Pennanen, S., Mussalo-Rauhamaa, H., Harju, A., Pippuri, M., Liesivuori, J., Elg, P., Hakala, K. & Haahtela, T. (2007). Exposure to mites, sensitisation and allergy to mites in moisture damaged buildings. *Indoor and Built Environment*, **16**(1), 19-27.

Penttinen, P., Pelkonen, J., Huttunen, K., Toivola, M. & Hirvonen, M.-R. (2005). Interactions between *Streptomyces californicus* and *Stachybotrys chartarum* can induce apoptosis and cell cycle arrest in mouse RAW264.7 macrophages. *Toxicology and Applied Pharmacology*, **202**, 278-288.

Penttinen, P., Tampio, M., Mäki-Paakkanen, J., Vähäkangas, K., Pelkonen, J. & Hirvonen, M.-R. (2007). DNA damage and p53 in RAW264.7 cells induced by the spores of co-cultivated *Streptomyces californicus* and *Stachybotrys chartarum*. *Toxicology*, **235**(1-2), 92-102.

Pessi, A. M., Suonketo, J., Pentti, M., Kurkilahti, M., Peltola, K. & Rantio-Lehtimäki, A. (2002). Microbial growth inside insulated external walls as an indoor air biocontamination source. *Applied and Environmental Microbiology*, **68**(2), 963-967.

Piecková, E. & Wilkins, K. (2004). Airway toxicity of house dust and its fungal composition. *Annals of Agricultural and Environmental Medicine*, **11**(1), 67-73.

Piipari, R. & Keskinen, H. (2005). Agents causing occupational asthma in Finland in 1986-2002: Cow epithelium bypassed by moulds from moisture-damaged buildings. *Clinical and Experimental Allergy*, **35**(12), 1632-1637.

Pollak, F. C. & Berger, R. G. (1996). Geosmin and related volatiles in bioreactor-cultured *Streptomyces citreus* CBS 109.60. *Applied and Environmental Microbiology*, **62**(4), 1295-1299.

Porter, J. N., Wilhelm, J. J. & Tresner, H. D. (1960). Method for the preferential isolation of actinomycetes from soils. *Applied Microbiology*, **8**, 174-178.

Portnoy, J. M., Barnes, C. S. & Kennedy, K. (2004). Sampling for indoor fungi. *Journal of Allergy and Clinical Immunology*, **113**(2), 189-198.

Portnoy, J. M., Kwak, K., Dowling, P., VanOsdol, T. & Barnes, C. (2005). Health effects of indoor fungi. *Annals of Allergy, Asthma & Immunology*, **94**(3), 313-320.

Prat, J., Xaubet, A. & Mullol, J. (1993). Immunocytologic analysis of nasal cells obtained by nasal lavage: A comparative study with standard method of cell identification. *Allergy*, **48**, 587-591.

Pridham, T. G. & Tresner, H. D. (1974). Family VII. Streptomycetaceae. 8th edition ed. In *Bergey's Manual of Determinative Bacteriology*, eds. R. E. Buchanan & N. E. Gibbons, The Williams & Wilkins Company. Baltimore, pp. 747-748.

Purokivi, M. K., Hirvonen, M.-R., Randell, J. T., Roponen, M. H., Meklin, T. M., Nevalainen, A. L., Husman, T. M. & Tukiainen, H. O. (2001). Changes in pro-inflammatory cytokines in association with exposure to moisture-damaged building microbes. *European Respiratory Journal*, **18**(6), 951-958.

Putus, T., Tuomainen, A. & Rautiala, S. (2004). Chemical and microbial exposures in a school building: adverse health effects in children. *Archives of Environmental Health*, **59**(4), 194-201.

Pyankov, O. V., Agranovski, I. E., Pyankova, O., Mokhonova, E., Mokhonov, V., Safatov, A. S. & Khromykh, A. A. (2007). Using a bioaerosol personal sampler in combination with real-

time PCR analysis for rapid detection of airborne viruses. *Environmental Microbiology*, **9**(4), 992-1000.

Ramachandran, G., Adgate, J. L., Banerjee, S., Church, T. R., Jones, D., Fredrickson, A. & Sexton, K. (2005). Indoor air quality in two urban elementary schools - measurements of airborne fungi, carpet allergens, CO_2, temperature, and relative humidity. *Journal of Occupational and Environmental Hygiene*, **2**, 553-566.

Rao, C. Y., Burge, H. A. & Chang, J. C. (1996). Review of quantitative standards and guidelines for fungi in indoor air. *Journal of the Air & Waste Management Association*, **46**(9), 899-908.

Raunio, P., Kärkkäinen, M., Virtanen, T., Rautiainen, J. & Pasanen, A.-L. (2001). Preliminary description of antigenic components characteristic of *Stachybotrys chartarum*. *Environmental Research Section A*, **85**(3), 246-255.

Raunio, P., Pasanen, A.-L., Husman, T. & Virtanen, T. (1999). Exposure to *Stachybotrys chartarum* induces immunoglobulin A antibody response in man. *Third International Conference on Bioaerosols, Fungi and Mycotoxins*, Saratoga Springs, New York. 174-178.

Rautiala, S., Reponen, T., Hyvärinen, A., Nevalainen, A., Husman, T., Vehviläinen, A. & Kalliokoski, P. (1996). Exposure to airborne microbes during the repair of moldy buildings. *American Industrial Hygiene Association Journal*, **57**, 279-284.

Rautiala, S., Reponen, T., Nevalainen, A., Husman, T. & Kalliokoski, P. (1998). Control of exposure to airborne viable microorganisms during remediation of moldy buildings; report of three case studies. *American Industrial Hygiene Association Journal*, **59**, 455-460.

Rautiala, S., Torvinen, E., Torkko, P., Suomalainen, S., Nevalainen, A., Kalliokoski, P. & Katila, M. L. (2004). Potentially pathogenic, slow-growing mycobacteria released into workplace air during the remediation of buildings. *Journal of Occupational and Environmental Hygiene*, **1**(1), 1-6.

Reeslev, M. & Miller, M. (2000). Mycometer-test: a new rapid method for detecting and quantification of mould in buildings. *Healthy Buildings 2000*, Espoo, Finland. Gummerus Kirjapaino Oy, Jyväskylä, Finland. 589-590.

Ren, P., Jankun, T. M. & Leaderer, B. P. (1999). Comparisons of seasonal fungal prevalence in indoor and outdoor air and in house dusts of dwellings in one Northeast American county. *Journal of Exposure Analysis and Environmental Epidemiology*, **9**(6), 560-568.

Reponen, T. (1995). Aerodynamic diameters and respiratory deposition estimates of viable fungal particles in mold problem dwellings. *Aerosol Science and Technology*, **22**(1), 11-23.

Reponen, T., Hyvärinen, A., Ruuskanen, J., Raunemaa, T. & Nevalainen, A. (1994). Comparison of concentrations and size distributions of fungal spores in buildings with and without mold problems. *Journal of Aerosol Science*, **25**(8), 1595-1603.

Reponen, T., Nevalainen, A., Jantunen, M., Pellikka, M. & Kalliokoski, P. (1992). Normal range criteria for indoor air bacteria and fungal spores in a subarctic climate. *Indoor Air*, **2**, 26-31.

Reponen, T., Willeke, K., Grinshpun, S. & Nevalainen, A. (2001). Biological particle sampling. 2nd ed. In *Aerosol Measurement*, eds. P. A. Baron & K. Willeke, John Wiley and Sons, Inc. New York, pp. 751-777.

Reynolds, S. J., Streifel, A. J. & McJilton, C. E. (1990). Elevated airborne concentrations of fungi in residential and office environments. *American Industrial Hygiene Association Journal*, **51**(11), 601-604.

Rho, Y. T. & Lee, K. J. (1994). Kinetic characterization of sporulation in *Streptomyces albidoflavus* SMF301 during submerged culture. *Microbiology* **140**, 2061-2065.

Rintala, H., Hyvärinen, A., Paulin, L. & Nevalainen, A. (2004). Detection of streptomycetes in house dust - comparison of culture and PCR methods. *Indoor Air*, **14**, 112-119.

Rintala, H. & Nevalainen, A. (2006). Quantitative measurement of streptomycetes using real-time PCR. *Journal of Environmental Monitoring*, **8**, 745-749.

Roe, J. D., Haugland, R. A., Vesper, S. J. & Wymer, L. J. (2001). Quantification of *Stachybotrys chartarum* conidia in indoor dust using real time, fluorescent probe-based detection of PCR products. *Journal of Exposure Analysis and Environmental Epidemiology*, **11**, 12-20.

Rogers, C. A. (2003). Indoor fungal exposure. *Immunology and Allergy Clinics of North America*, **23**(3), 501-518.

Roponen, M., Kiviranta, J., Seuri, M., Tukiainen, H., Myllykangas-Luosujärvi, R. & Hirvonen, M.-R. (2001a). Inflammatory mediators in nasal lavage, induced sputum and serum of employees with rheumatic and respiratory disorders. *European Respiratory Journal*, **18**, 542-548.

Roponen, M., Toivola, M., Alm, S., Nevalainen, A., Jussila, J. & Hirvonen, M.-R. (2003). Inflammatory and cytotoxic potential of the airborne particle material assessed by nasal lavage and cell exposure methods. *Inhalation Toxicology*, **15**, 23-38.

Roponen, M., Toivola, M., Meklin, T., Ruotsalainen, M., Komulainen, H., Nevalainen, A. & Hirvonen, M.-R. (2001b). Differences in inflammatory responses and cytotoxicity in RAW264.7 macrophages induced by *Streptomyces anulatus* grown on different building materials. *Indoor Air*, **11**, 179-184.

Ruoff, K. L. (1999). Leuconostoc, Pediococcus, Stomatococcus, and miscellaneous grampositive cocci that grow aerobically. 7. ed. In *Manual of Clinical Microbiology*, eds. P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover & R. H. Yolken, ASM Press. Washington, D.C., pp. 306-315.

Ruotsalainen, R., Jaakkola, N. & Jaakkola, J. J. K. (1993). Ventilation and indoor air quality in Finnish daycare centers. *Environment International*, **19**, 109-119.

Rusin, P., Orosz-Coughlin, P. & Gerba, C. (1998). Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaners. *Journal of Applied Microbiology*, **85**, 819-828.

Rutledge, R. G. (2004). Sigmoidal curve-fitting redefines quantitative real-time PCR with the prospective of developing automated high-throughput applications. *Nucleic Acids Research*, **32**(22), e178.

Rutledge, R. G. & Côté, C. (2003). Mathematics of quantitative kinetic PCR and the application of standard curves. *Nucleic Acids Research*, **31**(16), e93.

Rylander, R. (2004). Microbial cell wall agents and sick building syndrome. *Advances in Applied Microbiology*, **55**, 139-154.

Rylander, R. & Etzel, R. (1999). Introduction and summary: workshop on children's health and indoor mold exposure. *Environmental Health Perspectives*, **107**(Suppl. 3), 465-468.

Rylander, R., Norrhall, M., Engdahl, U., Tunsäter, A. & Holt, P. G. (1998). Airways inflammation, atopy, and (1->3)-β-D-glucan exposures in two schools. *American Journal of Respiratory and Critical Care Medicine*, **158**(5), 1685-1687.

Saad, D. S., Kinsey, G. C., Kim, S. & Gaylarde, C. C. (2004). Extraction of genomic DNA from filamentous fungi in biofilms on water-based paint coatings. *International Biodeterioration & Biodegradation*, **54**, 99-103.

Sait, M., Hugenholtz, P. & Janssen, P. H. (2002). Cultivation of globally distributed soil bacteria from phylogenetic lineages previously only detected in cultivation-independent surveys. *Environmental Microbiology*, **4**(11), 654-666.

Salo, P. M., Arbes, S. J., Sever, M., Jaramillo, R., Cohn, R. D., London, S. J. & Zeldin, D. C. (2006). Exposure to *Alternaria alternata* in US homes is associated with asthma symptoms. *Journal of Allergy and Clinical Immunology*, **118**(4), 892-898.

Samson, R., Flannigan, B., Flannigan, M., Verhoeff, A., Adan, O. & Hoekstra, E. (1994). Recommendations. In *Health implications of fungi in indoor environments*, Elsevier Science. Amsterdam, pp. 529-538.

Santilli, J. (2002). Health effects of mold exposure in public schools. *Current Allergy and Asthma Reports*, **2**, 460-467.

Santilli, J. & Rockwell, W. (2003). Fungal contamination of elementary schools: a new environmental hazard. *Annals of Allergy, Asthma, and Immunology*, **90**, 203-208.

SAS Institute Inc. (1999). SAS OnlineDoc. 8. ed, SAS Institute Inc. Cary, NC USA.

Savilahti, R., Uitti, J., Laippala, P., Husman, T. & Roto, P. (2000). Respiratory morbidity among children following renovation of a water-damaged school. *Archives of Environmental Health*, **55**(6), 405-410.

Schram-Bijkerk, D., Doekes, G., Boeve, M., Douwes, J., Riedler, J., Üblagger, E., von Mutius, E., Benz, M., Pershagen, G., Wickman, M., Alfvén, T., Braun-Fahrländer, C., Waser, M. & Brunekreef, B. (2006). Exposure to microbial components and allergens in population studies: a comparison of two house dust collection methods applied by participants and fieldworkers. *Indoor Air*, **16**(6), 414-425.

Schwab, M., McDermott, A. & Spengler, J. D. (1992). Using longitudinal data to understand children's activity patterns in an exposure context: Data from the Kanawha Country Health Study. *Environment International*, **18**, 173-191.

Schöller, C. E., Gürtler, H., Pedersen, R., Molin, S. & Wilkins, K. (2002). Volatile metabolites from actinomycetes. *Journal of Agricultural and Food Chemistry*, **50**(9), 2615-2621.

Scott, E., Bloomfield, S. F. & Barlow, C. G. (1982). An investigation of microbial contamination in the home. *The Journal of Hygiene*, **89**, 279-293.

Sebastian, A. & Larsson, L. (2003). Characterization of the microbial community in indoor environments: a chemical-analytical approach. *Applied and Environmental Microbiology*, **69**(6), 3103-3109.

Sebastian, A., Szponar, B. & Larsson, L. (2005). Characterization of the microbial community in indoor environments by chemical marker analysis: an update and critical evaluation. *Indoor Air*, **15**(Suppl. 9), 20-26.

Shelton, B. G., Kirkland, K. H., Flanders, W. D. & Morris, G. K. (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied and Environmental Microbiology*, **68**(4), 1743-1753.

Shen, H.-D., Lin, W.-L., Tsai, J.-J., Liaw, S.-F. & Han, S.-H. (1996). Allergenic components in three different species of *Penicillium*: crossreactivity among major allergens. *Clinical and Experimental Allergy*, **26**, 444-451.

Shinn, E. A., Griffin, D. W. & Seba, D. B. (2003). Atmospheric transport of mold spores in clouds of desert dust. *Archives of Environmental Health*, **58**(8), 498-504.

Shoemaker, R. C. & House, D. E. (2006). Sick building syndrome (SBS) and exposure to water-damaged buildings: Time series study, clinical trial and mechanisms. *Neurotoxicology and Teratology*, **28**(5), 573-588.

Sivasubramani, S. K., Niemeier, R. T., Reponen, T. & Grinshpun, S. A. (2004). Fungal spore source strength tester: laboratory evaluation of a new concept. *Science of the Total Environment*, **329**(1-3), 75-86.

Smedje, G. & Norbäck, D. (2001). Irritants and allergens at school in relation to furnishings and cleaning. *Indoor Air*, **11**(2), 127-33.

Solarz, K. (1998). The allergenic acarofauna of house dust from dwellings, hospitals, libraries and institutes in Upper Silesia (Poland). *Annals of Agricultural and Environmental Medicine*, **5**, 73-85.

Speirs, J. P., Anderton, A. & Anderson, J. G. (1995). A study of microbial content of the domestic kitchen. *International Journal of Environmental Health Research*, **5**, 109-122.

Spengler, J. D., Samet, J. M. & McCarthy, J. F. (2001). *Indoor air quality handbook*. The McGraw-Hill Companies, Inc., New York.

SPSS Inc. (1988). SPSS-X™ User's Guide. 3. ed, SPSS Inc. Chicago.

Spurgeon, J. C. (2003). A sampling method for comparing fungal concentrations in carpets. *American Industrial Hygiene Association Journal*, **64**(6), 842-845.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *International Journal of Systematic Bacteriology*, **47**(2), 479-491.

Stark, H., Roponen, M., Purokivi, M., Randell, J., Tukiainen, H. & Hirvonen, M.-R. (2006). *Aspergillus fumigatus* challenge increases cytokine levels in nasal lavage fluid. *Inhalation Toxicology*, **18**(13), 1033-1039.

Stark, P. C., Burge, H. A., Ryan, L. M., Milton, D. K. & Gold, D. R. (2003). Fungal levels in the home and lower respiratory tract illnesses in the first year of life. *American Journal of Respiratory and Critical Care Medicine*, **168**, 232-237.

Stetzenbach, L. D., Buttner, M. P. & Cruz, P. (2004). Detection and enumeration of airborne biocontaminants. *Current Opinion in Biotechnology*, **15**, 170-174.

Stewart, S. L., Grinshpun, S. A., Willeke, K., Terzieva, S., Ulevicius, V. & Donnelly, J. (1995). Effect of impact stress on microbial recovery on an agar surface. *Applied and Environmental Microbiology*, **61**(4), 1232-1239.

Strachan, D. P., Flannigan, B., McCabe, E. M. & McGarry, F. (1990). Quantification of airborne moulds in the homes of children with and without wheeze. *Thorax*, **45**, 382-387.

Su, H.-J., Wu, P.-C., Chen, H.-L., Lee, F.-C. & Lin, L.-L. (2001). Exposure assessment of indoor allergens, endotoxin, and airborne fungi for homes in Southern Taiwan. *Environmental Research*, **85**(Section A), 135-144.

Sunesson, A.-L., Nilsson, C.-A., Carlson, R., Blomquist, G. & Andersson, B. (1997). Production of volatile metabolites from *Streptomyces albidoflavus* cultivated on gypsum board and tryptone glucose extract agar - Influence of temperature, oxygen and carbon dioxide levels. *Annals of Occupational Hygiene*, **41**(4), 393-413.

Surowitz, K. G. & Pfister, R. M. (1985). Glucose metabolism and pyruvate excretion by *Streptomyces alboniger*. *Canadian Journal of Microbiology*, **31**(8), 702-706.

Suutari, M., Rönkä, E., Lignell, U., Rintala, H. & Nevalainen, A. (2002). Characterisation of *Streptomyces* spp. isolated from water-damaged buildings. *FEMS Microbiology Ecology*, **39**, 77-84.

Szponar, B. & Larsson, L. (2000). Determination of microbial colonisation in water-damaged buildings using chemical marker analysis by gas chromatography - mass spectrometry. *Indoor Air*, **10**, 13-18.

Szponar, B. & Larsson, L. (2001). Use of mass spectrometry for characterising microbial communities in bioaerosols. *Annals of Agricultural and Environmental Medicine*, **8**, 111-117.

Szponar, B., Pawlik, K. J., Gamian, A. & Dey, E. S. (2003a). Protein fraction of barley spent grain as a new simple medium for growth and sporulation of soil actinobacteria. *Biotechnology Letters*, **25**(20), 1717-1721.

Szponar, B., Szponar, A. & Larsson, L. (2003b). Direct assessment of microbial colonisation in damp houses by chemical marker analysis. *Indoor and Built Environment*, **12**, 251-254.

Taber, W. A. (1960). Evidence for the existence of acid-sensitive actinomycetes in soil. *Canadian Journal of Microbiology*, **6**, 503-514.

Teodoro, J. C., Baptista-Neto, A., Cruz-Hernández, I. L., Hokka, C. O. & Badino, A. C. (2006). Influence of feeding conditions on clavulanic acid production in fed-batch cultivation with medium containing glycerol. *Applied Microbiology and Biotechnology*, **72**(3), 450-455.

Terr, A. I. (2004). Are indoor molds causing a new disease? *Journal of Allergy and Clinical Immunology*, **113**(2), 221-226.

Tham, K. W. & Zuraimi, M. S. (2005). Size relationship between airborne viable bacteria and particles in a controlled indoor environment study. *Indoor Air*, **15** (Suppl. 9), 48-57.

Toivola, M., Alm, S. & Nevalainen, A. (2004). Viable fungi and bacteria in personal exposure samples in relation to microenvironments. *Journal of Environmental Monitoring*, **6**, 113-120.

Torvinen, E., Meklin, T., Torkko, P., Suomalainen, S., Reiman, M., Katila, M. L., Paulin, L. & Nevalainen, A. (2006). Mycobacteria and fungi in moisture-damaged building materials. *Applied and Environmental Microbiology*, **72**(10), 6822-6824.

Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E.-L., Lindroos, O., Kalso, S., Koukila-Kähkölä, P., Mussalo-Rauhamaa, H. & Haahtela, T. (2000). Mycotoxins in crude building materials from water-damaged buildings. *Applied and Environmental Microbiology*, **66**(5), 1899-1904.

Waegemaekers, M., van Wageningen, N., Brunekreef, B. & Boleij, J. S. M. (1989). Respiratory symptoms in damp homes, a pilot study. *Allergy*, **44**, 192-198.

Vaerewijck, M. J. M., Huys, G., Palomino, J. C., Swings, J. & Portaels, F. (2005). Mycobacteria in drinking water distribution systems: ecology and significance for human health. *FEMS Microbiology Reviews*, **29**(5), 911-934.

van Keulen, G., Jonkers, H. M., Claessen, D., Dijkhuizen, L. & Wösten, H. A. B. (2003). Differentiation and anaerobiosis in standing liquid cultures of *Streptomyces coelicolor*. *Journal of Bacteriology*, **185**(4), 1455-1458.

van Reenen-Hoekstra, E. S., Samson, R. A., Verhoeff, A. P., van Wijnen, J. H. & Brunekreef, B. (1991). Detection and identification of molds in dutch houses and nonindustrial working environments. *Grana*, **30**(2), 418-423.

Warner, A., Boström, S., Möller, C. & Kjellman, N.-I. M. (1999). Mite fauna in the home and sensitivity to house-dust and storage mites. *Allergy*, **54**, 681-690.

Verhoeff, A. P. & Burge, H. A. (1997). Health risk assessment of fungi in home environments. *Annals of Allergy, Asthma & Immunology*, **78**(6), 544-554.

Verhoeff, A. P., van Reenen-Hoekstra, E. S., Samson, R. A., Brunekreef, B. & van Wijnen, J. H. (1994). Fungal propagules in house dust. I. Comparison of analytic methods and their value estimators of potential exposure. *Allergy*, **49**, 533-539.

Vesper, S., Dearborn, D. G., Yike, I., Allan, T., Sobolewski, J., Hinkley, S. F., Jarvis, B. B. & Haugland, R. A. (2000). Evaluation of *Stachybotrys chartarum* in the house of an infant with pulmonary hemorrhage: quantitative assessment before, during, and after remediation. *Journal of Urban Health*, **77**(1), 68-85.

Vesper, S., McKinstry, C., Ashley, P., Haugland, R., Yeatts, K., Bradham, K. & Svendsen, E. (2007). Quantitative PCR analysis of molds in the dust from homes of asthmatic children in North Carolina. *Journal of Environmental Monitoring*, **9**, 826-830.

Vesper, S. J., Dearborn, D. G., Yike, I., Sorenson, W. G. & Haugland, R. A. (1999). Hemolysis, toxicity, and randomly amplified polymorphic DNA analysis of *Stachybotrys chartarum* strains. *Applied and Environmental Microbiology*, **65**(7), 3175-3181.

Vesper, S. J., McKinstry, C., Haugland, R. A., Iossifova, Y., Lemasters, G., Levin, L., Khurana Hershey, G. K., Villareal, M., Bernstein, D. I., Lockey, J. & Reponen, T. (2006a). Relative moldiness index as predictor of childhood respiratory illness. *Journal of Exposure Science and Environmental Epidemiology*, 1-7.

Vesper, S. J., McKinstry, C., Yang, C., Haugland, R. A., Kercsmar, C. M., Yike, I., Schluchter, M. D., Kirchner, H. L., Sobolewski, J., Allan, T. M. & Dearborn, D. G. (2006b). Specific molds associated with asthma in water-damaged homes. *Journal of Occupational and Environmental Medicine*, **48**(8), 852-858.

Vesper, S. J., Varma, M., Wymer, L. J., Dearborn, D. G., Sobolewski, J. & Haugland, R. A. (2004). Quantitative polymerase chain reaction analysis of fungi in dust from homes of infants who developed idiopathic pulmonary hemorrhaging. *Journal of Occupational and Environmental Medicine*, **46**(6), 596-601.

Vesper, S. J., Wymer, L. J., Meklin, T., Varma, M., Stott, R., Richardson, M. & Haugland, R. A. (2005). Comparison of populations of mould species in homes in the UK and USA using mould-specific quantitative PCR. *Letters in Applied Microbiology*, **41**, 367-373.

Wickman, M., Gravesen, S., Nordvall, L., Pershagen, G. & Sundell, J. (1992). Indoor viable dust-bound microfungi in relation to esidential characteristics, living habits, and symptoms in atopic and control children. *Journal of Allergy and Clinical Immunology*, **89**(3), 752-759.

Vidal, C., Boquete, O., Gude, F., Rey, J., Meijide, L. M., Fernández-Merino, M. C. & González-Quintela, A. (2004). High prevalence of storage mite sensitization in a general adult population. *Allergy*, **59**, 401-405.

Wieslander, G., Norbäck, D., Nordström, K., Wålinder, R. & Venge, P. (1999). Nasal and ocular symptoms, tear film stability and biomarkers in nasal lavage, in relation to building-dampness and building design in hospitals. *International Archives of Occupational and Environmental Health*, **72**, 451-461.

Wilkins, K. (1996). Volatile metabolites from actinomycetes. *Chemosphere*, **32**(7), 1427-1434.

Williams, R. H., Ward, E. & McCartney, H. A. (2001). Methods for integrated air sampling and DNA analysis for detection of airborne fungal spores. *Applied and Environmental Microbiology*, **67**(6), 2453-2459.

Wilson, S. C., Holder, W. H., Easterwood, K. V., Hubbard, G. D., Johnson, R. F., Cooley, J. D. & Straus, D. C. (2004). Identification, remediation, and monitoring processes used in a mold-contaminated high school. *Advances in Applied Microbiology*, **55**, 409-423.

Viollier, P. H., Minas, W., Dale, G. E., Folcher, M. & Thompson, C. J. (2001). Role of acid metabolism in *Streptomyces coelicolor* morphological differentiation and antibiotic biosynthesis. *Journal of Bacteriology*, **183**(10), 3184-3192.

Wójcik-Stopczyńska, B., Falkowski, J. & Jakubowska, B. (2003). Air microflora of university cafeteria. *Roczniki Państwowego Zakładu Higieny*, **54**, 321-328. (in Polish, abstract in English)

Vojdani, A., Thrasher, J. D., Madison, R. A., Gray, M. R., Heuser, G. & Campbell, A. W. (2003). Antibodies to molds and satratoxin in individuals exposed in water-damaged buildings. *Archives of Environmental Health*, **58**(7), 421-432.

Wu, P. C., Li, Y. Y., Chiang, C. M., Huang, C. Y., Lee, C. C., Li, F. C. & Su, H. J. (2005). Changing microbial concentrations are associated with ventilation performance in Taiwan's air-conditioned office buildings. *Indoor Air*, **15**(1), 19-26.

Wu, Z., Blomquist, G., Westermark, S.-O. & Wang, X.-R. (2002). Application of PCR and probe hybridization techniques in detection of airborne fungal spores in environmental samples. *Journal of Environmental Monitoring*, **4**, 673-678.

Vuorio, R., Andersson, M. A., Rainey, F. A., Kroppenstedt, R. M., Kämpfer, P., Busse, H.-J., Viljanen, M. & Salkinoja-Salonen, M. (1999). A new rapidly growing mycobacterial species, *Mycobacterium murale* sp. nov., isolated from the indoor walls of a children's day care centre. *International Journal of Systematic Bacteriology*, **49**, 25-35.

Würtz, H., Sigsgaard, T., Valbjørn, O., Doekes, G. & Meyer, H. W. (2005). The dustfall collector - a simple passive tool for long-term collection of airborne dust: a project under the Danish Mould in Buildings program (DAMIB). *Indoor Air*, **15**(Suppl. 9), 33-40.

Wålinder, R., Norbäck, D., Wieslander, G., Smedje, G. & Erwall, C. (1997). Nasal mucosal swelling in relation to low air exchange rate in schools. *Indoor Air*, **7**, 198-205.

Wålinder, R., Wieslander, G., Norbäck, D., Wessen, B. & Venge, P. (2001). Nasal lavage biomarkers: Effects of water damage and microbial growth in an office building. *Archives of Environmental Health*, **56**(1), 30-36.

Yao, M. S. & Mainelis, G. (2006). Utilization of natural electrical charges on airborne microorganisms for their collection by electrostatic means. *Journal of Aerosol Science*, **37**(4), 513-527.

Zar, J. (1996). *Biostatistical Analysis. 3 ed.* Prentice-Hall Inc. Simon & Schuster/Viacom Company, Upper Saddle River, New Jersey, USA.

Zeng, Q.-Y., Westermark, S.-O., Rasmuson-Lestander, Å. & Wang, X.-R. (2006). Detection and quantification of *Cladosporium* in aerosols by real-time PCR. *Journal of Environmental Monitoring*, **8**, 153-160.

Zeng, Q.-Y., Westermark, S.-O., Rasmuson-Lestander, Å. & Wang, X.-R. (2004). Detection and quantification of *Wallemia sebi* in aerosols by real-time PCR, conventional PCR, and cultivation. *Applied and Environmental Microbiology*, **70**(12), 7295-7302.

Zhou, G., Whong, W.-Z., Ong, T. & Chen, B. (2000). Development of a fungus-specific PCR assay for detecting low-level fungi in an indoor environment. *Molecular and Cellular Probes*, **14**, 339-348.

Åhman, M., Lundin, A., Musabašić, V. & Söderman, E. (2000). Improved health after intervention in a school with moisture problems. *Indoor Air*, **10**, 57-62.

OIRESEURANTALOMAKE

Nimi:_____ Työtehtävä: _____ Pvm:___/___ 200__

1. Onko Sinulla ollut

	viimeisen viikon aikana	viimeisen 3 vrk:n aikana	tänään
yskää	θ	θ	θ
käheyttä	θ	θ	θ
limannousua	θ	θ	θ
hengenahdistusta	θ	θ	θ
silmien ärsytysoireita	θ	θ	θ
hengityksen vinkuna	θ	θ	θ
kipua hengitysteissä	θ	θ	θ
kurkkukipua	θ	θ	θ
nuhaa, kirkasta eritettä nenästä	θ	θ	θ
nuhaa, sameaa eritettä nenästä	θ	θ	θ
veristä eritettä nenästä	θ	θ	θ
nenäverenvuotoa	θ	θ	θ
nenän tukkoisuutta	θ	θ	θ
poskiontelo-oireita	θ	θ	θ
aivastelua	θ	θ	θ
nenän kutinaa	θ	θ	θ
iho-oireita	θ	θ	θ
kuumetta	θ	θ	θ
päänsärkyä	θ	θ	θ
väsymystä	θ	θ	θ
pahoinvointia	θ	θ	θ

muuta, mitä ?_____

2. Oletko käyttänyt lääkkeitä viimeisen 7 vrk:n aikana ? Muista myös käsikauppalääkkeet !
En θ
Kyllä θ
Mitä ? _____

3. Oletko viimeisen 7 vuorokauden aikana tehnyt työssä tai vapaa-aikana mitään sellaista, joka voisi altistaa homepölylle ?
En θ
Kyllä θ
Kuvaa lyhyesti mitä ?_____

4. Oletko niistänyt nenääsi viimeisen tunnin aikana ?
En θ
Kyllä θ

5. Kysymykset vain naisille: Viimeisten kuukautisten alkamispäivä ____/_____ 200__.
Vuodon kesto yleensä_____ päivää.
Kuukautiskierron kesto päivissä (1. vuotopäivästä seuraavan kierron 1. vuotopäivään)_____päivää.

QUESTIONNAIRE

Name:_____ Work task: _____ Date:___/___ 200__

1. *Have you had*

	during the preceding 7 days	during the preceding 3 days	today
cough	θ	θ	θ
hoarseness	θ	θ	θ
upcoming phlegm	θ	θ	θ
dyspnea	θ	θ	θ
irritation of eyes	θ	θ	θ
wheezing	θ	θ	θ
painful breathing	θ	θ	θ
sore throat	θ	θ	θ
rhinorrhea, watery secretion	θ	θ	θ
rhinorrhea, purulent secretion	θ	θ	θ
blood in nasal secretion	θ	θ	θ
nasal bleeding	θ	θ	θ
blocked nose	θ	θ	θ
sinus symptoms	θ	θ	θ
sneezing	θ	θ	θ
itching of the nose	θ	θ	θ
skin symptoms	θ	θ	θ
fever	θ	θ	θ
headache	θ	θ	θ
fatigue	θ	θ	θ
nausea	θ	θ	θ

other symptoms ?_____

2. Have you used any medication during the previous 7 days ? Please report also the use of over the counter medication.

No θ
Yes θ
Label?_____

3. Have you been in contact with mold or moldy materials at work or during leisure time during the previous 7 days ?

No θ
Yes θ

4. Have you blown your nose during the previous hour ?

No θ
Yes θ

5. Questions for women only: Start of latest menstruation ____/____ 200__.
Duration of menorrhea _____ days.
Length of the menstrual cycle _____ days.

KANSANTERVEYSLAITOS **KOULUTUTKIMUS**

KYSELYLOMAKE ALA-ASTEEN OPPILAILLE

Päiväys: ___/___20___

Lapsen nimi: _____

Lapsen ikä: _____ **Sukupuoli**: poika 1
tyttö 2

1. Koulun nimi: _____

Luokka:_____

2. Kuinka kauan lapsenne on käynyt tätä koulua? _____ vuotta _____ kuukautta
(merkitkää kuukaudet vain, jos vähemmän kuin vuosi)

3. Millainen on lapsenne terveydentila mielestänne?
erinomainen 1
hyvä 2
tyydyttävä 3
huono 4

4. Onko lapsellanne ollut hengitystietulehduksia, "flunssaa" tai nuhakuumetta

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kertaa? _____ kertaa		- kuinka monta kertaa? _____ kertaa	

5. Onko lapsellanne ollut nielurisatulehdusta (angiinaa)

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kertaa? _____ kertaa		- kuinka monta kertaa? _____ kertaa	

6. Onko lapsellanne ollut välikorvatulehdusta

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kertaa? _____ kertaa		- kuinka monta kertaa? _____ kertaa	

7. Onko lapsellanne ollut poskiontelotulehdusta (sinuiittia)

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kertaa? _____ kertaa		- kuinka monta kertaa? _____ kertaa	

8. Onko lapsellanne ollut keuhkoputkentulehdusta tai keuhkokuumetta

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kertaa? _____ kertaa		- kuinka monta kertaa? _____ kertaa	

9. Oletteko käyttänyt lastanne lääkärin vastaanotolla edellä mainittujen hengitystietulehdusten vuoksi (kys. 4-8)

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kertaa? _____ kertaa		- kuinka monta kertaa? _____ kertaa	

10. Saiko lapsenne antibioottikuureja yllä mainittujen hengitystietulehdusten vuoksi (kys. 4-8)

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2
- kuinka monta kuuria?_____ kuuria		- kuinka monta kuuria? _____ kuuria	

11.A Onko lapsenne ollut poissa koulusta hengitystiesairauksien vuoksi

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2,____päivää	kyllä	2,____päivää

11.B Onko lapsenne ollut sairaalahoidossa tai -tutkimuksissa hengitystiesairauksien vuoksi

A. syyslukukaudella?		**B. kevätlukukaudella?**	
ei	1	ei	1
kyllä	2	kyllä	2

12. Onko lapsellanne ollut <u>toistuvasti tai usein</u> jokin tai joitakin alla olevia oireita tai vaivoja?

	A. syyslukukaudella?		**B. kevätlukukaudella?**	
	ei	kyllä	ei	kyllä
nenän tukkoisuutta	1	2	1	2
nuhaa	1	2	1	2
kuiva tai kipeä kurkku	1	2	1	2
äänen käheyttä	1	2	1	2
nenäverenvuotoa	1	2	1	2
kuivaa yskää	1	2	1	2
limaista yskää	1	2	1	2
hengenahdistusta tai hengityksen vinkunaa	1	2	1	2

	A. syyslukukaudella?		B. kevätlukukaudella?	
	ei	kyllä	ei	kyllä
vinkuvaa yskää	1	2	1	2
silmäoireita	1	2	1	2
kuumetta yli 37.5	1	2	1	2
lihaskipuja	1	2	1	2
nivelkipuja/-turvotusta	1	2	1	2
väsymystä	1	2	1	2
päänsärkyä	1	2	1	2
keskittymisvaikeuksia	1	2	1	2
virtsatievaivoja/-tulehdus	1	2	1	2
selkäkipuja	1	2	1	2
jotain muuta	1	2	1	2

- mitä? _____

13. Onko lapsellanne koskaan ollut <u>hengenahdistusta</u>, kun hän on liikkunut ulkona tai noussut portaita?

1 ei koskaan, **<u>siirtykää suoraan kys. 14</u>**

2 kyllä, **viimeisen 12 kuukauden aikana**
3 kyllä, **aikaisemmin**
Onko uloshengitykseen liittyviä hengitysvaikeuksia ollut
1 satunnaisesti
2 toistuvasti
3 vilustumisten yhteydessä
4 lapsen ollessa tekemisissä siitepölyn tai eläinpölyn kanssa
5 rasituksessa
6 koulussa

14. Onko lapsellanne koskaan ollut <u>hengityksen vinkunaa</u> muulloin kuin vilustumisen yhteydessä?

1 ei koskaan, **<u>siirtykää suoraan kys. 15</u>**

2 kyllä, **viimeisen 12 kuukauden aikana**
2 kyllä, **aikaisemmin**
Onko uloshengitykseen liittyvää hengityksen vinkunaa ollut
1 satunnaisesti
2 toistuvasti
3 vilustumisten yhteydessä
4 lapsen ollessa tekemisissä siitepölyn tai eläinpölyn kanssa
5 rasituksessa
6 koulussa

15. Onko lapsellanne koskaan ollut hengenahdistuskohtauksia, joihin liittyy hengityksen vinkunaa?

1 ei koskaan
2 kyllä, **viimeisen 12 kuukauden aikana**
3 kyllä, **aikaisemmin**

16. Onko lapsellanne ollut öisin kuivaa yskää viimeisen 12 kuukauden aikana (ei tarkoita vilustumisen tai infektion aiheuttamaa yskää)?

1 ei
2 kyllä

17. Onko lääkäri koskaan sanonut, että lapsellanne on...

	ei	kyllä	
astma	1	2	todettu vuonna _____
- astmaa tutkittu, mutta ei ole todettu		2	
allerginen nuha	1	2	
maitorupi, taiveihottuma eli atooppinen ihottuma	1	2	
allerginen silmätulehdus	1	2	
jokin pitkäaikaissairaus	1	2	

- mikä? _____

18. Onko lapsenne ollut astman takia lääkärin hoidossa viimeisen 12 kuukauden aikana?

1 ei
2 kyllä

19. Käyttääkö lapsenne päivittäin lääkkeitä hengitystiesairauden takia?

1 ei
2 kyllä, kausittain
3 kyllä, jatkuvasti

20. Onko lapsellanne <u>viimeisen 12 kuukauden aikana</u> ollut pitkäaikainen, yli kuukauden kestänyt nuha ja/tai toistuvia nuhakausia? Voitte rengastaa useita vaihtoehtoja.

0 ei
1 pitkäaikainen nuha vain vilustumisen yhteydessä
2 nuhakausia vain altistuessa siitepölylle tai eläinpölylle
3 koulussa
4 rasituksessa
5 nuhakausia ilman mitään selvää altistavaa syytä

21. Onko lapsellanne <u>viimeisen 12 kuukauden aikana</u> ollut pitkäaikainen, yli kuukauden kestänyt yskä ja/tai toistuvia yskäkausia? Voitte rengastaa useita vaihtoehtoja.

0 ei
1 pitkäaikainen yskä vain vilustumisen yhteydessä
2 yskäkausia vain altistuessa siitepölylle tai eläinpölylle
3 koulussa
4 rasituksessa
5 yskäkausia ilman mitään selvää altistavaa syytä

22. Onko lapsellanne ollut toistuvia silmän sidekalvon tulehduksia, punoitusta, turvotusta tai kutinaa?

0 ei
1 silmäoireita vain altistuessa siitepölylle tai eläinpölylle
2 silmäoireita tuulen tai roskien ärsyttäessä
3 koulussa
4 rasituksessa
5 silmäoireita ilman mitään selvää altistavaa syytä

23. Onko Teillä kotieläimiä? (Onko ollut aikaisemmin lapsen elinaikana?)

ei 1
kyllä, on nykyisin 2
mitä eläimiä? _____
on ollut aikaisemmin 3

24. Poltatteko talvisin kotonanne puulämmitteistä takkaa tai uunia?

ei koskaan 1
kyllä, kerran viikossa tai harvemmin 2
kyllä, useammin kuin kerran viikossa 3

25. Kuinka usein lapsenne hengittää muiden tupakansavua sisätiloissa?

A. kotona		**B. muualla**	
hyvin harvoin tai ei koskaan	1	hyvin harvoin tai ei koskaan	1
kuukausittain	2	kuukausittain	2
viikoittain	3	viikoittain	3
päivittäin	4	päivittäin	4
lapsi tupakoi itse	9		

26. Onko jokin seuraavista <u>koulun sisäilmatekijöistä</u> mielestänne aiheuttanut lapsellenne epämukavuutta tai haittaa viimeksi kuluneen lukuvuoden aikana? Rengastakaa sopivin vaihtoehto joka riviltä.

	ei koskaan	joskus	joka viikko	lähes päivittäin
veto	1	2	3	4
liian korkea huone-lämpötila	1	2	3	4
vaihteleva huonelämpötila	1	2	3	4
liian matala huonelämpötila	1	2	3	4
kylmä lattia	1	2	3	4
huono ilmanvaihto	1	2	3	4
liian kuiva ilma	1	2	3	4
liian kostea ilma	1	2	3	4
homeen haju	1	2	3	4
muu epämiellyttävä haju	1	2	3	4
sähköisyys, staattinen sähkö	1	2	3	4
melu	1	2	3	4
pöly tai likaisuus	1	2	3	4
muu	1	2	3	4

- mikä? _____

27. Missä koulun tiloissa yllä mainittuja haittoja mielestänne eniten esiintyy?

_____

28. Onko nykyisessä asunnossanne todettu kosteusvauriota, näkyvää homekasvua tai homeen hajua (tunkkaisuutta, kellarin hajua)?

ei	1
kyllä, kosteusvaurio	2
kyllä, näkyvää homekasvua	3
kyllä, tunkkaisuutta tai homeen hajua	4

Lisätietoja (esim. missä huoneissa?, milloin?, onko korjattu?):

_____

29. Montako aikuista ja lasta yhteensä asuu taloudessanne (samassa ruokakunnassa)?

_____ aikuista (16 v. tai yli)

_____ 7-15-vuotiasta lasta

_____ alle 7-vuotiasta lasta

30. Millaisella alueella kotinne sijaitsee?

kaupungin keskustassa	1
kaupungin reuna-alueella, lähiössä	2
taajamassa maaseudulla (kirkonkylä tms.)	3
haja-asutusalueella, maaseudulla	4

31. Asutteko

kerrostalossa	1
rivitalossa	2
omakotitalossa/paritalossa	3
maatilalla	4

- **Rakennusvuosi** _____ **ja asunnon pinta-ala** _____m^2

- **Jos kyseessä on maatila, mikä on tilan tuotantosuunta?**

_____

32. Isän (tai muun huoltajan) <u>ammatti</u>:_____

Äidin (tai muun huoltajan) <u>ammatti</u>:_____

TARKISTAKAA LOPUKSI, ETTÄ OLETTE VASTANNUT KAIKKIIN KYSYMYKSIIN.

KIITOS YHTEISTYÖSTÄ!