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Actinobacteria in indoor environments: exposures and respiratory health effects

Helena Rintala

National Institute for Health and Welfare, Department of Environmental Health, P.O. Box 95, 70100 Kuopio, Finland

TABLE OF CONTENTS

1. ABSTRACT

Actinobacteria are a large group of Gram-positive bacteria common in the environment, especially in the soil. They are morphologically diverse and extremely versatile in their metabolic activities. They produce tens of thousands of secondary metabolites with different biological activities. Exposure to actinobacteria in indoor environments is probably continuous, since they are both common environmental bacteria and human normal flora. However, the occurrence of some species of spore-forming filamentous actinomycetes has been associated with abnormal and health-hazardous situations, such as moisture damage of the building. The measured concentrations of actinobacteria indoors are low. Higher concentrations have been reported during the remediation work of moisture damaged buildings and in agricultural environments. Exposure to high concentrations of actinobacteria can cause allergic alveolitis. Other respiratory disorders have been reported, too and although the measured concentrations are low, the indoor exposure is always a mixture of many different agents, which may have synergistic effects. *In vitro* and *in vivo* studies have shown that actinobacteria are very immunoactive and hence, potential causative agents for respiratory and other disorders.

2. INTRODUCTION

Actinobacteria are a group of Gram-positive environmental bacteria comprising more than 230 genera, their normal habitat being mainly soil. However, some species belong to the human normal flora and some genera also include pathogenic or opportunistic pathogen species (1-3). Actinobacteria are a morphologically diverse group and also extremely versatile in their metabolic activities. They are able to utilize complex biological substances as their carbon and energy source, which makes them ubiquitous in natural and man-made environments (4). They also produce over 20 000 secondary metabolites having different biological activities (5-6). The taxonomy of actinobacteria has undergone many changes in the past years and the term “actinomycetes”, which has been used for spore-forming filamentous bacteria, is now often replaced with “actinobacteria”, which is the class name for a phylogenetically coherent group of Gram-positive, GC-rich bacteria, including the spore-forming filamentous species, but also many others (7).

Exposure to actinobacteria in indoor environments is probably continuous, at least for the most prevalent species. Some genera that are associated with

humans, such as *Corynebacterium* or *Propionibacterium,* or common in soil, such as *Mycobacterium* and *Streptomyces*, are also prevalent in "normal" indoor environments (3, 8-10). However, the reported indoor air concentrations are low, even in moisture damaged buildings. During remediation of such buildings, higher concentrations can occur (11). The group of spore-forming actinomycetes, as determined by culture methods, has been recognized as an indicator of conditions that promote the growth of abnormal microbial populations in buildings, such as moisture damage (12-13).

Exposure to high concentrations of some actinomycete species can cause allergic alveolitis (1,14-15). Such high concentrations mostly occur in occupational settings, for example in agriculture. In buildings having moisture and microbial damage, occupants are exposed to lower concentrations of actinobacteria. However, indoor exposure is always a mixture of many different agents, actinomycetes being one component, which makes it difficult to distinguish the health effects caused solely by actinobacteria. In the mixed exposure, the different components can also potentiate each other. Evidence exist that actinobacteria cause adverse health effects. The immunotoxic potential of *Mycobacterium* and *Streptomyces* species has been studied intensively and it has been shown that they are immunoactive, causing induction of proinflammatory cytokine production and cytotoxicity *in vitro* and systemic effects *in vivo* (16-20). Some *Nocardiopsis* and *Streptomyces* strains have also shown toxicity in other bioassays (21-23). Synergistic effects of mixed exposures have also been reported. Streptomycetes co-cultured with fungi or amoebae are more potent inducers of proinflammatory cytokines and cytotoxicity than those grown in monoculture (24-25). As other bacteria, actinomycete spores and cells are small; only few micrometers (26), hence they can be deposited deep in the airways by inhalation. Therefore, actinomycetes are important exposures in indoor environments and being potent inducers of inflammatory responses *in vitro* and *in vivo*, most probably contribute to our respiratory health.

This article summarizes the present knowledge of actinobacteria occurring in indoor environments – actinobacteria understood as a group comprising not only the spore-forming filamentous species, but the members of the order *Actinomycetales* of the class *Actinobacteria*.

3. ACTINOBACTERIA

3.1. Taxonomy

Actinomycetes is a term generally used to describe bacteria that have a characteristic growth cycle similar to fungi; they form substrate and aerial mycelium and spores. Taxonomic classification has been difficult, since many species don't form mycelium, form mycelium only in certain conditions or form mycelium that can fragmentate to single cells during growth (27). Phylogenetic information deduced from the ribosomal 16S rRNA gene and other gene sequences have aided the classification of actinobacteria (28). As a result new members have been included in this taxonomic group, while others have been excluded. For example *Thermoactinomyces* has been moved to the order *Bacillales* (29). Stackebrandt *et al.* (1997) proposed a new class called *Actinobacteria*, which consists of five subclasses, of which the subclass *Actinobacteridae* and more precisely the order *Actinomycetales* include the most relevant indoor species. The order *Actinomycetales* consists of ten suborders; *Actinomycineae, Micrococcineae, Corynebacterineae, Micromonosporineae, Propionibacterineae, Pseudonocardineae, Streptomycineae, Streptosporangineae, Frankineae* and *Glycomycineae*, which are in turn divided in 30 families (7). The number of individual genera is at the moment over 230 and growing continuously. Members of the suborders *Frankineae* and *Glycomycineae* have, to my knowledge, not been detected in indoor environments, however, that may only be due to the relative small number of studies done and the restrictions of the culture method, which would require the use of many special media and growth conditions to cover the whole diversity. Many members of the suborders *Actinomycineae, Micrococcineae, Corynebacterineae* and *Propionibacterineae* belong to the normal human flora and so are present in all indoor environments (3, 8). In addition, these suborders include human pathogens and opportunistic pathogens, as well as environmental bacteria. The species that have been isolated from moisture-damaged indoor environments are mostly members of the suborders *Pseudonocardineae, Streptomycineae* and *Streptosporangineae* (21-22, 30-34). In recent years there has been growing interest in actinobacteria present in indoor environments and many new species and even genera have been isolated and described, which do not all represent mycelium- and/or spore-forming species (35-38). Therefore, in this paper the term actinobacteria is used instead of actinomycetes, and the target group addressed with this term is the order *Actinomycetales*.

3.2. Ecology

Members of the order *Actinomycetales* are ubiquitous across many environments. They are commensals, symbionts or pathogens of human, animals and plants, and they live in natural environments including soil and water, as well as in man-made environments, such as drinking water distribution systems or built environment (3,12,39-43). Most species are aerobic; however, some are facultative anaerobic. Growth temperatures range from mesophilic to thermophilic (26). Most of environmental species are saprophytic and able to use many carbon and nitrogen sources. Characteristic of many filamentous actinobacteria is the ability to degrade complex organic materials, for example cellulose and lignin (4,26).

Many actinobacteria are characterized through their ability to produce secondary metabolites. Of the tens of thousands of known microbial secondary metabolites, more than the half is produced by actinobacteria (5-6). The metabolites have a large spectrum of biological activities; e.g. antibacterial (streptomycin, tetracycline), antifungal (nystatin), antiviral (tunicamycin), antiparasitic (avermectin), immunosuppressive (rapamycin), antitumor (actinomycin, mitomycin C), enzyme inhibiting (clavulanic acid) and diabetogenic (bafilomycin). Some of the metabolites are volatile, such as geosmin, giving soil its

characteristic smell (44). Geosmin is also produced on building materials (45) and an earthy odor indoors often indicates a moisture problem and unusual microbial growth in the building.

3.3. Isolation and identification

The *Actinomycetales* are such a diverse group that no single medium can be used for their isolation. Most of them will grow on common bacteriological media, such as nutrient agar, but some pathogens, such as *Mycobacterium* or *Nocardia* species may require more rich media, such as trypticase soy agar, blood agar or brain-heart infusion agar (46). The production of spores and pigments of sporoactinomycetes can be enhanced by using inorganic salts in the medium and oatmeal or starch as a carbon source (46). In studies done in indoor environments, tryptone-yeast extract-glucose agar is often used for the simultaneous determination of total bacteria counts and actinobacteria (12, 47). The use of several media increases the detected actinobacterial diversity (48), however, in routine analysis it is not feasible to use many different media. Many actinobacteria grow slowly, while other, more rapidly growing species may overgrow actinobacteria in culture. To overcome this problem, decontamination, *e.g.* treatment of the samples with chemicals that kill the non-desired bacteria more sensible to the agent, can be used for species that have a more rigid cell wall than other bacteria or for those that have spores (26). In addition, the use of low-nutrient media and specific substrates, such as starch may be useful. Cultivation time can be weeks or even months (26). In routine analysis of indoor air quality, incubation time of 2 weeks is commonly used (47).

This large taxonomic group is morphologically diverse, comprising cell forms from coccoid (*Micrococcus*) to the complex life cycles of mycelial growth and spore production (*Streptomyces*). During the life cycle of spore-forming actinomycetes, they form substrate mycelium during the vegetative growth, when there are enough nutrients and moisture present. When either of those becomes limited, they will start to form aerial mycelium and spores, which will help them to survive environmental stress (26,49). The morphological features of the colonies, production and possible fragmentation of mycelium and type of conidia can be helpful as the first steps of the identification. Determination of the cell wall composition; especially the presence/absence and type of diaminopimelic acid (DAP) in the peptidoglycan, the presence/absence of mycolic acid, and the proportion of long chain length (16 carbon atoms and more) and branched fatty acids can already be enough for the genus level identification (50-52). Biochemical tests, 16S rDNA sequencing and DNA-DNA hybridization are needed for species identification (53). The identification of actinobacteria to the species level requires expertise, and therefore in many indoor-relevant studies, no identification has been attempted and hence, only the total number of actinobacteria has been recorded.

3.4. DNA based detection methods

The problem of the tedious and often failing culture-based detection of actinomycetes can be overcome by using DNA-based detection methods, where identification is based on the detection of specific DNA sequences in the sample. One such method is the polymerase chain reaction (PCR). Actinomycete specific PCR primers have been published for investigation of actinobacterial diversity in soil and other environments (48,54-55). Although useful for diversity studies, these primers cannot be used for quantitative measurement of the amount of actinomycetes in the sample. Quantitative real-time PCR (qPCR) is a method, where the amount of PCR product is monitored continuously during the PCR reaction and the amount of target DNA in the sample can be calculated using standard curves (56). While species-specific qPCR methods exist for some clinically relevant actinobacterial species (57-58), PCR methods developed for clinical purposes might not be suitable for environmental samples because they have not been tested for non-specific background targets not often found in clinical samples. Indoor-related actinomycete species have become of interest only in the last few years; nevertheless, there are two genus-specific qPCR methods for indoor actinomycetes that have been published and applied for indoor samples; for streptomycetes and mycobacteria (10,59). For both methods, a good correlation between numbers of cultured and qPCR-detected bacteria was reported. The number of analyzed dust samples was not large; however, both mycobacteria and streptomycetes were readily detected. The results of further application of the methods to larger sample material still need to be published. The qPCR method for streptomycetes has been successfully applied to air and dust samples (60-61).

4 ACTINOBACTERIA IN INDOOR ENVIRONMENTS

The actual growth of microbes in indoor environments takes place on surfaces. If a building material is long enough moist, microbial colonization will occur. Even if a surface is moist or wet for a short time, small, invisible micro-colonies can be formed (62). Following desiccation of the material, microbial cells, spores and structural parts will be released into the air (63) and similar to mold spores, actinomycete spores are easily suspended into the air (64). It has been shown that microbial spores from colonized building materials can be found in the indoor air (34,65-66). For example, Schafer *et al.* (2010) isolated actinobacteria from building materials and indoor air and following rDNA sequencing found the same actinobacterial species inhabiting the material and the air sample of the same building (34). Pessi *et al.* (2002) showed that actinomycete concentrations in indoor air are higher in buildings that have actinomycete contamination in their wall insulation material (66). They did not see the same effect on fungi and suggested that because of their small size, actinomycete spores may aerosolize from the wall structure into the indoor air more easily than fungi. Therefore, it is necessary to consider materials and surfaces too when investigating actinomycete exposure in indoor environments, although the most important exposure route is inhalation. The actinobacteria genera detected in indoor environments and their potential health effects are listed in Table 1.

Table 1. Major actinobacteria genera detected indoors, their main habitats, occurrence in different indoor sample materials and reported health effects. (c), detected by culture; (d), detected by DNA-based methods

Genus	Main habitat	Where detected indoors	Reported health effects and other characteristics
Actinomadura	soil	indoor air (c) (34)	some species are opportunistic pathogens (15), produces bioactive compounds (6)
Amycolatopsis	soil	building materials (c,d) (34,48)	some species are potentially pathogenic (15), produces bioactive compounds (6)
Brevibacterium	cheese, human skin	building materials (c,d), indoor air (c) (34,48)	some species are opportunistic pathogens (67)
Cellulomonas	soil	building materials (c,d) (34,48)	some species are potentially pathogenic (68)
Corynebacterium	human, animals, plants	building materials (c), Indoor air (c), dust (c,d) (8,34,69,70)	some species are human, animal and plant pathogens (71)
Gordonia	soil	building materials (c) (34)	some species are opportunistic pathogens (72)
Janibacter	soil, waste water, insects	indoor air (c) (34)	some species are potentially pathogenic (73)
Kocuria	soil, human skin and oropharynx mucosa	building materials (c,d), indoor air (c), dust (d) (8,34,48)	some species are potentially pathogenic (74)
Kytococcus	mammalian skin	indoor air (c) (34)	some species are potentially pathogenic (75)
Micrococcus	mammalian skin	building materials (c), indoor air (c), dust (d) (8,34,69,76)	some species opportunistic pathogens (77)
Mycobacterium	soil, fresh water, human, animal	building materials (c,d), indoor air (c,d) (32,34,48,78)	some species are human and animal pathogens, others opportunistic pathogens (1)
Nocardia	soil	building materials (c,d), indoor air (c) (34,48,76)	some species are opportunistic pathogens of human and animal (15), produces bioactive compounds (6)
Nocardiopsis	soil	building materials (c,d), indoor air (c), dust (c,d) (8,34,48,70)	some species are opportunistic pathogens (15)
Promicromonospora	soil	building materials (c,d) (34,48)	none reported
Pseudonocardia	soil, compost	building materials (c,d), indoor air (c) (34,48)	produces bioactive compounds (6)
Rhodococcus	soil, fresh water, insects, animal feces	building materials (c,d), indoor air (c), dust (c) (34,48,70,76)	some species are opportunistic pathogens of human and animal (15)
Saccharomonospora	leaf litter, compost, heated grain or hay	building materials (c,d) (34,48)	none reported
Saccharopolyspora	grain, hay	building materials (c,d), indoor air (c) (34,48)	causes allergic alveolitis (14), produces bioactive compounds (6)
Streptomyces	soil	building materials (c,d) , indoor air (c), dust (c) (8,34,48,70,76)	some species cause allergic alveolitis or are opportunistic pathogens (15), produces bioactive compounds (6)

4.1. Indoor surfaces and building materials

Actinobacteria and other bacteria often co-colonize building materials with fungi. Actinobacterial genera that have been isolated from indoor surfaces or building materials include *Amycolatopsis, Cellulomonas, Gordonia, Micrococcus, Mycobacterium, Nocardia, Nocardiopsis, Promicromonospora, Pseudonocardia, Saccharomonospora, Saccharopolyspora, Streptomyces*, among others (21-22,30-34,79). In a large survey of 1140 building material samples, culturable actinomycetes were detected in 18-48% of the samples, depending on the material (47). In another study of 184 material samples culturable actinomycetes were detected in 34% of the samples, with densities ranging from 0 to 10^6 cfu g^{-1} (80). On heavily mold contaminated building materials, the concentrations of viable actinomycetes can range from 10^4 to 10^7 cfu g^{-1} (34). Torvinen *et al.* (2006) looked specifically at mycobacteria and found that 23% of visibly mold-damaged building materials also harbored cultivable mycobacteria in densities up to 10^7 cfu g^{-1} (32). Culture methods often underestimate the numbers of environmental microbes (81), which is also true in indoor environments (82-83). There is no universal qPCR method for actinomycetes, but qPCR for *Streptomyces* spp. of building material samples revealed concentrations from 0 to 10^7 cell equivalents g^{-1} for this genus alone (80). The detected concentrations were, on average, ten times higher than culture-based concentrations of spore-forming actinomycetes. Schafer *et al.* (2010) sequenced 16S rDNA clone libraries from contaminated building materials and detected 32 actinobacteria genera of which eight were detected more than once. The culture-independent approach revealed the occurrence of genera that have not been detected by culture, such as *Geodermatophilus*, *Knoellia* and *Marmoricola* (50).

4.2. Indoor air

DNA-based molecular investigations have shown that actinobacteria are constant members of urban and rural aerosols (78, 84-86). Additionally, knowing that outdoor air contributes heavily to the particle content of indoor air, it can be assumed that actinomycetes present in outdoor air are also present in indoor air. In addition, some human-associated actinomycete species are likely present whenever human beings are present. The true diversity of actinobacteria might, however, remain undetected by using the routine analysis method with one medium and one cultivation temperature.

In a Polish study conducted in 85 indoor spaces with and without mold problems, 17 of the more closely characterized homes harbored *Micrococcus* spp.; in addition, the genera *Streptomyces* (found in 29% of the samples), *Nocardia* (35%), *Corynebacterium* and *Rhodococcus* (less than 15%) were also detected (69). Other actinobacterial genera that have been isolated from indoor air are *Actinomadura, Arsenicicoccus, Brevibacterium, Janibacter, Kocuria, Kytococcus, Nocardiopsis, Pseudonocardia* and *Saccharopolyspora* (23,34,76). In a culture-independent investigation of bacterial diversity in the indoor air of two shopping centers in Singapore, the authors observed actinobacterial genera

such as *Agrococcus, Agromyces, Arthrobacter, Brachybacterium, Brevibacterium, Dermacoccus, Mycobacterium* and *Rhodococcus* (78).

The reported concentrations of culturable actinobacteria in air are low. In air samples collected with the Andersen six-stage impactor, concentrations < 200 cfu m^{-3} have been reported for residential settings, schools and day-care centers (12,87-90). It is generally assumed that actinomycetes in indoor air indicate moisture damage (13). For example, Nevalainen *et al.* (1991) reported that the occurrence of actinomycetes in indoor air is indicative of moisture damage of the building (12), which has been confirmed in other studies of the same group (88-89), but not in all (87). The present knowledge is, however, based on results obtained with culture-based methods. Further information based on qPCR and other DNA based methods will facilitate more conclusive generalizations concerning concentrations of the individual species or genera and their role in indoor air quality.

Using air filtration and longer sampling times slightly higher concentrations of airborne actinobacteria have been reported. Toivola *et al.* (2004) collected indoor aerosols on PVC filters using the Button inhalable aerosol sampler. They were able to cultivate actinomycetes from 23% of the samples. Actinomycetes were more common in samples collected from rural sites (40% of the samples were positive) compared with urban area (20% positive samples), the median concentration being <1 cfu m^{-3} and the maximum 637 cfu m^{-3} (82). Lee *et al.* (2006) used the Button inhalable aerosol sampler to investigate the indoor/outdoor ratio of airborne actinomycetes. In their study, the concentrations of cultivable actinomycetes ranged from 6 to 138 cfu m^{-3} indoors and 6 and 90 cfu m^{-3} outdoors (91). *Streptomyces*-specific qPCR from outdoor air samples collected with the Button sampler revealed somewhat, but not significantly higher concentrations in outdoor air depending on the season (60).

Concentrations of actinobacteria are often higher in agricultural settings. For example, concentrations up to 10^6 cfu m^{-3} have been reported (14, 92). Also during demolition work of moldy buildings the airborne concentration of cultivable actinobacteria in indoor air reached 10^4 cfu m^{-3} (11). Remediation of mold damaged and undamaged buildings can also release culturable mycobacteria into the indoor air, as concentrations up to 160 cfu m^{-3} of potentially pathogenic species of the *M. avium* complex, *M. scrofulaceum* and *M. fortuitum* have been reported (11). In outdoor samples collected simultaneously, no cultivable actinomycetes were detected.

4.3. Indoor dust

Only a few studies exist, where actinobacterial counts have been determined from indoor dust. Actinobacteria concentrations up to 10^6 cfu g^{-1} have been reported in studies using cultivation methods (69,83,93-94). A few studies have been published describing quantification of a single genus in indoor dust using qPCR. The concentration of *Streptomyes* spp. in indoor dust determined by qPCR was 0-10^7 cell equivalents g^{-1} dust (83). Torvinen *et al.* (2010) reported concentrations of *Mycobacterium* spp. from 10^4 to 10^6 cell equivalents g^{-1} dust in normal dwellings without any building-related problems (10). The authors were also able to cultivate mycobacteria from all dust samples, the median colony count being 2.1×10^3 cfu g^{-1} dust.

Culture independent studies have shown that actinomycetes are common members of microbial assemblages in indoor dust and can exhibit a large diversity in that environment. Rintala *et al.* (2008) sequenced clone libraries from the indoor dust of two buildings and reported that 55 (19% of total) distinct operational taxonomic units (OTUs) belonging to the class *Actinobacteria* were detected, the most abundant ones being human-related propionibacteria and corynebacteria (8). Some genera, *e.g. Micrococcus*, *Kocuria* and *Friedmanniella* occurred more frequently; with the majority of the OTUs occurring only once or twice in the clone library (8). In a similar study, Pakarinen *et al.* (2008) reported congruent results revealing that 20% of the clones affiliated with actinobacteria (95). In the clone libraries, sequences affiliating with the Gram-positive classes *Firmicutes* and *Actinobacteria* dominated indoor dust, *Firmicutes* being more dominant (8,95). Although not frequently encountered in the clone libraries, qPCR detection of streptomycetes and mycobacteria have shown that these genera are frequently present in indoor dust, in both moisture-damaged and undamaged buildings (10,59,93). This may be explained by the fact that clone library based diversity studies are biased through differential behavior of bacterial groups in DNA isolation and preferential amplification of certain groups in the PCR (96).

5. RESPIRATORY HEALTH EFFECTS

5.1. Immunotoxic potential

During the past years the possible mechanisms of the adverse health effects of actinobacteria were studied intensively using human and animal cell lines and animal models. First investigations revealed that bacteria are more potent inducers of proinflammatory mediators than fungi, and that actinobacteria were more potent than other Gram-positive species (17). Differences in induction of cytokine production between the species of the same genus were observed as mouse macrophage cells and human lung epithelial cells were exposed to five environmental mycobaterial strains isolated from moisture damaged indoor environments. The production of inflammatory mediators was measured, and all strains induced the production of nitric oxide, IL-6 and TNF-alpha in a dose- and time-dependent manner. In specific, the isolate representing the *M. terrae* complex was the most potent inducer, while the one representing the *M. avium* complex was the weakest (16,97). Congruent observations have been made with *Streptomyces* spp., the spores of which were potent inducers of proinflammatory cytokines and nitric oxide *in vitro* independent on the viability of the spores (98-99). The production was strongly dependent on the species and on the growth medium (99-100).

The immunoactive or –toxic effects of microbial cells or spores for mammalian cells observed *in vitro* have been confirmed with *in vivo* studies using mouse models. Spores of *S. californicus* isolated from the indoor air of a moisture-damaged building administered to mice via intratracheal instillation provoked acute inflammation in mouse lungs in addition to causing cytotoxicity (19). The effects included increased production of proinflammatory cytokines TNF-alpha and IL-6 and nitric oxide in the bronchoalveolar fluid (BALF). Moreover, repeated intratracheal instillation of *S. californicus* spores caused a dose-dependent inflammatory cell response, e.g. increased cell numbers of neutrophils, macrophages and lymphocytes in the airways as well as inflammation and cytotoxicity associated biomarkers, such as total protein, albumin and lactate dehydrogenase in BALF (20). By using a single dose of *S. californicus* spores as the exposure agent, measured proinflammatory mediators decreased to the control level in seven days (18). However, a single dose of *M. terrae* induced a sustained biphasic inflammation of the lungs which lasted over 28 days (19). When mice were exposed to *Mycobacterium immunogenum* isolated from metal-working fluid they developed hypersensitivity pneumonitis (101).

Exposures in indoor environments represent complex mixtures of different agents (102). This is logical, as it has been estimated that there are several hundreds to thousands of both bacterial and fungal species in indoor air and dust (8,78,103). The exposure to the spores of several microbial species at the same time may already trigger a synergistic inflammatory response (104). The synergistic effects are stronger if the microbes have been co-cultured (105), as is sometimes prevalent on moisture damaged building materials, where several fungal and bacterial species can grow together. In this competitive situation, both fungi and bacteria use their chemical weapons to gain advantage over others and start to produce secondary metabolites. Some of the immunotoxic effects of co-cultured *S. californicus* and *S. chartarum* resemble the effect of known cytostatic compounds produced by streptomycetes (24), so part of the adverse health effects of microbes might be explained by secondary metabolites produced by both bacteria and fungal strains. In addition to microbes, protists, such as amoebae can be present on building materials (25). Contact with amoebae increases the virulence and inflammatory potential of actinobacteria (25,106).

5.2. Allergic alveolitis

Some actinomycetes are known as the causal agents of allergic alveolitis or hypersensitivity pneumonitis. Development of this disease requires exposure to high concentrations of actinomycete spores, which can be present in occupational settings, especially in agricultural environments (14,92,107). The best known example is farmer's lung disease, which can be caused for example by *Saccharopolyspora (Faenia) rectivirgula*, *Saccharomonospora viridis* and *Streptomyces albus* (14). Recently, hypersensitivity pneumonitis has been reported among factory workers doing metal cutting, for example in the automobile industry, due to exposure to high concentrations of *Mycobacterium* species contaminating the metal cutting fluid (1). In a small community in Finland an "epidemic" of a respiratory disease resembling allergic alveolitis took place in late seventies. Investigations concluded that exposure to tap water heavily contaminated with actinomycetes was the reason for the disease and people were exposed to actinomycete aerosols during shower and sauna (108). Another case report of hypersensitivity pneumonitis from a moisture damaged home environment with co-contamination of fungi and *Saccharopolyspora rectivirgula*-like actinomycete indicates that this disease can also be acquired in home environments (109).

5.3. Asthma

Airborne allergens are the major cause of allergic rhinitis and asthma, and indoor exposure to biological factors plays an important role in the development of asthma (110). Moisture damage of a building is one situation, where elevated concentrations of microbial cells in addition to spores and fragments of those can occur in the indoor environment. Epidemiological studies have concluded that moisture damage of a building is a risk factor for asthma, both newly developed and exacerbation of existing asthma symptoms (111-116). In Finland, moisture damage is the most prevalent cause of occupational asthma since 2001 (117). The causative mechanisms and exact agents behind the adverse health effects of dampness or moisture damage are still unclear. The epidemiological studies have concentrated on showing the association of dampness and asthma (111-112) and the association of individual exposures caused by indoor dampness to asthma has still to be more thoroughly explored. Nevertheless, in the study of Hyvarinen *et al.* (2006), the authors found that exposure to actinomycetes, determined as cultivable mesophilic actinomycetes in house dust, may increase the risk of asthma in children (118). Actinobacteria are present in about 30% of moisture and mold damaged buildings (47,80), which clearly are a risk factor of asthma and they also have considerable immunotoxic potential (111-112). However, more research is needed to establish the association of indoor actinobacteria exposure and asthma.

5.4. Pulmonary infections

Some actinobacterial genera harbor species that are opportunistic pathogens, which can cause infections mainly in immunocompromised people, although infections also in immunocompetent patients have been reported. Genera such as *Gordonia, Mycobacterium, Nocardia, Rhodococcus, Streptomyces* have been isolated in connection with these infections (2,15,119-120). The lung is often the affected organ, but infections of skin and other organs have been reported, too (15). In the USA, most of the *Nocardia* infections are caused by the inhalation of environmental bacteria (2,121). *M. tuberculosis*, the well-known causative agent of tuberculosis will not be discussed here, but the incidence of pulmonary infections caused by environmental mycobacteria (non tuberculous mycobacteria, NTM) is increasing steadily, especially among immunocompromised and elderly people (43). The source of mycobacteria can be soil and natural waters as

well as drinking water distribution systems. The incidences of pulmonary infections caused by actinobacteria that have been reported indoors are linked with exposure to water aerosols, such as shower, hot tubs and swimming pools (1,122-124). This does not rule out the existence of other indoor sources for pulmonary actinobacterial infections.

6. SUMMARY

Actinobacteria are ubiquitous in the environment, including the indoor environment. As a morphologically diverse and a versatile group in their metabolic capacities they inhabit ecological niches from human body to soil. Sources of actinobacteria in indoor environments are occupants, pets, outdoor air and outdoor dust or dirt carried in with clothing or feet, but also biofilms on indoor surfaces such as moist building materials. Several actinobacterial genera can grow on moist building materials together with fungi and the occurrence of cultivable spore forming actinomycetes in indoor air or material samples has been accepted as an indicator of moisture damage of the building.

The immunotoxic potential of some actinobacteria has been shown in intensive *in vitro* and *in vivo* studies. Respiratory health effects caused by actinobacteria include hypersensitivity pneumonitis, mostly reported in occupational settings, although some case reports from residential settings also exist. Some actinobacteria are known pathogens and the number of diagnosed environmentally acquired pulmonary infections caused by actinobacteria is increasing. The connection between asthma and actinobacteria exposure needs more research.

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Abbreviations: DAP: diamino pimelic acid, PCR: polymerase chain reaction, qPCR: quantitative polymerase chain reaction, OTU: operational taxonomic unit, IL: interleukin, TNF: tumor necrosis factor, BALF: broncho alveolar lavage fluid, NTM: non tuberculous mycobacteria

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Send correspondence to: Helena Rintala, P.O.Box 95 70701 Kuopio, Tel: 358 50 4345416, Fax: 358 20 6106497, E-mail: helena.rintala@thl.fi

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