Actinomycetes in building materials

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ABSTRACT

Most indoor pollution experts only investigate fungi. But bacteria also grow in humid building materials. Our own statistics show that 83.3% of 561 samples with high amounts of microorganisms contain bacteria. In about 61% of these samples actinomycetes were present. Through a polyphasic taxonomic approach using morphological, chemotaxonomical and molecular biological methods, actinomycetes isolated from building material could be identified as *Nocardia carnea*, *Nocardiopsis exhalans*, *Streptomyces griseus*, *Streptomyces violaceoruber*. The others belong to new until now un-described species of the following genera: *Nocardiopsis*, *Promicromonospora*, *Pseudonocardia and Streptomyces.*

Species of actinomycetes, isolated from building materials, are potential producers of toxic metabolites and some may be pathogenic. So bacteria and especially actinomycetes should no longer be ignored.

INDEX TERMS

Nocardia, *Nocardiopsis*, *Pseudonocardia*, *Promicromonospora*, *Streptomyces*

INTRODUCTION

In water-damaged buildings microorganisms will grow in and on moist building materials. Although bacteria are present in addition to fungi, in most studies only the presence of fungi was discussed regarding the health problems of the residents. The main reason of the ignorance of bacteria is the much simpler cultivation and differentiation of fungi compared to those of bacteria. But if bacteria are ignored during the investigation of building related health complaints and discussing the presence of toxins in dust, airborne particles or building materials, the wrong conclusions will be drawn. The lack of correlation between quality and quantity of fungi and toxic effects may be explained by an influence of bacterial toxins in many cases.

Streptomycetes are known as potent producer of secondary metabolites. About 6000 of the 8000 known antibiotics are produced by them. Species of this genus can often be found in building materials, house dust or indoor air. Andersson (1999) and Peltola (2001) showed that in addition to streptomycetes other actinomycetes belonging to the families *Corynebacteriaceae*, *Mycobacteriaceae*, *Nocardiaceae* can be found in house dust and building materials of water-damaged buildings. In this habitat strains of new species or genera of actinomycetes could be isolated, i.e. a strain of a new *Dietzia* species (Andersson *et al*., 1998), *Williamsia muralis* (Kämpfer *et al*., 1999), *Mycobacterium murale* (Vuorio *et al.*, 1999), *Nocardiopsis exhalans* and *N. umidischolae* (Peltola, 2001).

Scientists in search for new substances for pharmaceutical use are looking for actinomycetes species in extreme habitats, e.g. in maritime habitat. But obviously there are some new actinomycetes in our dwellings. This we could see during our own investigations.

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The importance of bacteria depends on the following aspects:
(a) Will they cause a hazardous effect on our health?
(b) How often do bacteria occur in buildings?

(a) Biologically active bacterial substances will be present in water-damaged buildings (Andersson *et al.*, 2001; Roponen *et al.*, 2001; Hirvonen *et al.*, 2001) and some actinomycetes may be pathogenic (Kroppenstedt, 1992; Andersson, 1999; Yassin *et al.*, 2001). Additionally, there are clear indications that bacterial growth in building materials is the causative agent of rheumatoid symptoms (Husmann *et al.*, 2002; Lorenz *et al.*, 2002; Seuri *et al.*, 2002).
(b) Bacteria are predominantly present in building materials, see Table 1. In 612 samples taken from building materials we detected microbial growth in more than 90% and additional bacteria in about 75% of all samples. Actinomycetes were found in more than 50% of all samples.

Table 1 Fungi and bacteria in water-damaged building materials

Samples	Solely fungi	Solely Act.	Solely bact. without Act.	Fungi and Act.	Fungi + bact. without Act.
561	94	4	10	340	113
100%	16.7%	<1%	1.8%	60.6%	20.1%

Act. = Actinomycetes, bact. = bacteria.

In selected cases we isolated actinomycetes from building materials. The isolated strains were analysed with different methods, to get new information about building-related actinomycetes.

METHODS

Cultivable bacteria were analysed by the dilution plate method. Parts of the samples were suspended in a buffer solution (peptone with 0.05% Tween 80), then shaken and diluted. From the original suspension up to the fourth dilution step 0.1 ml were plated on casein soy peptone agar (CASO) with cycloheximide. The plates were incubated at 24 ± 0.5°C and analysed after 3, 6 and 12 days. Pure cultures of actinomycetes were obtained by isolation on CASO agar at 24 ± 0.5°C.

The actinomycetes were identified by their morphological characters using the methods described by Shirling and Gottlieb (1966), by their fatty acid and mycolic acid patterns using the Microbial Identification System (Rainey *et al.*, 1995) and by 16S rDNA sequencing (Rainey *et al.*, 1996; Stackebrandt *et al.*, 1997).

In seven different cases 13 actinomycetes strains were isolated from water-damaged building materials. In all seven cases the inhabitants suffered from different complaints, see Table 2. Under the microscope morphologically different strains were isolated and analysed with the methods described above.

RESULTS

The 13 analysed strains contain seven different species. All analysed strains are Gram-positive aerobic bacteria belonging to the class *Actinobacteria,* to the Subclass *Actinobaceridae* and to the order *Actinomycetales* (Stackebrandt *et al.*, 1997). The genera belong to different suborders.

Four species are unknown and belong to four different genera: *Nocardiopsis* sp. nov., *Pseudonocardia* sp. nov., *Promicromonospora* sp. nov., *Streptomyces* sp. nov. The same *Pseudonocardia* sp. nov. and *Streptomyces* sp. nov. could be found in materials from two

different buildings and the same *Nocardiopsis* sp. nov. in two different materials from one building.

Table 2 Cases with health complaints and isolated Actinomycetes

Case	Symptoms	Material	Fungi	Actinomycetes
Dwelling	Bronchitis, rheumatoid complaints, head aches, skin problems, drowsiness	Wall paper	*Penicillium* spp., *Aspergillus versicolor*, *Acremonium* sp., *Stachybotrys chartarum*, *Acremonium* sp., *Chaetomium* sp.	*Nocardia carnea*, *Nocardiopsis* sp. nov., *Promicromonospora* sp. nov.
Ditto	Ditto	Plaster	Not analysed	*Nocardiopsis exhalans*, *Nocardiopsis* sp. nov.,
Apartment	Respiratory complaints	Loam rendering	Sterile mycelia	*Nocardiopsis exhalans*
Dwelling	Sinusitis, rheumatoid complaints, head aches, skin problems, drowsiness	Concrete	*Aspergillus versicolor*, *Acremonium* sp.	*Nocardia carnea*
Dwelling (old building)	Respiratory infections, dizziness, drowsiness	Plastic foil	*Stachybotrys chartarum*, *Trichoderma* sp., *Cladosporium* sp., *Penicillium* sp.	*Streptomyces griseus*
Ditto	Ditto	Gypsum board	*Penicillium* spp., *Cladosporium* spp., yeasts, *Stachybotrys chartarum*, *Aphanocladium* sp., *Trichoderma*	*Streptomyces* sp. nov.
Ddwelling for two families	Rhinitis, bronchitis, neurodermatitis, susceptibility to infections	Plaster	*Penicillium* spp., *Trichoderma* sp.	*Streptomyces griseus*
Apartment	Respiratory problems, rheumatism	Concrete and organic insulation material	*Aspergillus versicolor*, *Penicillium* spp., *Cladosporium* sp., yeasts	*Streptomyces* sp. nov., *Pseudonocardia* sp. nov.
Dwelling	Respiratory problems, pseudo-croup, otitis media, asthma	Plaster and some wall paper	*Scopulariopsis* sp., *Engyodontium album*, *Aspergillus versicolor*, *Acremonium* sp.	*Pseudonocardia* sp. nov.

Genus *Nocardia* belongs to the family *Nocardiaceae* (together with *Rhodococcus*). The family *Nocardiaceae* belongs to suborder *Corynebacterineae*.
Pseudonocardia sp. belongs to the family *Pseudonocardiaceae* together with the genera *Actinopolyspora*, *Actinosynnema*, *Amycolatopsis*, *Kibdelosporangium*, *Kutzneria*, *Lentzea*, *Saccharomonospora*, *Saccharopolyspora*, *Saccharotrix*, *Streptoalloteichus*, *Thermocrispum.* The family *Pseudonocardiaceae* belongs to the suborder *Pseudonocardineae*.
Genus *Nocardiopsis* belongs to the family *Nocardiopsaceae*. This family belongs to the suborder *Streptosporangineae* together with *Streptosporangiaceae* and *Thermomonosporaceae.*
Genus *Streptomyces* belongs to the family *Streptomycetateae* and to the suborder *Streptomycineae*.
Promicromonospora sp. belongs to the family *Promicromonosporaceae* and together with *Micrococcaceae*, *Cellulomonadaceae*, *Dermatophilaceae*, *Brevibacteriaceae*,

Dermabacteraceae, *Ontrasporangiaceae*, *Jonesiaceae*, *Microbacteriaceae* to the suborder *Micrococcineae*.

DISCUSSION

Surprisingly, four of the seven different species detected were unknown. When investigating building materials also other scientists often found new species. Because of this it may be not rare to find new species in building materials. Although we analysed only a few strains besides the new species we found two species as described in the other works (Andersson, 1999; Peltola, 2001), which are *Nocardiopsis exhalans* and *Streptomyces griseus.* This cannot be a coincidence. The reason must be that water-damaged buildings were not investigated as a habitat for actinomycetes up until now.

Nearly all *Streptomyces* can use chitin as C- and N-source, probably other actinomycetes can also do so. Chitin is a cell wall substance of fungi. Because actinomycetes mainly were detected at old water damages and because growth of *Streptomyces* is observed on dead fungal hyphae (Williams *et al.*, 1989) it is possible that some actinomycetes are secondarily growing microorganisms and eat the fungi as carrion (Zeltins and Schrempf, 1997).

All actinomycete isolates produced substrate and aerial mycelium. The diameter of the hyphae ranged roughly from 0.5 to 1.5 µm. On this marker they could easily be differentiated from fungi which also produce mycelium but with hyphae of a diameter of about 5 µm. In addition the hyphae of fungi are septated whereas in actinomycetes septa are absent. Colonies of *Streptomyces* and *Nocardiopsis* show a very well developed substrate and aerial mycelium whereas in *Pseudonocardia* and *Nocardia* the development of aerial mycelium is often poor. In *Promicromonospora* the scant aerial mycelium can only be seen by using a microscope. Strains of *Streptomyces griseus* and some *Nocardiopsis* species are often mixed up because the colonies of both taxa are grey–yellow. But in contrast to *Streptomyces*, which produce chains of arthrospores at the end of the aerial hyphae in *Nocardiopsis*, the aerial mycelium disintegrate into masses of spore-like structures during ageing. Most species of the genera *Pseudonocardia* und *Nocardia* produce a scant white to dirty white aerial mycelium. In some *Nocardia* strains the production of aerial mycelium is lost during sub-cultivation.

Until now *Nocardia* were found in soil or clinical specimens like sputum. In the literature the genera *Nocardia asteroides* and *N. brasiliensis* are described very often. These species and *N. caviae* can cause pulmonary infections in humans called nocardiosis and cutaneous infections (matura foot, granulotomata, abscesses with soft tissue and bone involvement) (Murray, 2001). Maybe *N. nova* is also pathogenic (Tsukamura, 1982). It is reported that 80–90% of the pulmonary nocardiosis cases are caused by *N. asteroides* and about 5% by *N. brasiliensis* and that *N. brasiliensis* is the main causative agent of cutaneous infections (Murray, 2001). In the same paper it is described that nocardial infections mainly occur at immunocompromised individuals. But we have information that 50% of the infected people were not immunocompromised (Schaal, 2001). *N. uniforms* can produce different beta-lactam antibiotics called nocardicins (Anonymous, 1995a). These substances inhibit the building of bacterial cell walls and are related to penicillin substances. *Nocardia lactamdurans* is a potential producer of cephamycins. These substances are beta lactam antibiotics and also related to penicillin. They are effective against Gram-negative bacteria. Genus Nocardia belongs to the CMN taxons and the cell wall components of these CMN taxons are stimulators of the immune system (Andersson, 2001). Reports about other antibiotic or toxic metabolites produced from *Nocardia* spp. were not found. Infections by *N. carnea* are not described.

Nocardiopsis exhalans was described a short time ago as a new species (Peltola, 2001). The same work describes other *Nocardiopsis* species in building materials, e.g. *N. umidischolae*, *N. alba*. The toxicity of these isolated species was tested with boar spermatoza. As a new feature of this genus toxic metabolites were detected. Some *Nocardiopsis* species are pathogenic. For example, *N. dassonvillei* is classified as hazard category 2 in European legislation, which means that this species may cause infectious disease in humans (Anonymous, 1995b).

No pathogenic *Pseudonocardia* species are described in literature. Thermophilic species of this genus are described as a causative agent of allergic disease which occurs among persons working with material, where such actinomycetes are growing (Greiner-Mai *et al.*, 1987).

Streptomyces are well-known producers of a variety of different antibiotics. In a children's day care centre a valinomycin producing *Streptomyces griseus* was isolated (Andersson, 1999; Peltola, 2001). The valinomycin was very toxic to sperm cells. The cause of the ability of *Streptomyces* to produce a lot of different antibiotics is that there may be many other metabolites of *Streptomyces* in buildings. Roponen (2001) showed that *S. anulatus* can induce inflammatory responses and cytotoxicity in macrophages. The assumption is that building materials may provide different conditions to induce production of biological active substances by streptomycetes. This is probably not only an assumption. As shown previously (Puder, 1998) the parameters temperature, ventilation, oxygen partial pressure, containers for culturing, pH-value, light, agar-substrates, anorganic salt, adsorbing materials, detergents, etc., have an influence on kind of metabolite substances and their quantity. Pathogenic species of genus *Streptomyces* are not known.

Neither toxic metabolites nor pathogenic effects of *Promicromonospora* spp. are known.

CONCLUSION AND IMPLICATIONS

Actinomycetes surely can be considered being a health hazard. It may be similar to that of fungi. We assume that it is enhanced. Actinomycetes that grow in building materials can produce toxic metabolites, and possibly some of these species are pathogenic.

In hazard group 2 of BioStoffV (BioStoffV = German legislation for handling of biological materials), *Aspergillus fumigatus* is the only building related fungus but there are various species of actinomycetes. Peltola (2001) demonstrates that certain bacteria may be more potent in causing adverse health effects than fungi considering their higher toxin content and smaller size. Actinomycetes must be considered more than in the past as a causative agent for health complaints. Because they may be pathogenic and since they can cause infections that can lead to death, such as nocardiosis, one must be very careful when handling contaminated materials, e.g. when taking samples or during renovation. Therefore, the potential effects of actinomycetes must also be taken into account when assessing mould damages.

The detection of the species is difficult and may be too expensive in practical work. But besides fungi, bacteria should be cultivated routinely so that it can be shown if there are actinomycetes or not.

Obviously, buildings are a very interesting habitat for searching for new actinomycetes as potential producers of active substances (in our investigations four of seven analysed species were unknown!).

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