PCR DETECTION AND DIVERSITY OF STREPTOMYCETES IN WATER-DAMAGED BUILDINGS

H Rintala[*], M Suutari and A Nevalainen

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

ABSTRACT
Streptomycetes are actinobacteria that are common in soil, but also found in buildings indicating moisture conditions favorable for both fungal and bacterial growth. In addition, streptomycetes are potential causes of adverse health effects on human. Current detection methods are based on cultivation, which is time-consuming and detects only viable microbes. A Polymerase chain reaction (PCR) based method for the specific detection of streptomycetes was developed. DNA isolated from two moisture damaged building materials served as a template for PCR amplification with two *Streptomyces*-specific primer pairs targeting the 16S rRNA gene. The PCR amplification products were sequenced and the sequences were analyzed. Both primer pairs were specific for streptomycetes and several different sequence types were detected in both materials suggesting the presence of several species. The results of this work confirm the presence of streptomycetes in moisture-damaged buildings and provide a more quick and accurate method for their determination.

INDEX TERMS
Streptomyces, PCR, Water-damaged buildings

INTRODUCTION
Streptomycetes are a group of bacteria belonging to the class *Actinobacteria* and common in soil. They are also found in material and air samples in buildings with moisture problems, indicating moisture conditions that are favorable for microbial growth (Hyvärinen *et al*, 2001, Nevalainen *et al*, 1992). These organisms are interesting, not only as indicators for moisture damage in buildings, but also for their huge metabolic capacities. Streptomycetes are known as producers of many secondary metabolites, antibiotics and toxins (Williams *et al*, 1989). Few species are pathogenic (Mishra *et al*, 1980), however, some species have been associated with development of chronic diseases, like farmer's lung disease (Lacey and Crook, 1988). In addition, the spores of streptomycetes cause inflammatory responses in mouse macrophages and human lung epithelial cells *in vitro* (Hirvonen *et al*, 1997, Jussila *et al*, 1999).

Current detection methods for streptomycetes are based on cultivation and take quite a long time, even fourteen days. In addition, a part of the microbes remains undetected by cultivation (Ward *et al*, 1980). Thus, new methods for the determination streptomycetes in indoor environments are needed. Polymerase chain reaction (PCR) based methods for the detection and identification of microbes have advantages compared to cultivation. Non-cultivable microorganisms are detected, the time needed for the analysis is shorter, and PCR method is more sensitive. Sequencing of the PCR amplified DNA fragments gives information about the diversity of *Streptomyces* species present in moisture-damaged buildings.

[*] Contact author email: helena.rintala@ktl.fi

METHODS

Building materials were collected from moisture-damaged buildings and the amount of culturable fungi and mesophilic actinobacteria was determined as described earlier (Hyvärinen *et al*, 2001). Two materials were used in this study, painted plaster from an interior wall of a dwelling and linoleum carpet from the ground floor of an office building. Visible signs of moisture and microbial damage were observed on both materials.

DNA isolation

DNA was isolated from the building materials as described elsewhere (Rintala *et al*, 2001). Shortly, 2 g of building material was wetted with 600 µl of Crombach buffer, 2 g of glass beads (Merck, Darmstadt, Germany) and 2.1 ml of lysis solution were added. The sample was incubated at 65°C for 20 min and then vigorously vortexed for 30 minutes. The cell debris was removed by centrifugation (15 min, 10 000 *g*, room temperature), and the supernatant was treated with an equal volume of chloroform-isoamylalcohol (24:1 v/v). The DNA was purified with Wizard DNA Clean up spin column (Promega, Madison, WI, USA) and agarose gel electrophoresis. The DNA was extracted from the agarose with QIAquick gel extraction kit (Qiagen Inc., Valencia, CA, USA).

PCR amplification

Two primer pairs StrepB/StrepE and StrepB/StrepF (Rintala *et al*, 2001, Suutari *et al*, 2001) were used for the specific amplification of streptomycete 16S rDNA sequences from building materials. The forward primer StrepB 5'-ACAAGCCCTGGAAACGGGGT-3' and the reverse primers StrepE 5'-CACCAGGAATTCCGATCT-3' and StrepF 5'-ACGTGTGCAGCCCAAGACA-3' target the regions nt 139-158, 640-657 and 1194-1212, respectively, of the 16S rRNA gene (*S. ambofaciens* numbering (Pernodet *et al*, 1989). The 50 µl PCR reaction mixture contained 1× *Pfu* reaction buffer (Stratagene, Amsterdam, The Netherlands), 0.1 mM of deoxynucleoside triphosphates (Finnzymes, Espoo, Finland), 0.2 µM of phosphorylated primers, 5-20 ng of template DNA, and 1U of *Pfu* DNA polymerase (Stratagene). The cycling was performed in a PTC-100 Programmable Thermal Controller (MJ Research, Inc., Watertown, MA, USA). The cycling parameters were: an initial denaturation of 5 min at 98°C, and 30 cycles of 45 s denaturation at 95°C, 40 s primer annealing, and 2 min primer extension at 72°C. Final extension at 72°C for 10 min followed. The annealing temperature was 54°C for the primers StrepB/StrepE and 58°C for the primer pair StrepB/StrepF. A positive control and a tube containing no nucleic acid were included in each set of PCR reactions. The amplification products were analyzed by agarose (1.5%) gel electrophoresis, visualized by UV transillumination and photographed.

Cloning and analysis of the clones by *Bst*YI restriction enzyme digestion

The PCR products were purified from 1 % agarose gels with the QIAquick gel extraction kit (Qiagen). The fragments were ligated into *Sma*I digested and dephosphorylated pUC19 vector and transformed into *Escherichia coli*. Plasmid DNA from transformants was isolated with the Wizard Plus SV minipreps kit (Promega). The plasmid DNA was digested with the restriction enzyme *Bst*YI, which cuts the StrepB/StrepF PCR amplicons originating from streptomycetes in fragments of specific length. 2 U of BstYI (New England Biolabs) were used under conditions recommended by the supplier in a reaction volume of 10 µl and incubated at 60°C for 2h. The results were analyzed in agarose (1.5%) gel electrophoresis.

Phylogenetic analysis

The PCR amplified 16S rRNA gene fragments were sequenced and the sequences were compared to other prokaryotic sequences in the EMBL database using the fasta-algorithm

(Pearson and Lipman, 1988). The sequences of the PCR fragments were aligned with 15 sequences from other streptomycetes obtained from GenBank, and one sequence from *Arthobacter globiformis* by using GCG-Pileup. Phylogenetic analysis was performed with the PHYLIP package (Felsenstein, 1989). Bootstrap analysis (1000 replicates) was done to assess the significance of the obtained tree.

RESULTS

Detection of streptomycetes

Both primer pairs amplified a PCR fragment of expected size from both building materials. The amplification products were cloned in pUC19-vector and 20 fragments amplified with StrepB/StrepE, as well as 29 fragments amplified with StrepB/StrepF were sequenced. All sequenced PCR amplification products obtained with the primer pair StrepB/StrepE showed over 96.1 % sequence similarity to streptomycete 16S rDNA sequences and thus, most probably originate from streptomycetes.

24 of the 29 sequenced PCR fragments amplified with StrepB/StrepF showed greater than 95.5 % similarity to *Streptomyces* and 5 fragments over 97.9 % similarity to *Cellulomonas* 16S rDNA sequences. The StrepB/StrepF amplification products were digested with the restriction enzyme *Bst*YI, and all fragments having sequence similarity to streptomycetes were *Bst*YI positive. The five fragments showing similarity to *Cellulomonas* species were *Bst*YI negative.

Table 1. Results of the sequence analysis of PCR fragments amplified with primer pairs StrepB/StrepE and StrepB/StrepF from DNA isolated from painted plaster and linoleum carpet.

Sample	Primer pair	Number of sequences	Sequence similarity
Plaster	StrepB/StrepE	10	99.6% *Streptomyces* spp.
	StrepB/StrepF	17	99.4-100% *Streptomyces* spp.
Linoleum	StrepB/StrepE	10	96.1-99.8% *Streptomyces* spp.
	StrepB/StrepF	7	95.5-99.7% *Streptomyces* spp.
	StrepB/StrepF	5	97.9-98.5% *Cellulomonas* spp.

Diversity of streptomycetes

The phylogenetic affiliations of the PCR amplified *Streptomyces* 16S rDNA sequences obtained from two building materials are shown in Figure 1. The sequences amplified from the painted plaster grouped together with sequences from *S. griseus*, *S. setonii* and *S. coelicolor*. The sequences amplified from the linoleum carpet affiliated with *S. griseus*, *S. coelicolor*, *S. virginiae*, *S. sampsonii* and *S. rimosus*.

DISCUSSION

In this work, a PCR based method for the specific detection of streptomycetes in environmental samples was developed and tested with building materials. The PCR primer pairs StrepB/StrepE and StrepB/StrepF in combination with *Bst*YI restriction enzyme digestion proved to be specific for streptomycetes. Several different sequences were found in both materials indicating the presence of several species.

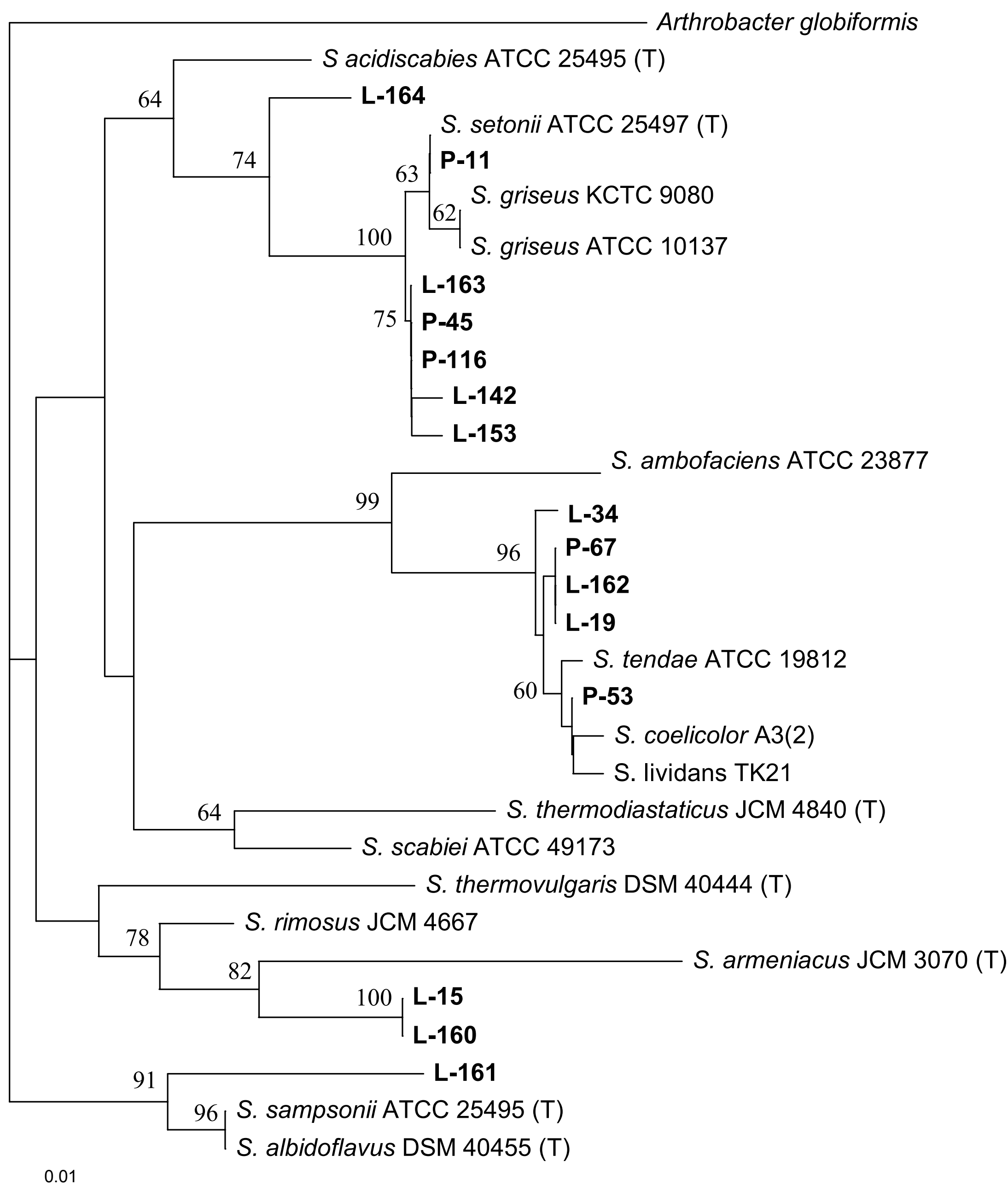

Figure 1. Neighbor-joining tree of a 330 bp alignment of 16 Streptomyces 16S rDNA sequences obtained from GenBank and PCR amplified sequences obtained from two building materials with *Streptomyces*-specific primers. The sequence of Arthrobacter globiformis was used as an outgroup. Bootstrap values over 50 are indicated. P, sequences amplified from plaster; L, sequences amplified from linoleum carpet.

Streptomycetes are non-fastidious organisms able to degrade complex biological materials like cellulose and lignin among others and they are satisfied with an inorganic nitrogen source (Williams *et al*, 1989). Their role in biodeterioration has been investigated earlier (Williams, 1985). Therefore, it is not surprising that they are also found in buildings. By investigations of moisture-damaged buildings, mesophilic actinobacteria can be cultivated from material or air samples, and their presence indicates moisture conditions that also enable fungal growth (Hyvärinen *et al*, 2001, Nevalainen *et al*, 1991). Actinobacteria have been cultured from all

kinds of materials, most commonly from ceramic materials, like concrete and bricks (Hyvärinen *et al*, 2001).

CONCLUSION AND IMPLICATIONS

The results of this work confirm the presence of streptomycetes among the actinobacteria in buildings. Several different *Streptomyces* 16S rDNA sequences were amplified from DNA isolated from building materials. The role of streptomycetes in moisture damaged buildings should be further investigated.

ACKNOWLEDGEMENTS

The work was supported by the Finnish Work Environment Fund (grants no. 96028, 98103 and 101053).

REFERENCES

Felsenstein J. 1989. PHYLIP-phylogeny interference package (version 3.2). *Cladistics.* Vol. 5, pp 164-166.

Hirvonen MR, Nevalainen A, Makkonen N, *et al*. 1997. Streptomyces spores from moldy houses induce nitric oxide, TNF and IL-6 secretion from RAW264.7 macrophage cell line without causing subsequent cell death. *Environmental Toxicology and Pharmacology.* Vol. 3, pp 57-63.

Hyvärinen A, Meklin T, Vepsäläinen A, *et al*. 2001. Fungi and actinobacteria in moisture-damaged building materials - concentrations and diversity. *International Biodeterioration & Biodegradation.* In press.

Jussila J, Ruotsalainen M, Komulainen H, *et al*. 1999. *Streptomyces anulatus* from indoor air of moldy houses induce NO and IL-6 production in a human alveolar epithelial cell-line. *Environmental Toxicology and Pharmacology*. Vol. 7, pp 261-266.

Lacey J and Crook B. 1988. Fungal and actinomycete spores as pollutants of the workplace and occupational allergens. *Annals of Occupational Hygiene.* Vol. 32, pp 515-533.

Mishra SK, Gordon RE, and Barnett DA. 1980. Identification of nocardiae and streptomycetes of medical importance. *Journal of Clinical Microbiology*. Vol. 11, pp 728-736.

Nevalainen A, Pasanen AL, Niininen M, *et al*. 1991. The indoor air quality in Finnish homes with mold problems. *Environment International*. Vol. 17, pp 299-302.

Pearson WR, and Lipman DJ. 1988. Improved tools for biological sequence analysis. *Proceedings of the National Academy of Sciences of the United States of America.* Vol. 85, pp 2444-2448.

Pernodet JL, Boccard F, Alegre MT, *et al*. 1989. Organisation and nucleotide sequence analysis of a ribosomal RNA cluster from *Streptomyces ambofaciens*. *Gene*. Vol. 79, pp. 33-46.

Rintala H, Nevalainen A, Rönkä E, *et al*. 2001. PCR primers targeting the 16S rRNA gene for the specific detection of streptomycetes. *Molecular and Cellular Probes*. In press.

Suutari M, Rintala H, Rönkä E, *et al*. 2001. Oligonucleotide primers for detection of Streptomyces species. Patent no FI20011004.

Ward DM, Weller R, and Bateson MM. 1990. 16S rRNA sequences reveal numerous uncultured inhabitants in a natural community. *Nature*. Vol. 345, pp 63-65.

Williams ST, Goodfellow M, and Alderson G. 1989. Genus *Streptomyces* Waksman and Henrici 1943, 399AL. In *Bergey's manual of systematic Bacteriology*, Williams ST, Sharpe ME, Holt JG, eds. Williams & Wilkins, Baltimore, Vol. 4, pp 2452-2492.

Williams ST. 1985. Streptomycetes in biodeterioration - Their relevance, detection and identification. *International Biodeterioration.* Vol. 21(3), pp 201-209.