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CHEMOTAXONOMICAL IDENTIFICATION OF ACTINOBACTERIAL STRAINS ISOLATED FROM DAMP-AFFECTED OFFICE BUILDING

Four actinobacterial sporulating strains have been isolated from two damp-affected office buildings. They have been identified as Streptomyces sp. (three strains) and cell wall type IV actinobacteria (one strain) by the chemotaxonomical approach.

K e y w o r d s: Actinobacteria, Streptomyces, chemotaxonomy, cell wall, indoor air.

Harmful microbes in indoor environments are a cause of public concern. Bacteria and fungi infest house and office buildings affected by dampness and the environmental conditions and microbial communities undergo continual changes resulting in different microbial populations. Microbial growth on moisture-damaged building materials is commonly associated with adverse health effects in the occupants. Microorganisms, their spores and cell wall components are biologically active agents which may cause and trigger allergy and other respiratory diseases, i.e. chronic airways inflammation [1]. Although the microbial diversity in indoor environment is high, the filamentous and sporulating fungi dominate on indoor microbiota screenings [2, 3]. Recently actinobacteria involved in biological infestation of damp buildings attract increasing interest [4].

Actinobacteria are Gram-positive bacteria, with high G+C content, filamentous microorganisms, majority of which are saprophytic soil and other environmental inhabitants. As common soil organisms they easily colonize water-damaged building materials and may emit toxic-metabolites isolated from the indoor environment of a building where the occupants suffered building-related ill-health symptoms [5].

The aim of present studies was identification of sporulating bacterial strains isolated from wall surface of damp-affected office building in Warsaw (Poland), by means of chemotaxonomical approach.

Materials and methods

Bacterial strains L1, L2, W3, and W4 were isolated from the wall surface of damp-affected office building in Warsaw, Poland.

The isolates were cultivated aerobically on medium “79” in 37 °C for 48 hours. Bacterial biomass was collected by centrifugation in 7000 rpm, after repeated washing

with PBS and MilliQ water. The wet biomass was suspended in MilliQ water (1:1, v:v) and frozen in –70 °C prior to X-press (AB Biox, Sweden) cell disruption.

The crude cell wall extract was prepared by centrifugation (6000 rpm, 20 min, 4 °C) of disintegrated cells. Supernatant containing crude bacterial cell walls was collected and frozen dried. The cell wall preparations were frozen dried and subjected to hot SDS extraction according to [6]. Proteins and nucleic acids remaining in preparations were removed by using enzymes and dialyzed to deionized water [6].

Sugars were analysed after derivatisation according to [7]; amino acids and fatty acids were derivatised according to [5] and analysed by gas chromatography-mass spectrometry [8].

LL- and *meso* diaminopimelic acid (DAP) isomers were determined by thin-layer chromatography [8].

Bacterial glycolipids were analysed in crude lipid extracts of bacterial biomass by use of thin layer chromatography and visualized by orcinol reagent [8].

An inflammatory potential of the cell wall constituents after purification has been examined by stimulation of cytokines TNF-α and interleukin 1β (IL-1β) in vitro in isolated human leukocytes by commercial ELISA tests (BD Biosciences).

Results and discussion

All four studied strains formed dry, lathery colonies producing white spores (Fig. 1). Microscopically, extensively branched Gram-positive pseudomycelium was observed.

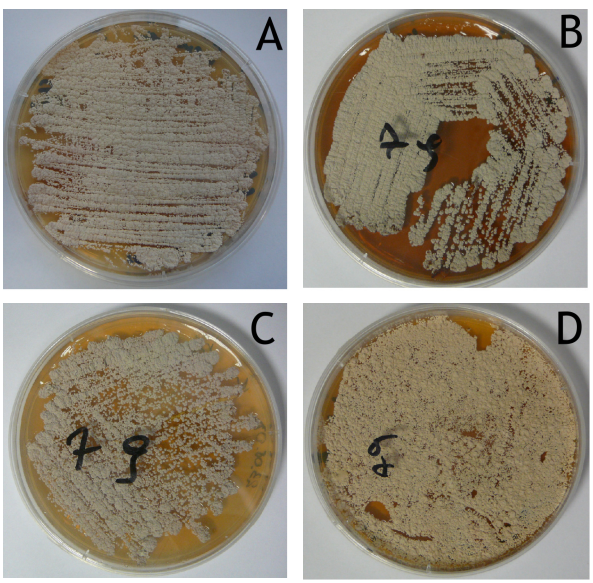

Fig. 1. Sporulating strains L1(a), L2 (b), W3 (c), W4 (d) cultivated on "79" medium

Fatty acids profile of strains L2, W3 and W4 consisted of saturated, branched forms of different chain length *iso*C12, *iso*C16, *aiso*C16, *iso*C17, *iso*C18, and *aiso*C18.

L1 strain is characterized by the least number of fatty acids only *iso*C17:0, *iso*C18:0, and *aiso*C18:0 were present (tab. 1).

Table 1

Fatty acid composition and relative percentage in studied strains

Strain	iso C12:0	iso C16:0	aiso C16:0	iso C17:0	n C17:0	iso C18:0	aiso C18:0
L1	-	-	-	76%	-	9.5%	14.5%
L2	-	10%	25%	25.5%	12.5%	7%	20%
W3	4%	11%	21%	31%	10%	10%	13%
W4	4%	10%	20%	31%	9.5%	10.5%	15%

Alanine, glycine, glutamic acid and diaminopimelic acid (DAP) were detected in the preparations of the cell wall of all investigated strains. LL-Diaminopimelic acid (LL-DAP), marker of *Streptomyces* spp., has been detected in strains W3, W4 and L2. Mezo-DAP was present in L1 strain.

Taxonomically important sugars present in studied strains are presented in Table 2.

Table 2

Sugar composition in cell biomass and in cell wall preparations of *Streptomyces* isolates

Strain	Mannose		Glucose		Galactose		N-acetyl glucosamine	
	total cell	cell wall	total cell	cell wall	total cell	cell wall	total cell	cell wall
L2	+	+	-	-	-	-	-	+
L1	-	+	-	-	+	-	-	+
W3	+	+	-	-	-	-	-	+
W4	-	+	+	+	-	-	-	+

Three strains: L2, W3 and W4 represent I-type of bacterial cell wall, with mannose and N-acetylglucosamine (strain W4 contained mannose and glucose). The L1 strain differed from these strains by the type of growth, and sugar profile which comprised mannose, N-acetylglucosamine and galactose. Fatty acids were represented only by iso C17, and *meso*-DAP was detected. Due to this characteristics strain L1 cannot be classified as belonging to *Streptomyces* genus.

Three of studied strains (L2, W3, W4) revealed similar glycolipid profile with one domination glycolipid of similar retention time to *Streptomyces* sp., while L1 strain did not contain major glycolipid (Fig. 2).

An increasing potency of bacterial cell wall constituents of strain L2 has been shown to stimulate the human blood cells to TNF-α and IL-1β production and was concentration-dependent.

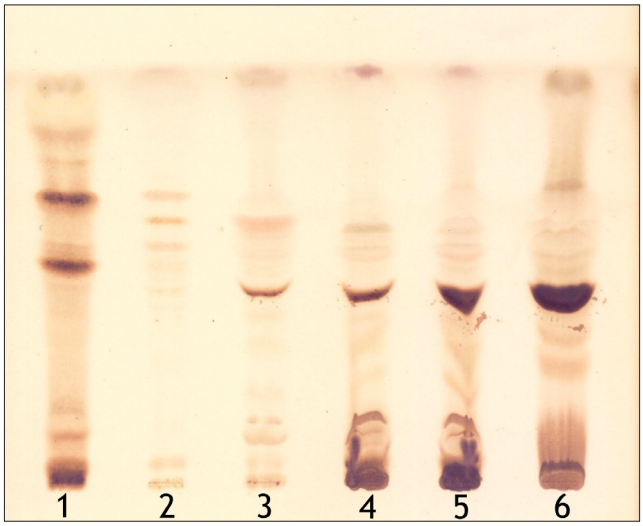

Fig. 2. Glycolipids of studied strains, TLC chromatogram visualized with orcinol reagent

1 — *Nocardiopsis dassonvillei*; 2 — strain L1; 3 — strain L2; 4 — strain W3; 5 — strain W4; 6 — *Streptomyces sp.*

Streptomyces are Gram-positive filamentous bacteria of the class *Actinobacteria* [9]. They are predominantly soil bacteria, but are also present in other habitats [10]. *Streptomyces* have been cultivated from dust samples collected from schools, day-care facilities and private houses [11,12,13]. *Streptomyces* are not demanding in their growth requirements; they metabolise biological polymers, i.e. cellulose, lignin, or chitin as their carbon source, and they do not need organic nitrogen for growth. Excepting a few thermophilic species, the optimal growing temperature is 25–28 °C, as it was observed also for our strains. These features make building materials suitable for their growth and proliferation [14]. *Streptomyces* are also highly potent producers of secondary metabolites of diverse biological activities, such as antibiotic, immunosuppressive, or antitumor [15]. Characteristic volatile metabolite of *Streptomyces* is geosmin, a volatile degraded sesquiterpene, responsible for odor of moist soil [16]. Cytotoxic potential of streptomycetal spores in cocultivation with moulds was reported [17]. Inflammatory and toxic potential of *Streptomyces* spores, not dependent on their viability, has also been confirmed [18].

Three investigated strains: W2, W3 and L2 belong to *Streptomyces* genus, while sporulating L1 strain characterized by cell wall type IV, is a member of *Actinobacteria* class, and further analysis is needed for complete determination of the taxonomical position.

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ХЕМОТАКСОНОМИЧЕСКАЯ ИДЕНТИФИКАЦИЯ ШТАММОВ АКТИНОБАКТЕРИЙ, ИЗОЛИРОВАННЫХ ИЗ ПОРАЖЕННЫХ СЫРОСТЬЮ ОФИСНЫХ ЗДАНИЙ

Реферат

Выделены четыре спорообразующих штамма бактерий из двух офисных зданий поврежденных сыростью. С помощью хемотаксономического подхода они идентифицированы как *Streptomyces sp.* (3 штамма) и актинобактерии с клеточной стенкой IV типа (1 штамм).

К л ю ч е в ы е с л о в а: *Actinobacteria, Streptomyces sp.*, хемотаксономия, клеточная стенка, воздух помещений.

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ХЕМОТАКСОНОМІЧНА ІДЕНТИФІКАЦІЯ ШТАМІВ АКТИНОБАКТЕРІЙ, ІЗОЛЬОВАНИХ З УРАЖЕНИХ ВОЛОГОЮ ОФІСНИХ БУДІВЕЛЬ

Реферат

Виділено чотири штами споротвірних бактерій з двох офісних будівель уражених вологою. За допомогою хемотаксономічного підходу штами ідентифіковані як *Streptomyces sp.* (3 штами) і актинобактерії з клітинною стінкою IV типу (1 штам).

К л ю ч о в і с л о в а: *Actinobacteria, Streptomyces,* хемотаксономія, клітинна стінка, повітря приміщень.