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Identification of *Thermobifida fusca* as a main causative agent of mushroom compost worker's lung – case series

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Abstract

Introduction and Objective. Mushroom compost worker's lung (MCWL) is a hypersensitivity pneumonitis (HP) entity occurring in workers employed at preparing compost for the cultivation of white button mushrooms. The aim of the study is to identify the strains of thermophilic actinobacteria as the potential causative agents of MCWL symptoms in workers.

Materials and Method. Clinical examinations included chest X–ray, high-resolution computed tomography (HRCT), bronchoscopy with bronchoalveolar lavage (BAL), pulmonary function tests comprising spirometric measurements, DLCO measurements, body pletysmography, and arterial – blood gas tests. Identification of thermophilic actinobacteria was performed by microscopy, culture and genetic methods. Allergological tests included the agar gel precipitation test and inhalation challenge with actinomycetal antigens.

Results. Among a group of 20 patients, 15 showed in HRCT ground-glass attenuations solely and were diagnosed with acute HP, 3 showed ground-glass attenuations with pulmonary fibrosis and were diagnosed with subacute HP, while 2 patients were diagnosed with Organic Dust Toxic Syndrome (ODTS). *Thermobifida fusca* and *Laceyella sacchari* strains were isolated from the symptom-causing mushroom compost. In 3 workers, strong precipitin reactions to *Th. fusca* were found, whereas there were no reactions to *L. sacchari*. Four workers showed a positive inhalation response to small doses of *Th. fusca* antigen.

Conclusions. The results of this study indicate that *Th. fusca* is most probably a major causative agent of MCWL and should be considered in the diagnosis of HP in workers of the mushroom compost facility.

Key words

occupational exposure, hypersensitivity pneumonitis, inhalation challenge, mushroom compost worker's disease, thermophilic actinobacteria, *Thermobifida fusca*, *Laceyella sacchari*

INTRODUCTION

Hypersensitivity pneumonitis (HP) is an infrequent, immune-mediated disease of the lower parts of the lungs initiated by the interaction of immunocompetent cells with the large quantities of offending allergens, mostly belonging to fungi, actinobacteria, non-tuberculous mycobacteria, and bird proteins, which are repeatedly inhaled at exposure to various organic dusts. This interaction leads to lymphocytic and often granulomatous inflammation of the peripheral airways, alveoli, and surrounding interstitial tissue and, for long-lasting exposure, to fibrosis [1–9]. The immunopathogenic allergens are usually inhaled at work, hence the entities (subunits) of the disease are named after occupation at risk, such as farmer's lung or bird breeder's lung [1, 4, 6].

One of the occupational groups under a high risk of HP are workers engaged in the industrial process of the cultivation of edible mushrooms, usually white button mushrooms (*Agaricus bisporus*). The HP subunit in this group has been described by Sakula [10] as the "mushroom worker's lung" (MWL). However, a more accurate name for this disease seems to be the "mushroom compost worker's lung" (MCWL) proposed by Philips et al. [11], and adopted in the present article, as the majority of hitherto reported cases occurred among the workers employed at the initial stage of the cultivation process, namely, at the production of compost used as the growing medium for white button mushrooms, followed by mixing of the compost with mushroom spawn, described as "spawning" [12].

Agaricus bisporus is the most common among edible mushrooms cultivated on compost with a world yearly production of nearly 12 million metric tonnes (data for 2019). Production is dominated by China with nearly 9 million metric tonnes, Poland is in the 4th place with a yearly production of circa 0.36 million metric tonnes. The spores of this mushroom species themselves have not been implicated in the etiology of MCWL and HP, although they could be a rare cause of IgE-dependent allergy, such as asthma [13] or anaphylactic reaction after ingestion of mushrooms [14].

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In the 21st century, the common occurrence of actinobacteria in mushroom compost has been confirmed by molecular methods. Song et al. [15] found an abundant occurrence and a high biodiversity of thermophilic actinobacteria in mushroom compost in Korea. The most common were strains of *Streptomyces* and *Thermoactinomyces* (the latter included more recently into *Bacillaceae*), the others represented most often the genera *Pseudonocardia*, *Saccharomonospora*, *Saccharopolyspora*, and *Thermobifida*. In the study performed in North Ireland, Xu et al. [16] identified by PCR four strains of thermophilic bacteria associated with MCWL as belonging to *Thermoactinomyces sacchari*, *Thermoactinomyces vulgaris*, and *Saccharomonospora viridis*. In the mushroom compost in Hungary, Székely et al. [17] found the prevalence of Proteobacteria with the dominant genus *Pseudoxanthomonas*, followed by Actinobacteria, among which the most common were *Thermobifida* and *Thermomonospora* genera.

The aim of the present study is to examine the potential role of the *Thermobifida fusca* strain, isolated as a dominant actinomycete species from the sample of MCWL-causing mushroom compost, as a specific etiological factor of this disease.

MATERIALS AND METHOD

Patients. The study group consisted of 20 workers employed in a mushroom compost facility in eastern Poland, admitted to the Clinic of Pneumonology, Oncology, and Allergology at the Medical University of Lublin, Poland, in 2009–2019 with symptoms corresponding to hypersensitivity pneumonitis (HP). As all the workers were diagnosed as having HP (18 individuals) or ODTS (two individuals) we described them further as "patients". ODTS (Organic Dust Toxic Syndrome) is a clinical entity similar to the acute stage of HP that is caused by the immunotoxic activity of some microbial substances present in organic dusts, such as endotoxin, glucans and peptidoglycan. It differs from HP by lack of specific allergic reaction and absence of changes in radiological or HRCT examination. All patients were men at the age ranging from 23.0–55.0 years (median 33.0 years).

Clinical examination. Clinical examination comprised: 1) detailed anamnesis concerning symptoms appearing at exposure to dust from mushroom compost, 2) physical examination, 3) routine laboratory examination, 4) chest X-ray, 5) high resolution computed tomography (HRCT), 6) bronchoscopy with bronchoalveolar lavage (BAL) described in detail below, 7) pulmonary function tests comprising: spirometric measurements (FVC, FEV_1, FEV_1%FVC) with the LUNGTEST 1000 (MES, Kraków, Poland), measurements of diffusion lung capacity for carbon monoxide (DLCO), and body pletysmography, 8) arterial-blood gas test with the RAPIDLAB 348 (Siemens, Erlangen, Germany).

Bronchoalveolar lavage (BAL). Bronchoalveolar lavage was performed in accordance with the ATS guidelines [18]. During flexible bronchoscopy with the Olympus BF-1T180 videobronchoscope, the device was placed in a wedge position in middle lobe: lateral (RB4) or medial (RB5). 150 ml normal saline was instilled through an endoscope, divided into seven aliquots. Negative suction pressure was performed. From total volume retrieved, 20 ml was sent for cellular analysis. The data on particular cell subpopulations were obtained during regular diagnostic BAL analysis. We used a two-colour (FITC and PE) BD Simultest IMK Plus (BD Biosciences, San Jose, USA) to analyze T-cell subpopulations on a BD FACSCalibur cytometer, using CellQuest Pro analysis software. For proper analysis of cell populations, lymphocyte purity was kept at >85% and recovery within gate at >90%.

Allergological tests. The agar-gel precipitation test and inhalation challenge were performed with the antigens derived from two thermophilic actinobacteria strains isolated from the samples of mushroom compost. Antigens preparation and agar-gel precipitation test by the Ouchterlony double diffusion method were performed according to the unified procedure used in routine diagnostics by the Medical Diagnostic Laboratory of the Department of Health Biohazards and Parasitology at the Institute of Rural Health in Lublin, as described earlier [19]. The study was conducted in a subgroup of six patients marked with the numbers 4, 5, 12, 13, 14, 15, selected based on the severity of symptoms.

Inhalation challenge. The test was performed according to the method described earlier [19]. All patients were informed in detail about the aim and possible effects of the test and gave written consent for its performance. The tests were performed with antigen of *Th. fusca* strain isolated from the mushroom compost, chosen on the base of positive precipitin reactions with patients' sera. The antigen was dissolved in 0.85% NaCl at a very low concentration of 20 μg/ml, sterilized, and checked for sterility and lack of toxicity. The antigenic solution was administered to patients by the LUNGTEST 1000 device (Inhalation Allergological Provocation System, MES, Poland) during 20 breaths. The absorbed dose of allergen was about 2.16 μg. The tests with bacterial allergen were preceded with the control test with saline (PBS), applied for the measurement of diurnal variability of spirometric values. At each test, spirometry, blood pressure, pulse rate, body temperature, peripheral blood analyses, auscultation, and observations for the appearance of general and respiratory symptoms were performed before the test (at 8 a.m.) and after 2 min, 30 min, 2 h, 4 h, 8 h and 24 h post- inhalation challenge. The spirometric measurements comprised determination of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1). The decrease of FVC and/or FEV_1 by 10% of the initial value at any time interval(s) was considered as a positive result of the test. Peripheral blood analyses comprised erythrocyte sedimentation rate, concentration of leukocytes, blood morphology, oxygen saturation, procalcitonin (PCT) level, C Reactive Protein (CRP) level, and interleukin 6 (IL-6) level.

The use of the inhalation challenge as well as all other diagnostic procedures used in the present study were approved by the Bioethics Commission at the Medical University in Lublin (Decision No. KE-0254/258/2017, dated 26 October 2017).

Identification of Thermophilic actinobacteria strains Thermophilic actinobacteria were identified by microscopy and with genetic methods. Mushroom compost samples weighing 10 g each were used to perform microbiological cultures on tryptic soy agar plates (Difco, Sparks, USA), according to the method described previously [19]. Chemotaxonomic characteristics of the strains were

determined as described in [20]. DNA isolation from the cultures was performed using Qiamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol for Gram-positive bacteria. Thermophilic actinobacteria DNA was detected by amplification of 16S rDNA gene fragment according to method by Xu et al. [16]. Sequencing was performed with ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Inc., Foster City, USA) using Abi Prism Big Dye Terminator v. 3.1. Cycle Sequencing Kits. The results were compared with sequences in GenBank database using the BLAST software at the National Center for Biotechnology Information (Bethesda, USA).

Statistical analysis. The occurrence of work-related symptoms depending on job duration were analyzed with Mann-Whitney test. The results of inhalation challenge were analyzed with chi-square test. All tests were performed using STATISTICA v. 6.0 package (Statsoft, Tulsa, USA).

RESULTS

Clinical examination. All patients experienced work-related respiratory and general symptoms. Their number was greater in the subgroup of 8 workers with a job duration above 1 year compared to that below 1 year (Mann-Whitney, P = 0.0337). The symptoms reported most commonly were: cough (in 19 out of 20 workers), dyspnea (in 18) and chest tightness (in 14). Physical examinations by auscultation found inspiratory crackles (rales) in 16 patients, and vesicular murmur in 4. In HRCT, ground-glass attenuations solely were observed in 15 patients diagnosed with acute hypersensitivity (Fig. 1). Ground-glass attenuations with pulmonary fibrosis were found in 3 patients diagnosed with subacute hypersensitivity (Fig. 2–3). Two patients did not show any changes in the HRCT examination and were diagnosed with Organic Dust Toxic Syndrome (ODTS). Generally, the cases of subacute HP were diagnosed in the middle-age (≥40 years) workers with a long job duration. By contrast, the cases of ODTS occurred in younger people (≤30 years) with a short job duration (Tab. 1).

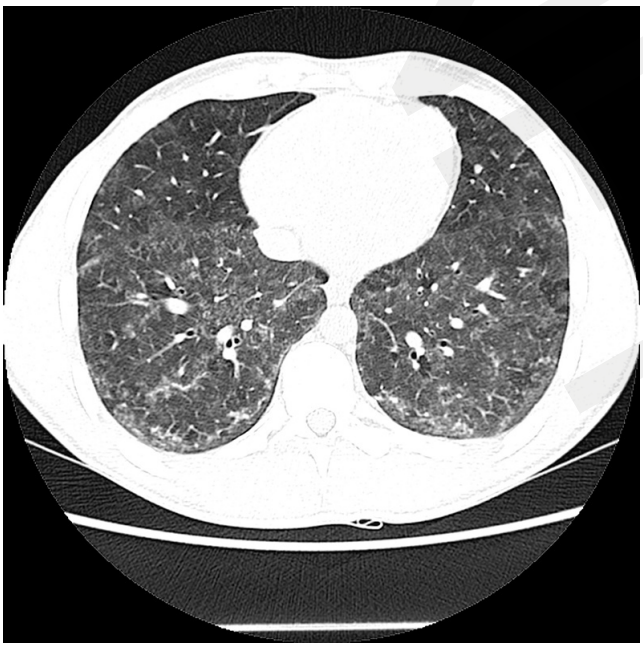


Figure 1. HRCT of patient No. 2. with the diagnosis of acute HP. Areas of mosaic attenuation are visible

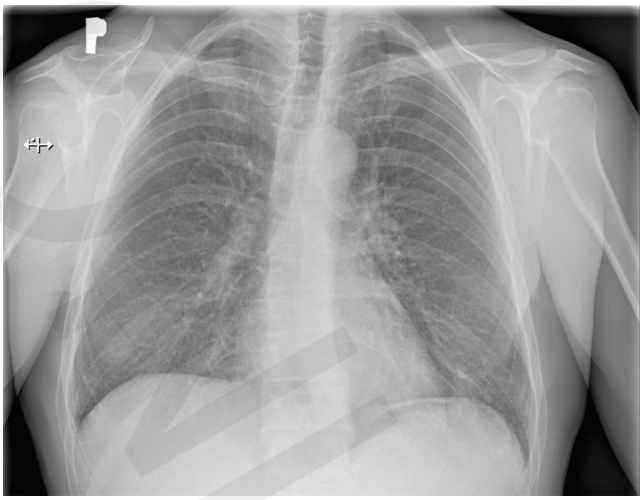


Figure 2. Chest radiogram of patient No. 5 with the diagnosis of subacute HP. Bilateral interstitial diffuse changes visible

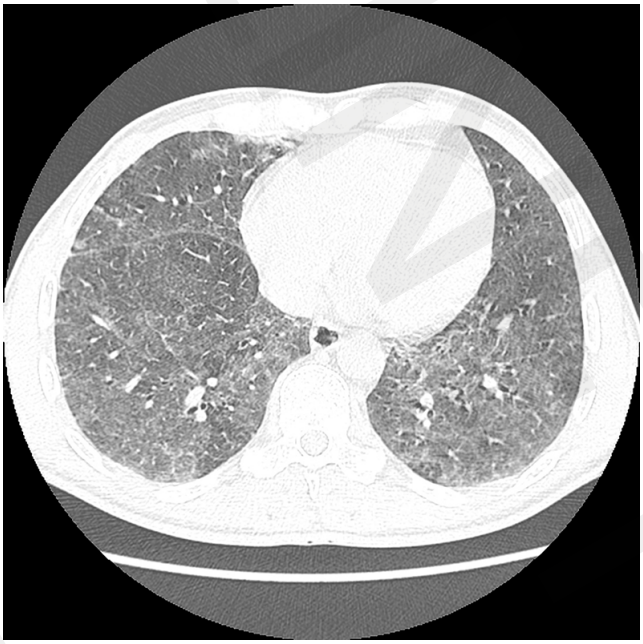


Figure 3. HRCT of patient no. 5. Areas of ground glass with reticular subpleural fibrosis are visible

Bronchoalveolar lavage (BAL). Examination of bronchoalveolar lavage (BAL) demonstrated a typical lymphocytic alveolitis in HP patients, with prevalence of CD4+ T helper cells, and low proportion of neutrophils. Interestingly, the situation was reverse in 2 patients diagnosed with ODTS, where neutrophils clearly dominated over lymphocytes (Suppl. Tab. 1S). Pulmonary function tests were normal in 16 patients. In the remaining 4 patients, reductions were stated in FVC (in 1 patient), of FEV_1 (in 2), of FEV_1%FVC (in 3), and of diffusion lung capacity for carbon monoxide (DLCO) (in 2) (Tab. 2).

In an arterial-blood gas test, abnormalities were found in 6 patients who showed a joint significant decrease of pO_2. value and saturation (Suppl. Tab. 2S). In these cases, abnormalities were not related to job duration of the patients.

Table 1. Clinical symptoms and HRCT examination results of patients employed at processing mushroom compost

Patient No.	Age (years)	Job duration	Symptoms	Auscultations	HRCT	Diagnosis
1	32	9 months	C, CT, D	crackles	GG	Acute HP
2	23	3 months	C, GW, F, FA, PD, SW	crackles	GG	Acute HP
3	26	3.5 years	BN, CT, D, EI, F, FA, GW, H, N, PD, S, SW	crackles	GG	Acute HP
4	23	1 year	BN, CT, D, F, GW, GM, H, HE, PD, V, SW	crackles	GG	Acute HP
5	54	10 years	BN, C, D, FA, GM, H, S	crackles	GG+FB	Subacute HP
6	31	1 month	BN, C, D, F, H, GM, GW, S	crackles	GG	Acute HP
7	40	1 month	C, D, F, GM, GW, H, HE, PD, S, SW	vesicular murmur	GG	Acute HP
8	27	3 months	BN, C, CT, D, EI, GW, H, HE, J, N	crackles	GG	Acute HP
9	27	2 months	CT, D, F, GW, S	crackles	GG	Acute HP
10	40	4.5 years	PD, CT, D, EI, F, GM, GW, H, HE, J, SW	crackles	GG+FB	Subacute HP
11	38	1 month	C, D, F, GM	crackles	GG	Acute HP
12	50	11.5 years	BN, C, CT, D, F, GW, H, HE, N, S, V	crackles	GG+FB	Subacute HP
13	34	3.5 years	C, CT, D, GM, GW, H, HE, J, SW	crackles	GG	Acute HP
14	23	2 months	C, CT, D, GW, H, HE	vesicular murmur	GG	Acute HP
15	41	1 month	CT, D, EI, F, GW, GM, HE, PD, S, V	crackles	GG	Acute HP
16	50	1 month	C, FA, GM, GW	crackles	GG	Acute HP
17	34	1 month	BN, C, CT, D, EI, GM, GW, SW	crackles	GG	Acute HP
18	55	1 month	C, CT, D, F, GM, H, S	crackles	GG	Acute HP
19	29	1 year	C, CT, D, EI, FA, GW, H, HE, J, PD	vesicular murmur	Normal	ODTS
20	29	3 years	BN, C, CT, D, F, GW, H, N	vesicular murmur	Normal	ODTS

ODTS – Organic Dust Toxic Syndrome. Symptoms: BN – blockade of the nose; C – cough; CT – chest tightness; D – dyspnea; EI – eyes itching; F – fever; FA – fatigue; GW – general weakness; H – hoarseness; HE – headache; J – joint and muscle aching; N – nausea; PD – productive cough; S – shivering; SW – sweating; V – vomiting. HRCT results: GG – ground glass opacities; GM – general malaise; FB – fibrosis

Table 1S. Results of the analysis of BAL fluid from patients employed at processing mushroom compost

Patient	Lymphocytes (%)	Macrophages (%)	Neutrophils (%)	CD4	CD8	CD4/CD8
1	69.7	25.12	0.3	60.22	19.49	3.09
2	32.74	7.02	18.97	34.33	29.8	1.15
3	61.4	32.37	0.58	41.12	18.7	2.19
4	45.36	52.7	0.51	38.02	21.99	1.72
5	86.04	8.04	0.21	45.67	37.41	1.22
6	74.29	11.29	0.82	76.61	7.26	10.55
7	89.3	2.16	0.37	25.64	30.77	0.83
8	42.38	40.44	5.54	22.63	41.33	0.54
9	37.63	28.30	1.49	27.96	24.44	1.14
10	78.8	7.42	0.35	37.18	19.94	1.87
11	66.7	2.47	27.76	27.77	45.84	0.56
12	31.1	58.48	4.97	20.13	35.54	1.04
13	44.85	31.35	0.62	22.26	32.49	0.68
14	60.88	37.48	0.34	58.4	21.02	2.78
15	32.93	66.32	0.3	68.61	8.8	7.08
16	36.46	50.7	1.2	38.14	20.46	1.86
17	68.39	26.53	0.77	60.37	25.75	2.34
18	40.08	58.73	0.9	68.23	14.78	4.61
19*	15.79	28.53	40.26	23.41	50.02	0.57
20*	10.38	30.18	31.79	38.21	30.76	1.22

*ODTS diagnosis (Organic Dust Toxic Syndrome)

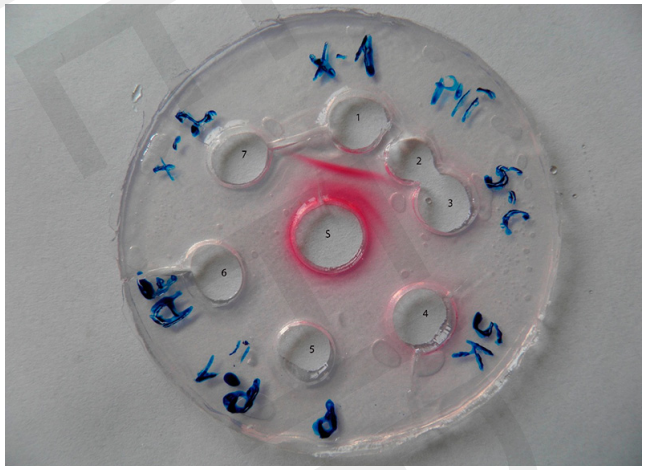


Figure 4. Agar-gel precipitation test with serum of patient No. 5. Centre well marked "S" contained patient's serum. Peripheral wells contained following antigens: 1) *Thermobifida fusca* strain X-1 isolated from compost, 2) grain dust extract, 3) duck serum, 4) chicken serum, 5) *Penicillium* spp., 6) *Aspergillus fumigatus*, 7) *Laceyella sacchari* strain X-2 isolated from compost

ALLERGIC REACTIONS IN SYMPTOMATIC WORKERS

Agar-gel precipitation test. In 3 serum samples of symptomatic workers (patients No. 5, 12, 13), strong or very strong precipitin reactions to *Th. fusca* antigenic extract were detected, whereas no precipitins against *L. sacchari* extract were found (Fig. 4). The positive precipitin reactions to *Th. fusca* antigen were found only in patients with a long job duration of between 3.5 – 11.5 years, and all were strong or very strong. In contrast, no positive reactions to this antigen appeared in patients with a short job duration from 1 month to 1 year.

Table 2. Pulmonary function tests of patients employed at processing mushroom compost

Patient No.	FVC (L) (%N)	FEV1 (L) (%N)	FEV1%FVC	DLCO (%N)	TLC (L) (%)	RV (L) (%N)	RV%TLC
1	4.42 (85%)	3.84 (88%)	86.8%	96%	5.36 (87%)	2.13 (120%)	39.7%
2	4,78 (79%)	83.3 (89%)	82.7%	112%	5.02 (105%)	3.86 (126%)	41.7%
3	3.81 (85%)	3.31 (89%)	86.8%	101%	4.91 (127%)	3.94 (132%)	37.9%
4	5.78 (110%)	4.10 (88%)	68.91%	119%	8.48 (118%)	2.55 (155%)	30.07%
5	4.81 (112%)	3,56 (101%)	74.01%	99%	6.80 (106%)	2.14 (98%)	31.4%
6	4.79 (90%)	3.49 (78%)	70.7%	124%	8.12 (145%)	3.98 (157%)	45.6%
7	**2.92 (63%)[1]**	**1.66 (43%)[2]**	**53%[3]**	103%	6.78 (119%)	4.16 (154%)	38.2%
8	5.53 (116%)	4.32 (109%	78.1%	151%	8.52 (134%)	3.95 (142%)	46.2%
9	4.92 (80%)	4.13 (78%)	83.9%	**41%[4]**	7.31 (91%)	1.83 (99%)	25.03%
10	4.87 (88%)	4.09 (89%)	83.9	142%	9.94 (131%)	4.22 (185%)	42.4%
11	5.18 (98%)	3.31 (75%)	**61.9%[3]**	88%	8.45 (123%)	3.41 (179%)	39.1%
12	4,88 (93%)	**2.64 (66%)[2]**	**54.1%[3]**	**64%[4]**	9.68 (129%)	4.27 (186)	44.1%
13	5.49 (110%)	4.34 (104%)	79.05%	107%	8.54 (123.5%)	3.16 (176%)	37%
14	4.83 (88%)	4.03 (82%)	83.4%	102%	7.13 (96%)	2.04 (120%)	28.6%
15	3.66 (85%)	2.61 (73%)	71.3%	126%	6.55 (106%)	2.56 (138%)	39.08%
16	4.59 (111%)	3.13 (92%)	68.19%	99%	8.35 (135%)	3.87 (190%)	46.3%
17	4.21 (98%)	2.98 (89%)	74.1%	N. D.	N. D.	N. D.	N. D.
18	3.76 (100%)	3.03 (94%)	80.3%	78%	6.29 (104%)	2.12 (100%)	33.7%
19	4.94 (94%)	3.89 (88%)	78.7%	113%	6.75 (97%)	1.99 (116%)	29.4%
20	5.86 (106%)	4.77 (107%)	81%	83%	8.71 (122%)	2.97 (180%)	34.0%

FVC – forced vital capacity in **litres** (% of the norm); FEV_1 – forced expiratory volume in one second in **litres** (% of the norm); DLCO – diffusion lung capacity for carbon monoxide; TLC – total lung capacity in **litres** (%); RV – residual volume in **litres** (% of the norm);
[1] reduction of FVC; [2] reduction of FEV_1; [3] reduction of FEV_1%FVC (obstruction); [4] reduction of DLCO (% of the norm); N.D. – not done

Specific inhalation challenge with the extract of *Th. fusca* strain. Four out of 6 examined patients showed a positive response to the challenge with allergenic extract of *Th. fusca* strain. The decrease of spirometric values (FVC and FEV_1) below 10% of initial value, assumed as a threshold level, was well expressed after 8 h post-challenge, but some of the patients showed a significant decrease of these values at earlier (4 h) or later (24 h) measurements (Fig. 5). The results were confirmed statistically by χ^2 test that showed highly significant decrease ($P<0.0001$) both of FVC and FEV_1 after inhalation challenge with inciting allergen. Four out 6 patients tested also showed a highly significant decrease of FEV_1 ($P<0.0001$). The results of spirometric measurements were supported by the occurrence of subjective respiratory symptoms after the inhalation challenge with *Th. fusca* allergenic extract. Three patients responded with cough and 1 with dyspnea. At the auscultation examination after challenge, rales were noted in 4 out of 6 examined persons (Tab. 3).

From among the results of laboratory blood analysis, the most remarkable were those concerning the interleukin 6 (IL-6) level. Three out of 6 patients responded after challenge with a positive increase of IL-6 level which proved to be significant in 2 persons when assessed by χ^2 test ($P<0.05$ and $P<0.0001$). The increase of C-reactive protein (CRP) after challenge was noted in 5 out of 6 persons; however, only in 2 cases the value considered as normal (3 mg/l) was exceeded, although this increase was not statistically significant. Slight increases, below the normal values, were recorded in the erythrocyte sedimentation rate (ESR) and leukocyte concentration in peripheral blood (Tab. 3).

Summarizing, the distinct decrease of vital capacity and the presence of subjective respiratory symptoms after the inhalation challenge with the extract of *Th. fusca* univocally support the positivity of the test, and indicate the most probable role of this bacterium as a main disease agent causing symptoms in exposed mushroom compost workers.

Table 2S. Arterial-blood gas test of patients employed at processing mushroom compost

Patient No.	pO_2 mmHg	pCO_2 mmHg	Saturation (%)	pH
1	81.2	38.7	95	7.442
2	**64.9[1]**	40.6	**93[2]**	7.436
3	83.0	36.3	96	7.436
4	**65.1[1]**	40.2	**93[2]**	7.433
5	84.1	35.3	96	7.431
6	**61.8[1]**	34.0	**91[2]**	7.455
7	78.9	40.4	95	7.424
8	83.7	38.1	96	7.422
9	**57.7[1]**	39.7	**90[2]**	7.417
10	73.0	34.4	95	7.461
11	**62.1[1]**	39.0	**90[2]**	7.408
12	**55.1[1]**	41.7	**90[2]**	7.452
13	76.3	43.0	95	7.439
14	70.5	39.5	94	7.417
15	69.1	33.7	94	7.483
16	75.2	41.0	96	7.427
17	72.1	38.2	95	7.431
18	74.1	36.3	96	7.451
19	81.8	34.0	96	7.447
20	83.2	39.6	96	7.403

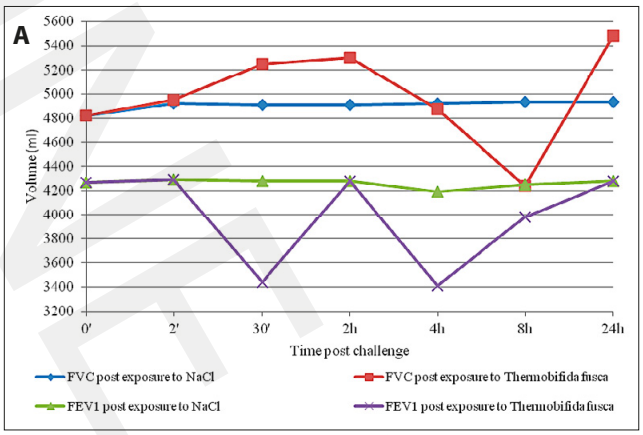
[1]significant decrease of pO_2;
[2]significant decrease of saturation

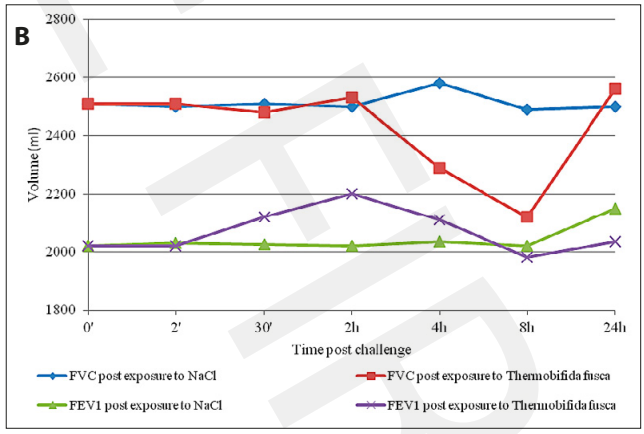
Table 3. Results of inhalation challenge with the extract of *Thermobifida fusca* (8 h post-challenge)

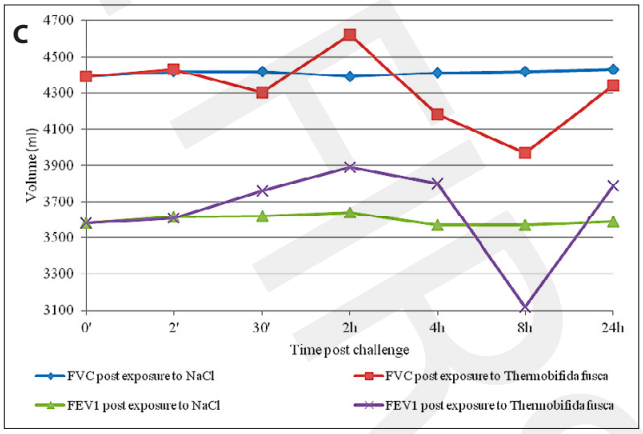
Patient No.	Lung function tests #		Laboratory blood analysis ##				Subjective symptoms	Auscultation
	FVC	FEV_1	IL-6	CRP	ESR	LC		
4	neg. ↑ 1.64%	neg. ↑ 1.75%	neg. ↔ 0%	neg. ↓ 0.04%	neg. ↓ 16.6%	neg. ↓ 2.81%	none	normal
5	**pos. **** **↓ 9.57%**	**pos. **** **↓ 12.8%**	**pos.** **↑20.0%**	**pos.** **↑ 67.8%**	neg. ↓ 50%	**pos.** **↑ 20.4%**	**Yes** **cough**	**rales**
12	**pos. **** **↓ 9.62%**	**pos. **** **↓ 5.92%**	neg. ↔ 0%	**pos.** **↑ 7.46%**	neg. ↓ 28.5%	**pos.** **↑ 16.3%**	**Yes** **cough**	**rales**
13	**pos. **** **↓15.53%**	**pos. *** **↓ 1.98%**	**pos. *** **↑ 20.0%**	**pos.** **↑ 960.9%**	**pos.** **20.0%**	neg. ↔ 0%	**Yes** **dyspnea**	**rales**
14	**pos. **** **↓12.03%**	**pos. **** **↓ 6.79%**	neg. ↔ 0%	**pos.** **↑ 20.6%**	**pos.** **85.7%**	**pos.** **↑ 30.4%**	**Yes** **intensive cough**	normal
15	neg. ** ↑ 9.49%	neg. ** ↑ 18.94%	**pos. **** **↑ 80.0%**	**pos.** **↑ 2835.48%**	neg. ↓ 19.23%	**pos.** **↑ 12.12%**	none	**rales**
Mean decrease (-) or increase (+) (in %)	**-11.69%**	**-6.87%**	**+40.0%**	**+778.4%**	n. c.	+19.8%	n. a.	n. a.

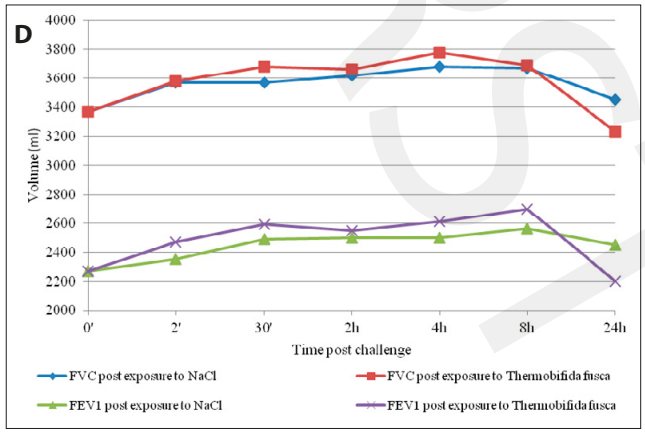
pos. = result of the test assessed positive by decrease or increase of initial value (marked in bold); neg. = result negative;
↓ = decrease of initial value (in percent); ↑ = increase of initial value (in percent); ↔ = no change in measured value (<1%);
* – ** = changes in the course of test statistically significant (χ^2); * = P<0.05, ** = P<0.0001;
IL-6 = interleukin 6; CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; LC = concentration of leukocytes in peripheral blood; n. c. = not calculated (when a majority of results was not positive); n. a. = not applied;
= final positive result was assumed at the fall equal to or below 10% considering all time intervals and both tests;
= final positive result was assumed at the rise equal to or above 50% considering all time intervals.
Results of patient # 15 are noteworthy. In spite of a negative spirometric response, the patient showed a very high increase in pathologic blood markers which seem to indicate the HP pathologic process(es) probably run by different immunopathologic pathways.

Identification of thermophilic actinobacteria strains. Two species of thermophilic actinobacteria were identified in mushroom compost samples: *Th. fusca* strain X-1 forming small, light, convex colonies and *Laceyella sacchari* strain X-2 forming large, flat, brownish colonies. *Th. fusca* isolates appeared microscopically as filaments with dichotomously branched sporophores carrying spore clusters, whereas *L. sacchari* strains occurred in the form of filaments with irregularly distributed sessile, single spores. *Th. fusca* and *L. sacchari* strains were isolated in the concentrations of 2.45 CFU × 10^6 g^{-1} and 0.1 CFU × 10^6 g^{-1}, respectively. Thus, the concentration of *Th. fusca* was nearly 25 times greater compared to *L. sacchari*. Both strains exhibited distinct chemotaxonomic profiles. Strain X-1 was characterized by the presence of phospholipid type I, one major glycolipid, branched-chain fatty acids from C14:0 to C18:0 (with









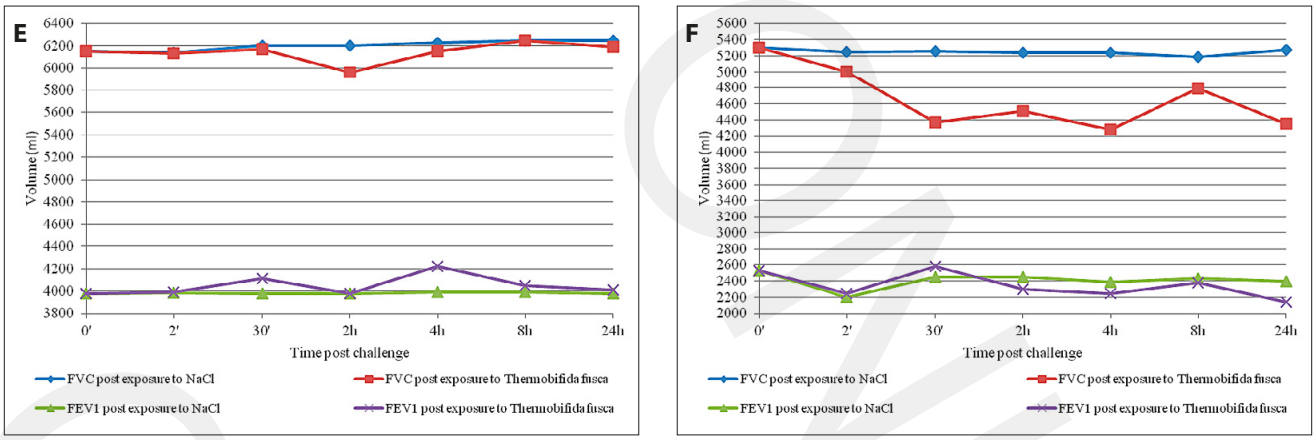


Figure 5. Results of inhalation challenge with the extract of *Thermobifida fusca* X1 in patients No. 14 (A), 13 (B), 5 (C), 15 (D), 4 (E), and 12 (F).
A) Patient No. 14. Note the distinct drop of FVC 8 h post-exposure and dual response in FEV_1 by the drop at 30 min and 4 h post-exposure.
B) Patient No. 13. Note the distinct drop of FVC 8 h post-exposure and lack of significant response in FEV_1.
C) Patient No. 5. Note the distinct drop of FVC and FEV_1 8 h post-exposure.
D) Patient no. 15. Note the weak drop of FVC and FEV_1 24 h post-exposure.
E) Patient no. 4. Note lack of significant response of FVC and FEV_1.
F) Patient no. 12. Note the drop of FVC 30 min – 4 h and 24 h post-exposure, and lack of significant response in FEV_1

major iso C16:0), and no DAP isomer in peptidoglycan. In contrast, strain X-2 possessed phospholipid type II with phosphatidylethanolamine, two major glycolipids, branched-chain fatty acids (major fatty acid iso C15:0), and meso DAP isomer in peptidoglycan (data not shown). The obtained sequences were deposited in the GenBank under Accession Nos. PV111331.1 (*Thermobifida fusca* strain X-1 16S ribosomal RNA gene, partial sequence), and PV111330.1 (*Laceyella sacchari* strain X-2 16S ribosomal RNA gene, partial sequence).

DISCUSSION

The diagnosis of HP is usually based on clinical, functional and radiological criteria supplemented by additional tests such as inhalation challenge and/or bronchoalveolar lavage. As much as 18 out of 20 our patients revealed signs and symptoms complying to these criteria, except for 2 with normal HRCT who were diagnosed with ODTS.

For establishing a proper diagnosis, a detailed anamnesis aimed at identifying exposure agents suspected to cause the disease, characteristic symptoms and type of relation between the potential etiological agents and particular symptoms, is very important. Eighteen patients who developed disease after occupational exposure to the inhalation of dust from mushroom compost displayed dyspnea, cough, fever and malaise, reported as typical symptoms for the acute stage of HP [1–6]. Physical examination revealed crackles (rales) over lung fields, regarded as characteristic for HP, in 16 out of 20 patients. For example, Lacasse et al. [21] expressed an opinion that finding crackles in patients with documented exposure, and positive immunologic tests allow for the diagnosis of HP in 80% of cases.

In lung function tests, a reduction of FEV_1, FVC, FEV_1%FVC and/or DLCO were found in 4 patients, whereas others did not show abnormalities. Though restrictive changes are reported as typical for HP, they occur usually in the chronic stage of the disease [21, 22], hence, the lack of such changes does not abolish the diagnosis of acute HP.

Radiological examination of the chest revealed pathologic changes in the lungs of 18 out of 20 patients. Similarly, the HRCT examination, regarded as very important in the HP diagnosis [22], displayed an abnormal picture in 18, except 2 diagnosed with ODTS (Organic Dust Toxic Syndrome). This is a clinical entity similar to the acute stage of HP caused by immunotoxic activity of some microbial substances present in organic dusts, such as endotoxin, glucans and peptidoglycan. ODTS differs from HP by lack of specific allergic reaction and absence of changes in radiological or HRCT examination. The 2 cases of ODTS mentioned above, did not affect the main conclusions concerning the HP (MCWL) case series.

The HRCT examination enabled us to differentiate between the acute and subacute stages of the disease. In 15 patients with the acute HP, ground-glass attenuations with features of air trapping dominated. In 3 patients with the subacute HP, a fine reticular fibrosis was found in addition to ground-glass changes. On the basis of the obtained results, we presume that the clinical course of the MCWL could be dependent on human genetics and ontogenesis, appearing as subacute HP in middle-aged or elderly patients, and as acute HP in young people.

Fibrobronchoscopic (BAL) examination performed in our 18 HP patients for estimation of the lung cell composition and lymphocyte subpopulations revealed lymphocyte level ranging from 31.1–89.3%. Such a level is regarded by Lacasse et al. [23] as significant for HP diagnosis, whereas Selman [22] proposed values exceeding 40% or even 50% as significant lymphocytosis. These levels were also exceeded in our patients with HP diagnosis, respectively, in 13 and 9 persons. Interestingly, in 2 patients with ODTS diagnosis, lymphocytes occurred in very low levels of 10.38% and 15.74%. The ratio of CD4/CD8 in our HP patients ranged from 0.54–10.55, and in 14 out of 18 exceeded the threshold of 1.0 regarded by Girard et al. [24] as significant for HP diagnosis. In conclusion, the results of bronchoalveolar lavage examination confirm the diagnosis of HP in our patients.

The identification of agent(s) inciting the symptoms of HP is crucial for the successful therapy and prevention of the disease [25]. In this study, of the 6 examined mushroom compost workers with HP symptoms, 3 showed the presence of specific precipitins to inciting allergen. The reactions appeared only in the patients with a long job duration,

which seems to indicate that this test is inferior to inhalation challenge which is a more reliable diagnostic tool in the early, acute stage of disease. The precipitin test may also be very helpful in diagnosis, but usually does not reveal whether or not the patient has HP [3, 6, 26]. Therefore, the inhalation challenge, regarded by some authors [6, 27] as a "gold standard", represents the greatest value for establishing the diagnosis of HP [6, 28, 26].

Immunopathologic reaction in HP is initiated by exposure to large amounts of adverse factors, usually microorganisms [28], hence, the organisms occurring abundantly in the material associated with evoking symptoms are almost surely the disease-causing agents. This has been demonstrated many times, beginning from the classic works by Towey et al. [29] and Pepys et al. [30]. Accordingly, in the present study we have chosen as test allergens the extracts of the biomass of two strains of thermophilic actinobacteria, *Th. fusca and L. sacchari*, which occurred abundantly in the mushroom compost associated with the MCWL cases in the orders of 10^6 CFU g^{-1} and 10^5 CFU g^{-1}, respectively.

It is noteworthy that the authors of review articles on HP [1, 3–6, 9] list thermophilic actinobacteria as the causative agents of the Mushroom Worker's Lung (MWL), based on numerous reports on the isolation of these bacteria from mushroom compost and/or positive allergological tests in exposed workers [10, 11, 20, 31]. Nevertheless, until recently, none of the species have been identified as a single or main cause of this disease. Our results, however, indicate that *Th. fusca* could be regarded as such a species, although the potential etiological role of *L. sacchari* and other thermophilic actinomycetes must also be taken into consideration.

Thermobifida fusca develops in various self-heating organic materials. It produces multiple extracellular enzymes including cellulases and lignocellulases, and is a major degrader of plant cell wall [32]. Because of strong enzymatic activity this species could be used in various biotechnological facilities. Considering its potential role in induction of HP documented in this study, this organism should be regarded as a factor of occupational risk, not only for mushroom compost workers, but also for workers employed at waste removal, agricultural workers exposed to various self-heating materials, such as piles of rotting hay and manure, and potentially also for biotechnology workers at preparing its enzymes. Thus, the antigens of *Th. fusca* should be included into a battery of allergens produced by various companies for the diagnostics of HP at mushroom compost production, and similar facilities.

CONCLUSIONS

The results of our study confirm the common occurrence of the mushroom compost worker's lung (MCWL) in Poland. The study also confirmed the etiological role of thermophilic actinobacteria in causing this disease, simultaneously determining the species *Th. fusca* as a main causative agent of MCWL. Thus, we believe that this species should be included into the batteries of antigens produced by various companies for diagnostics of MCWL and other entities of hypersensitivity pneumonities (HP).

To prevent further cases of this disease in Poland and abroad, a range of prophylactic measures should be applied, such as the installment of efficient ventilation systems for trapping small, often submicron, particles of actinobacteria, providing efficient and comfortable respirators and/or masks, and, in the case of increasing hazard, the application into compost of bacteriocides, such as propionic acid, which are safe for humans.

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REFERENCES

1. Selman M, Pardo A, King Jr. TE. Hypersensitivity pneumonitis: insights in diagnosis and pathobiology. Am J Respir Crit Care Med. 2012;186(4):314–324. https://doi.org/10.1164/rccm.201203-0513CI
2. Spagnolo P, Rossi G, Cavazza A, et al. Hypersensitivity pneumonitis: a comprehensive review. J. Investig. Allergol Clin Immunol. 2015;25(4):237–250.
3. Quirce S, Vandenplas O, Campo P, et al. Occupational hypersensitivity pneumonitis: an EAACI position paper. Allergy. 2016;71(6):765–779. https://doi.org/10.1111/all.12866
4. Riario Sforza GG, Marinou A. Hypersensitivity pneumonitis: a complex lung disease. Clin Mol Allerg. 2017;15:6. http://doi.org/10.1186/s12948-017-0062-7
5. Greenberger PA. Hypersensitivity pneumonitis: a fibrosing alveolitis produced by inhalation of diverse antigens. J Allergy Clin Immunol. 2019;143(4):1295–1301. https://doi.org/10.1016/j.jaci.2018.09.040
6. Nogueira R, Melo N, Novais e Bastos H, et al. Hypersensitivity pneumonitis: Antigen diversity and disease implications. Pulmonology. 2019;25(2):97–108. https://doi.org/10.1016/j.pulmoe.2018.07.003
7. Barnes H, Lu J, Glaspole I, et al. Exposures and associations with clinical phenotypes in hypersensitivity pneumonitis: A scoping review. Respir Med. 2021;184:106444. https://doi.org/10.1016/j.rmed.2021.106444
8. Akkale T, Sari G, Şimşek C. Occupational hypersensitivity pneumonia. Tuberk Toraks. 2023;71(1):94–104. http://doi.org/10.5578/tt.20239911
9. Calaras D, David A, Vasarmidi E, et al. Hypersensitivity pneumonitis: Challenges of a complex disease. Can Respir J. 2024; e4919951. https://doi.org/10.1155/2024/4919951
10. Sakula A. Mushroom-worker's lung. Brit Med J. 1967;3:708–710. http://doi.org/10.1136/bmj.3.5567.708
11. Phillips MS, Robinson AA, Higenbottam TW, et al. Mushroom compost worker's lung. J R Soc Med. 1987;80(11):674–677. http://doi.org/10.1177/014107688708001105
12. Derikx PJ, Op Den Camp HJ, van der Drift C, et al. Biomass and biological activity during the production of compost used as a substrate in mushroom cultivation. Appl Environ Microbiol. 1990;56(10):3029–3034. https://doi.org/10.1128/aem.56.10.3029-3034.1990
13. Venturini M, Lobera T, Blasco A, et al. Occupational asthma caused by white mushroom. J Investig Allergol Clin Immunol. 2005;15(3):219–221.
14. Cunha IM, Marques ML, Abreu C, et al. Anaphylaxis to *Agaricus bisporus* ingestion. Einstein (São Paulo) 2020;18:eRC5478. https//doi.org/10.31744/einstein_journal/2020RC5478
15. Song J, Weon HY, Yoon SH, et al. Phylogenetic diversity of thermophilic actinomycetes and *Thermoactinomyces* spp. isolated from mushroom composts in Korea based on 16S rRNA gene sequence analysis. FEMS Microbiol Lett. 2001;202(1):97–102. https://doi.org/10.1111/j.1574-6968.2001.tb10786.x
16. Xu J, Rao JR, Millar BC, et al. Improved molecular identification of *Thermoactinomyces* spp. associated with mushroom worker's lung by 16S rDNA sequence typing. J Med Microbiol. 2002;51(12):1117–1127. https://doi.org/10.1099/0022-1317-51-12-1117
17. Székely AJ, Sipos R, Berta B, et al. DGGE and T-RFLP analysis of bacterial succession during mushroom compost production and sequence-aided T-RFLP profile of mature compost. Microb Ecol. 2009;57(3):522–533. http://doi.org/10.1007/s00248-008-9424-5
18. Meyer KC, Raghu G, Baughman RP, et al. An official American Thoracic Society clinical practice guideline: the clinical utility of bronchoalveolar lavage cellular analysis in interstitial lung disease. Am J Respir Crit Care Med. 2012;185(9):1004–1014. https://doi.org/10.1164/rccm.201202-0320ST
19. Mackiewicz B, Dutkiewicz J, Siwiec J, et al. Acute hypersensitivity pneumonitis in woodworkers caused by inhalation of birch dust contaminated with *Pantoea agglomerans* and *Microbacterium barkeri*. Ann Agric Environ Med. 2019;26(4):644–655. https://doi.org/10.26444/aaem/114931

20. Paściak M, Pawlik K, Gamian A, et al. An airborne actinobacteria *Nocardiopsis alba* isolated from bioaerosol of a mushroom compost facility. Aerobiologia (Bologna). 2014;30(4):413–422. http://doi.org/10.1007/s10453-014-9336-4
21. Lacasse Y, Selman M, Costabel U, et al. Classification of hypersensitivity pneumonitis: a hypothesis. Int Arch Allergy Immunol. 2009;149(2):161–166. https://doi.org/10.1159/000189200
22. Selman M. Hypersensitivity pneumonitis. In: Schwarz MI, King Jr, TJ, editors. Interstitial Lung Disease. Hamilton (ON): BC Decker Publications; 2003. p. 454–484.
23. Lacasse Y, Selman M, Costabel U, et al. Clinical diagnosis of hypersensitivity pneumonitis. Am J Respir Crit Care Med. 2003;168(8):952–958. http://doi.org/10.1164/rccm.200301-137OC
24. Girard M, Lacasse Y, Cormier Y. Hypersensitivity pneumonitis. Allergy. 2009;64:322–334. https://doi.org/10.1111/j.1398-9995.2009.01949.x
25. Dutkiewicz J, Mackiewicz B. Hypersensitivity pneumonitis: correct identification of causative microbial agents is essential for efficient diagnosis and prevention. Am J Respir Crit Care Med. 2023;208(2):213–214. https://doi.org/10.1164/rccm.202211-2182LE
26. Pereira C, Gimenez A, Kuranishi L, et al. Chronic hypersensitivity pneumonitis. J Asthma Allergy. 2016;9:171–181. http://doi.org/10.2147/JAA.S81540
27. Masuo M, Miyazaki Y, Suhara K, et al. Factors associated with positive inhalation provocation test results in subjects suspected of having chronic bird-related hypersensitivity pneumonitis. Respir Investig. 54(6):454–461. http://doi.org/10.1016/j.resinv.2016.05.002
28. Milanowski J, Dutkiewicz J, Potoczna H, et al. Allergic alveolitis among agricultural workers in eastern Poland: A study of twenty cases. Ann Agric Environ Med. 1998;5(1):31–43.
29. Towey JW, Sweany HC, Huron WH. Severe bronchial asthma apparently due to fungus spores found in maple bark. JAMA. 1932;99:453–459. http://doi.org/10.1001/jama.1932.02740580021005
30. Pepys J, Jenkins PA, Festenstein GN, et al. Farmer's lung thermophilic actinomycetes as a source of "farmer's lung hay" antigen. Lancet. 1963;282(7308):607–611. http://doi.org/10.1016/s0140-6736(63)90398-2
31. Van den Bogart HG, Van den Ende G, Van Loon PC, et al. Mushroom worker's lung: serologic reactions to thermophilic actinomycetes present in the air of compost tunnels. Mycopathologia. 1993;122(1):21–28. http://doi.org/10.1007/BF01103705
32. Gomez del Pulgar EM, Saadeddin A. The cellulolytic system of *Thermobifida fusca*. Crit Rev Microbiol. 2014;40(3):236–247. http://doi.org/10.3109/1040841X.2013.776512