Microbial colonization and biodeterioration of plasticized polyvinyl chloride plastics

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Significant substratum damage can occur when microorganisms colonize plasticized polyvinyl chloride (pPVC). We investigated microbial colonization of pPVC in an *in situ*, longitudinal study. Pieces of pPVC containing the plasticizer dioctyl phthalate (DOP) and dioctyl adipinate (DOA) were exposed to the atmosphere for up to 8 years. The fungal and bacterial populations were quantified, and the colonizing fungi were classified on the basis of morphology. Bacteria *Pseudomonas aeroginosa* and yeast-like fungus *Aureobasidium pullalans* were the principal colonizing microorganisms establishing themselves on the pPVC between 30 and 60 weeks of exposure. Yeast *Rhodotorula rubra*, yeast-like fungus *Geotrichum candidum* and fungi, including *Alternaria alternaria*, *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp. and *Ulocladium atrum*, established themselves on the pPVC much later (after 60 weeks of exposure). Negligible bacterial colonization was observed during 54–202 weeks of exposure. The deteriogenic properties of fungi isolated from the pPVC were investigated. All strains of *A. alternata*, *Aureobasidium pullulans, Cladosporium herbarum, Mortierella isabellina and Aspergillus ruber* could grow on PVC powder and DOP or DOA as the sole sources of carbon, and cause weight loss of the substratum during growth on its surface *in vitro*. In contrast, several yeast isolates did not grow on PVC powder agar or degrade plasticizers. They degraded DOA plasticizer only when investigated in association with bacteria and fungus.

Key words: biodeterioration, fungal colonization, plasticized polyvinyl chloride

INTRODUCTION

Deterioration is a natural phenomenon that affects plastic materials due to the effect of humidity, rain, wind, UV irradiation and thermal excursion. Chemical air pollution and microbiological attack can accelerate the degradation process [1, 10, 24]. Problems of substratum damage arise when microorganisms in different environmental situations colonize plasticized polyvinyl chloride. Extensive investigations by Rodriquez [29], Potts, Clendinning and Ackart [26] have shown that the major commercial vinyl plastics, polyethylene, polypropylene, polystyrene, polyvinyl chloride, and aromatic polyesters are particularly resistant to microbial attack. Polyvinyl chloride plastic materials were inspected for the molecular weight distribution in the polymer matrix. No changes were observed in the aged samples, indicating that PVC polymer is not degradable [14, 21]. Plasticizers are organic additives that are added to the PVC polymer in order to make flexible products [4, 24, 25, 32, 33]. A plasticizer is not chemically bound to a polymer and it can exude or be leached from a PVC compound. The predominant plasticizers used for PVC products are phthalic acid esters (phthalates). Under simulated landfill conditions, the behavior of plasticizers in flexible PVC compounds varied considerably [20, 21]. Findings by Eljertson et al. [9] detected that losses of plasticizers increased at elevated temperatures and under aerobic conditions. Plasticized PVC (pPVC) is highly susceptible to microbial attack in many different environmental situations. PVC plastics are used in everything from plumbing pipes and bags, to credit cards and diaper liners. The problems were identified and subsequent reports described defacement and deterioration of commercial pPVC products [4, 12, 35, 38]. Biodeterioration of pPVC is now known to occur in a wide range of industrial, commercial, and structural applications [10, 11, 13]. Both bacteria [3] and fungi [2, 8, 28] can degrade ester-based plasticizers. Plasticizers can be utilized as a carbon source by the yeast-like fungus *Aureobasidium pullulans* [32]. Loss of plasticizers from pPVC due to microbial degradation results in brittleness, shrinkage, and ultimately failure of the pPVC in its intended application. Research has determined biodegradability by measuring changes in the physical properties of

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pPVC, such as changes in tensile strength [37], mass [5], or electrical property [31] during biodegradation.

Colonization processes occurring on pPVC in the environment have received comparatively little attention. Nothing is known about the temporal sequence of microbial colonization of pPVC *in situ*. The existing studies have examined fungal defacement of pPVC in tropical or subtropical climates [12, 30]. In both studies fungal growth was evaluated with a subjective, visual assessment of defacement of the pPVC. The initial adhesion of the deteriogenic fungus *A. pullulans* has been studied *in vitro* by Webb et al. (1999) [32] as part of a long-term study of microbial colonization processes occurring on pPVC.

The aim of this work was to investigate the microbial origin of deterioration of pPVC materials exposed to the environment. The principal objectives of our study were: (i) to investigate colonization processes, (ii) to identify the probable microorganisms present in the altered areas, (iii) to investigate the effect of plasticizers on the deteriorating fungi, and (iiii) to determine if there is a relationship between the microbial colonization sequence observed *in situ* and the ability of microorganisms to cause pPVC biodeterioration in an *in vitro* study.

MATERIALS AND METHODS

Culture media. Fungi and yeast isolated from pPVC exposed to the atmosphere were maintained on malt extract (MEA) (Oxoid, Basingstone, United Kingdom). Bacteria were maintained on bacteriological agar (BA) (Oxoid). The basal mineral salts medium (MSM) used for determining the deteriogenic properties of the organisms contained the following compounds (in grams/litre of distilled H_2O): K_2HPO_4, 7; KH_2PO_4, 3; $MgSO_4 \cdot 7H_2O$, 0.1; and $(NH_4)_2SO_4$, 1. DOP or DOA agar, used for the isolation of organisms able to degrade the plasticizers DOP and DOA, contained MSM supplemented with 2 ml of DOP (or DOA) litre^{-1} and 15 g of bacteriological agar litre^{-1}. For preparation of DOP agar, the medium containing DOP was autoclaved at 121 °C for 15 min and allowed to cool to approximately 50 °C. An emulsion of a plasticizer was then created within the medium using a homogenizer at full power for 2 min (260 W, 25,000 rpm). DOP (or DOA) plates were poured immediately after homogenization.

Plasticized PVC. The investigation was conducted on two polyvinyl chloride (PVC) plastic materials whose main components were:

1. PVC film – polyvinyl chloride and the additives dibutyl phthalate and calcium stearate;
2. PVC belt – polyvinyl chloride and the additives dioctyl adipinate and calcium stearate.

Exposure of pPVC *in situ*. The site for material exposure was arranged in the coastal region of Lithuania with a temperate maritime climate. Three replicate support racks were constructed *in situ*. Each rack was positioned 1.5 m above ground level. Nylon cable ties were inserted through the rope windings to support pPVC pieces at numbered locations on the rack. Each rack supported up to 50 pPVC pieces. The materials were affected by all climatic factors (air humidity, temperature, direct solar radiation and precipitation).

At each sampling time, three replicate pPVC pieces were selected at random, one from each rack. Each piece was cut into three 4.5-by-2.5 cm sections, and a separate analysis was carried out on each section [16].

Viable counts of fungi, bacteria, and DOP- (or DOA)-degrading microorganisms. Fungi were isolated from PVC materials by direct isolation or by washing of PVC samples with appropriate amount of sterile water. pPVC sections (4.5 by 2.5 cm) were placed into 25 ml universal tubes containing 10 ml of sterile distilled H_2O. The tubes were shaken for 3 min. Plasticized PVC samples were transferred to a petri dish containing 5 ml H_2O and scraped heavily three times on both sides using the flat edge of a sterile scalpel blade. The pPVC and H_2O were then returned to the universal tubes and shaken for 1 min. A dilution series to 10^{-3} was prepared from each universal tube. Aliquots of 0.1 ml from each dilution series were spread onto three replicate plates each of malt extract, bacteriological agar, DOP, and DOA agar. Viable counts were performed on MEA plates after 5 days of incubation at 24 °C, on BA after 2 days of incubation at 32 °C, and on plasticizer agar plates after 14 days of incubation at 24 °C. To investigate whether statistically significant changes in CFU counts occurred between sample times, overall mean viable for three replicate pPVC pieces at each time point were compared using analysis of variance.

Enrichment culture and microbial screening. Garden soil (200 g) from a local site near Vilnius was dried and passed through a 16 mesh (1 mm opening) wire screen. Screened soil (50 g) was added to 100 mm Petri dishes to a depth of 10 mm. The dry soil layer was moistened with plasticizer DOP containing MSM medium. The moistened soil plates were then sealed in plastic bags and incubated at room temperature. Sterile distilled water was added as required to compensate for evaporation during the extended incubation period. At 20-day intervals, 1 g of the soil was added to 9 ml of sterile distilled water, mixed vigorously in a vortex mixer and 0.1 ml of this suspension was inoculated onto DOP-MSM agar. Colonies demonstrating the clearing reaction

were picked and transferred to standard media to establish pure cultures, which were subsequently maintained on DOP-MSM slants. Bacteria and yeast contaminating DOP were also isolated in pure cultures.

Identification of fungi isolated from pPVC materials and from soil. For evaluation of fungal growth on plastics, scanning electron microscopy was used. Standard agar media for the isolation and identification of fungi were used.

***In vitro* tests for biodeterioration of pPVC**. Samples of polymer film (25 × 50 mm) were placed on the surface of fresh MSM agar in Petri dishes and sprayed with a spore mixture with a hand atomizer. The inoculated samples were then placed in an incubation room at 28 °C. The extent of fungal growth on the films was observed visually at 1 week intervals and rated on a scale according to ISO 4607 [16] (see footnote, Table 3).

Weighed pPVC pieces (25 × 50 mm) were placed into Petri dishes containing 30 ml of MSM liquid. Three replicate Petri dishes were inoculated with 0.2 ml of spore or yeast suspension from each organism and incubated at 28 °C for 6–10 weeks. Fungal biomass was removed from pPVC pieces by washing, and samples were air-dried at room temperature until a constant weight was reached.

Clear-zone production and colony growth on plasticizer agar. Clear-zone production on agar plates containing emulsified DOP or DOA as the sole carbon source was used to test the ability of fungi to degrade plasticizers. Procedures were essentially the same as those previously described (2, 3, 30). Plates containing 20 ml of DOP or DOA agar were inoculated with 50 μl of spore or yeast suspension placed into 5-mm-diameter wells cut at the center of each plate. Three replicate plates were inoculated for each organism and incubated at 24 °C for 14 days. Clear-zone production on DOP or DOA-agar was scored according to the following criteria: 0, no visible growth; 1, slight growth within inoculation well; 2, colony diameter < 2 cm; 3, colony diameter > 2 cm. Tests for clear-zone production and growth on DOP or DOA agar were replicated on three separate occasions.

RESULTS

During the exposure one of the greatest difficulties encountered in the study of biological deterioration of pPVC was the identification of microorganisms, which first of all requires their isolation in culture. This took time and even gave negative results when a particular microorganism was not grown in the medium it requires. Several strains were not identified because of their association with other molds and the difficulty in obtaining pure cultures. At each sampling time many procedures were made. Viable microorganisms on pPVC determinations were carried out at varied intervals in the course of the trial. Throughout the exposure period, microbial community structure on pPVC samples was monitored. Plasticizer DOP- or DOA-degrading microorganisms were screened throughout the *in situ* trial. After a one-month exposure in the atmosphere all microbial types colonized pPVC samples. Bacteria *Pseudomonas aeruginosa* (Schroeter) Migula, *Actinobacter calcoaceticus* Beijerinck and the yeast-like fungus *Aureobasidium pullulans* (de Bary) Arnaud., common soil isolates, were the primer invaders on the pPVC samples. Fungi were also established on pPVC surface after 4 weeks of exposure (Table 1). A significant increase in fungal viable counts occurred during the next 16 weeks (200 CFU cm^{-2}). After the next 24 weeks the fungal population more than doubled (1,200 CFU cm^{-2}), but by week 56 the population significantly decreased (675 CFU cm^{-2}) and did not change by week 68. During weeks 68–106 the fungal viable count increased to 2.300 ± 120 CFU cm^{-2}, and no significant increase in fungal viable counts occurred during the next 96 weeks. Different fungi contaminated plasticized PVC materials exposed to the environment. We isolated in pure culture 78 fungal species. Only few of fungal species (8 on DOP and 11 on DOA agar) have been implicated in the biodegradation of the plasticizers. A small part of the fungal strains isolated from the surface of pPVC samples grew on plasticizers as a sole source of carbon in MSM medium (Table 1). The percentage of the active fungal strains varied from 3 to 49% and depended on the exposure time.

Plasticized PVC film was more damaged than plasticized PVC belt. PVC film has 35% of plasticizer DOP, while PVC belt has only 5% of plasticizer DOA. Perhaps the biodegradation rate depended on the plasticizer and its concentration in the pPVC material composition. The number of fungi isolated from pPVC film and pPVC belt in this study was different (Figure). Sixty-two fungal species were isolated in pure culture from pPVC film. Twelve species from the 58 identified were frequent on pPVC film (frequency of occurrence >25%). A lower number of fungal species was isolated from the pPVC belt and only 6 species were frequent on it. Webb et al. [32] found that DOA and DOP could increase adhesion of fungi indirectly by stimulating metabolic activity and the synthesis of adhesive cell surface structures. The ability of plasticizers to leach from pPVC into an aqueous environment is also well established [23, 36]. A leached plasticizer could serve as a carbon source and affect colonization and fungal viability on pPVC samples.

Table 1. **Viable counts of fungi isolated from pPVC exposed *in situ* for a period of over 202 weeks and their activity (% of active strains) in degradation of plasticizers dioctyl phthalate (DOP)**
1 lentelė. **Gyvybingų mikromicetų, išskirtų nuo 202 savaites eksponuotų pPVC, skaičius ir jų aktyvumas (aktyvių kamienų %) dioktilftalato (DOF) ir dioktiladipinato (DOA) destrukcijoje**

Sample (weeks)	All fungi on MEA (CFU cm^{-2})[a]	% DOP–degrading fungi[b]	% DOA-degrading fungi[b]
4	110 ± 17	3	5
20	200 ± 29	6	7
44	940 ± 80	11	14
56	1.200 ± 150	18	24
68	675 ± 109	28	31
106	850 ± 230	35	42
154	2.300 ± 120	41	46
202	1.980 ±	44	49
Number of fungal species isolated from pPVC	78	8	11

[a] Mean viable counts ± 1 standard error of the mean from three replicate pPVC pieces, one taken from each *in situ* rack, are shown. [b] Numbers of fungi recovered on DOP- or DOA-MSM agar expressed as a percentage of all fungi recovered on MEA.

The bacteria *Pseudomonas aeroginosa* and the yeast-like fungus *Aureobasidium pullulans* were the principal pPVC colonizing microorganisms after a 12-week exposure to the environment. After a 20-week exposure the viable count of bacteria was 1.500 CFU cm^{-2} and increased to 2.400 CFU cm^{-2} after 44-weeks of exposure (Table 2). During this exposure period fungal spoilage of pPVC materials was also large (Table 1). The percentage of the active bacterial strains in the microbial community on pPVC increased during exposure, but their total count decreased to 8 CFU cm^{-2} after a 154-week exposure. Bacteria colonizing pPVC exposed to the environment and able to utilize plasticizer DOP on DOP-MSM medium were identified as *Pseudomonas aeroginosa.* They were strongly related to the yeast population, which was also dominant on the pPVC samples after a 68-week exposure and was identified as *Rhodotorula rubra* (Demme) Lodder. Throughout the exposure period we isolated in pure culture 8 morphologically different bacteria. Only 3 of them grew on DOP-MSM agar (Table 2) *in situ* trials. Two bacteria were not identified to species. A low concentration of the viable bacteria (8–23 CFU cm^{-2}) was detected on pPVC after a 68-week exposure. Their concentration on pPVC samples did not change from 68 to 202 weeks of exposure. Bacterial growth could be inhibited by desiccation, solar irradiation, or acidification of pPVC. Acidification of pPVC could inhibit bacterial growth. Fungi tolerate lower pHs than bacteria [15]. After 8 years of exposure *P. aeroginosa* was not isolated from pPVC in this study, suggesting that environmental factors limited the establishment of these organisms on the pPVC.

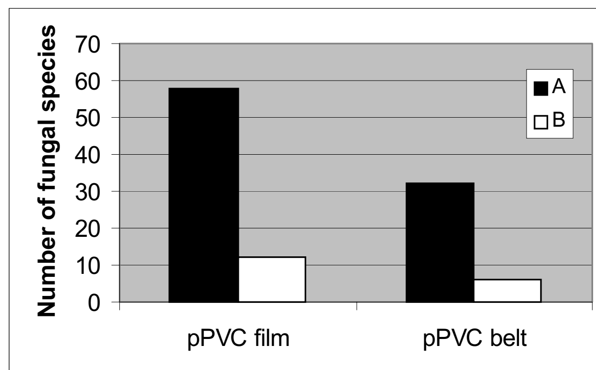

Figure. Total number of fungal species (A) isolated from different pPVC (film and belt) and number of fungal species (B) most frequently observed (frequency of occurrence > 25%)
Pav. Nuo skirtingų pPVC (plėvelė ir juosta) medžiagų išskirtų mikromicetų rūšių bendras (A) ir dažniau aptinkamų (dažnis > 25%) (B) skaičius

Most yeast and yeast-like fungi became established on the pPVC much later than *P. aeroginosa, A. pullulans* and some fungi such as *Aspergillus ruber* Thom et Church and *Ulocladium atrum* Preuss (after 26–30 weeks of exposure). Therefor, these microorganisms probably play a secondary role in the colonization of pPVC. Yeast are not generally considered as important deteriogenic organisms on artificial surfaces [18]. Counts of viable yeast on pPVC in our study slightly increased from 22 to 490 CFU cm^{-2} after a 106-week exposure and then decreased to 280 CFU cm^{-2} after 202 weeks of exposure (Table 2). The most common yeast on pPVC in this study was identified as *Rhodotorula rubra.* It was the only yeast detected on pPVC after 202 weeks of exposure and the only yeast able to grow on DOP-MSM agar. The present study suggested that high *Rhodotorula rubra* counts resulted from a small number of rapidly multiplying yeast colonies. A few other yeast strains degraded DOP very weakly. *Rhodotorula rubra* was more active in association with *Pseudomonas aeroginosa* and *Aspergillus puniceus* Kwon-Chung et Fennell [25].

Table 2. **Viable counts of bacteria and yeast isolated from pPVC exposed *in situ* for a period of over 202 weeks and their activity (% of active strains) in degradation of plasticizers dioctyl phthalate (DOP) and dioctyl adipinate (DOA)**
2 lentelė. **Bakterijų ir mielių, išskirtų nuo 202 savaites eksponuotų pPVC, skaičius ir jų aktyvumas (aktyvių kamienų %) dioktilftalato (DOF) destrukcijoje.**

Sample (weeks)	All bacteria on BA (CFU cm^{-2})[a]	All yeast on MEA (CFU cm^{-2})[a]	% DOP–degrading bacteria[b]	% DOP-degrading yeast[b]
4	94 ± 26	25 ± 5.2	56	2
20	1.500 ± 480	28 ± 3.4	68	1
44	2.400 ± 320	80 ± 12.6	77	8
56	1.110 ± 290	115 ± 16.2	84	16
68	64 ± 15	269 ± 27.5	92	23
106	23 ± 5	491 ± 24.9	88	20
154	8 ± 3	350 ± 67.6	95	9
202	10 ± 4	280 ± 54.7	96	4
Number of morphologically distinct colony types of bacteria and yeast	6	8	2	1

[a] Mean viable counts ± 1 standard error of the mean from three replicate pPVC pieces, one taken from each in situ rack, are shown. [b] Numbers of bacteria or yeast recovered on DOP or DOA agar expressed as a percentage of all bacteria or yeast recovered on MEA.

From the soil we have screened and isolated in pure culture 9 fungal species that degrade plasticizers DOP and DOA and were able to exert a clearing effect on PVC powder agar (Table 3). The filamentous fungi isolated from the soil included representatives of the genera *Alternaria* Nees ex Fr., *Aspergillus* Mich. ex Fr., *Cladosporium* Link ex Fr., *Emericella* Berk & Br., *Paecilomyces* Bain., *Penicillium* Link ex Fr. and *Ulocladium* Preuss. Yeast and bacteria were also screened from the soil as able to utilize pPVC components. The primary source of contaminating microorganisms is airborne dust. Species of *Penicillium, Aspergillus, Aureobasidium* and *Paecilomyces* Viala & Boyer have been isolated as the prevalent fungi causing the spoilage and damage of pPVC.

Fungal and bacterial strains more active on DOP-MSM agar were investigated to compare their activity on various media. It was found that certain bacteria and fungi were able to grow and form clear zones around their growing colonies when inoculated onto agar with PVC powder (at a concentration of 2%). *Pseudononas aeruginosa* and yeast *Rhodotorula rubra* were shown to produce clearing zones in PVC powder agar (Table 3). Of 24 fungi tested, 11 gave a positive clearing effect when tested in the same way. Fungi *Mortierella isabellina* (Oud.) Zycha, *Paecilomyces varioti* Bain., *Penicillium expansum* Link ex Gray and *Geotrichum candidum* Link colonized pPVC in the environment but showed no clearing effect on PVC powder agar. They also did not grow on the DOP agar. Their viability on pPVC exposed to the environment perhaps was due to metabolites of the other microorganisms. Fungi *Aspergillus ruber, Cladosporium* spp., *Aureobasidium pullulans, Phoma* sp. and bacteria *P. aeroginosa* exerted a positive clearing effect on PVC powder agar and grew well on DOP-MSM agar. These fungal species were dominant on pPVC samples exposed to the atmosphere. They were also isolated during enriched culture and microbial screening in the soil and showed the same clearing and activity effects in PVC powder and DOP agar (Table 3). Fungi *Alternaria alternata* (Fr.) Keissel. and *Aspergillus niger* Tiegh. grew on PVC powder agar, but did not exhibit a clearing effect. Agar persisting in the PVC powder agar medium perhaps was a sufficient carbon source for the colony growth of these fungi. *Alternaria alternata* and *Aspergillus niger* colonized pPVC film in the environment and an *in situ* experiment. The success of these fungi in colonizing pPVC *in situ* is probably due to their ability utilize a plasticizer or exogenous carbon sources that accumulate on the pPVC during long periods of exposure *in situ*.

Plasticized PVC film samples placed on MSM agar and inoculated with different microorganisms were incubated for 4 weeks at 28 °C. pPVC samples after incubation were visually examined for evidence of microbial growth. In Table 3 samples of pPVC film are rated for microbial growth according to the scale as shown in the footnote of Table 3. After 4 weeks of exposure, the highly degraded pPVC samples were completely covered with fungi *Aspergillus ruber*, *A. niger, Aureobasidium pullulans,*

Table 3. **Deteriogenic properties of microorganisms isolated from the pPVC materials exposed to the environment and screened in the soil emended with plasticizer dioctyl phthalate (DOP)**
3 lentelė. **Nuo gamtinėmis sąlygomis eksponuotų pPVC medžiagų išskirtų ir iš plastifikatoriumi dioktilftalatu (DOF) praturtinto dirvožemio atrinktų mikroorganizmų destrukcinės savybės**

Microorganisms (*)	Percent weight loss of pPVC	Growth on pPVC film in agar culture[a]	Growth in PVC powder agar[b]	Clearing on PVC powder agar[c]	Growth on DOP agar[d]
**Alternaria alternata* (Fr.) Keissl.	1.2 ± 0.1	1	11.8 ± 3.5	0	1
Alternaria alternata	1.4 ± 0.3	1	16.7 ± 2.5	0	2
**Aspergillus niger* Tiegh.	0.8 ± 0.1	3	12.3 ± 1.2	0	1
Aspergillus niger	0.7 ± 0.2	2	16.5 ± 2.0	0	1
**Aspergillus ruber* Thom & Church	3.4 ± 0.6	3	22.4 ± 2.6	17.8	3
Aspergillus ruber	3.9 ± 0.5	3	18.1 ± 2.5	16.3	3
**Aureobasidium pullulans* (de Bary) Arnaud	4.9 ± 0.7	3	10.6 ± 1.9	11.2	3
Aureobasidium pullulans	3.2 ± 0.5	3	8.5 ± 0.5	10.4	3
**Cladosporium herbarum* (Pers.) Link ex Gray	0.5 ± .05	1	2.3 ± 0.1	2.0	1
**Cladosporium cladosporioides* (Fres.)	0.6 ± 0.1	0	1.6 ± 0.3	2.1	1
Cladosporium cladosporioides	1.1 ± 0.3	1	2.4 ± 0.2	2.4	1
**Emericella nidulans* (Eidam) Viull.	0	1	5.6 ± 0.5	5.0	0
Emericella nidulans	0	1	6.3± 0.4	5.6	0
**Mortierella isabellina* (Oud.) Zycha	0	0	21.3 ± 2.7	0	0
**Paecilomyces varioti* Bainier	1.2 ± 0.2	1	28.7 ± 5.2	0	1
Pseudomonas aeruginosa (Schroeter)	1.1 ± 0.4	0	2.3 ± 0.2	1.6	3
Migula					2
**Rhodotorula rubra* (Demme) Lodder	0.1 ± 0.4	2	0.6 ± 0.2	0	2
Rhodotorula rubra	0.6 ± 0.7	2	1.8 ± 0.3	0	1
**Phoma* sp.	2.0 ± 0.5	3	16.9 ± 2.7	17.6	0
**Geotrichum candidum* Link.	0	1	14.7 ± 3.8	0	0
Geotrichum candidum	0	1	19.4 ± 2.5	0	0
**Penicillium expansum* Link ex Gray	0.4 ± 0.1	2	5.7 ± 0.8	0	0
Ulocladium atrum Preuss	3.7 ± 0.6	3	8.8 ± 1.2	8.0	0
**Ulocladium atrum*	4.2 ± 0.5	3	6.2 ± 0.9	5.8	

* – microorganisms isolated from the pPVC exposed to the environment, without * – microorganisms isolated from the soil.
Test methods used were measurement of growth on PVC surface, clear zone production and growth on PVC powder agar, growth on DOP agar. [a] Scores for growth on PVC surface: 0, no visible growth; 1, slight growth, barely visible; 2, growth clearly visible around the edges of the PVC; 3, strong growth visible around the edges and in the agar above the pPVC. [b] Colony diameter (mm) after 21 days of cultivation. [c] Clear zone diameter (mm) after 21 days of cultivation. [d] Scores for growth on DOP agar: 0, no visible growth; 1, slight growth within inoculation well; 2, colony diameter $<$ 1 cm; 3, colony diameter $>$ 1 cm.

Ulocladium atrum and *Rhodotorula rubra.* Only several isolated fungal colonies were observed on some pPVC samples inoculated with *Alternaria alternata, Geotrichum candidum* and *Emericella nidulans* (Eidam) Vuill. No microbial growth was observed on pPVC in Petri dishes inoculated with *Cladosporium cladosporioides* (Fres.), *Mortierella isabellina* and *Pseudomonas aeroginosa*.

Microorganisms isolated from the pPVC and from the soil were grown on pPVC film. The ability of *in situ* isolates to cause weight loss from pPVC was determined after incubation with pPVC for 8 weeks in a sterile MSM medium. In Table 3, percent weight losses of the washed and dried film samples are listed. Such weight change was used as an indication of degradation, because Wendt, Kaplan and Greenberg [34] had previously demonstrated that weight loss is a reliable and sensitive quantitative measure of decomposition extent. The net weight loss in all cases was very low (Table 3). The greatest weight reduction (4.9%) was caused by *Aureobasidium pullulans*. Fungi *Aspergillus ruber* and *Ulocladium atrum* demonstrated 3.4–3.9% and 3.7–4.2% weight losses of pPVC film, respectively. *Geotrichum candidum* and *Emericella nidulans* did not grow in the liquid MSM medium with pPVC film as sole carbon source. Very low pPVC film weight losses were detected when inoculated with *Aspergillus ni-*

ger, Cladosporium cladosporioides and *Rhodotorula rubra*.

PVC film and belt samples exposed to the environment were periodically observed under light and scanning electron microscope. Irregular fungal black and fox spots were noticeable on PVC samples exposed to the environment. At low magnification the surface of the pPVC specimens appeared as a rough layer of granular material (pigment). Immediately below this layer, large cavities of about 0.1 mm in diameter were observed. Their side walls were covered by mycelium of fungi and formation of conidial chains. At higher magnification, the fungal ultrastructure as well as some bacilli were clearly visible (arrow). Yeast-like cells occurred occasionally either singly or in clumps of two-three cells, both associated with fungal colonies and on uncolonized areas of the plastic. The specimens also showed the presence of numerous fungal spores of different morphology. The spot usually contained a large number of conidia and some conidiophores. Almost all spots contained combinations of two-three fungal types.

DISCUSSION

The range of materials suffering from biotic attack is very wide. Alexander [1] has contributed much to the appreciation of microbiological degradation of chemical compounds degraded by microorganisms. He stated that material must be considered together with its temporary environment. Some long-term studies of PVC in nature were conducted, during which PVC samples were inspected for molecular weight distribution in the polymer matrix [14, 32]. No changes were observed in the aged samples, indicating that PVC polymer was not degradable. Whilst the microorganisms are generally unable to degrade PVC as showed extensive investigations by Rodriquez [29] and by Potts, Clendinning and Ackart [26], they can utilize additives from plasticized PVC [32, 33]. Plasticized PVC materials in our study were exposed to the environment and were affected by all climatic factors (air humidity, temperature, direct solar radiation and precipitation). Breaking of pPVC film was observed only after three years of exposure. A large number of microbial species contaminated pPVC. The results suggested that plasticizers provide a suitable medium for the growth of some microorganisms. DOP- and DOA-MSM agar was useful in selecting strains capable of utilizing plasticizers as a source of carbon. In this study, pPVC film containing higher concentrations of plasticizer (35%) was more intensively colonized by microorganisms than pPVC belt containing lower plasticizer concentrations (3.4%). Microbial contamination was observed from the first 4 weeks of exposure. Fungi, bacteria and yeasts were established on the pPVC samples. Bacteria *Pseudomonas aeroginosa* and the yeast-like fungus *Aureobasidium pullulans* were detected after a 4-week exposure and dominated in microbial population. Fungi were prevalent in the later period and dominated on pPVC after a 56-week exposure. The success of some fungi in colonizing pPVC *in situ* is probably due to a combination of several factors. The most frequently isolated ones were, in decreasing order, *Aureobasidium pullulans, Aspergillus ruber, Ulocladium atrum* and *Paecilomyces varioti*. The genus *Aureobasidium* was detected in almost all the pPVC areas sampled. *A. pullulans,* which usually colonizes the phyloplane [7], and *U. atrum* can withstand periods of desiccation and high temperatures and produce highly melanized hyphae that protect against UV exposure [6]. *Aureobasiodium pullulans* also produces extracellular polysaccharides that may facilitate permanent adhesion to the surface. Microbial adhesion is the first in a series of events that occur in the colonization of a solid substratum [4, 10, 33]. Adhesion to the materials such as plastics or glass is caused by nonspecific interactions between the cell surface and the substratum [32]. Some research has focused on bacterial adhesion [32]. Little was known about fungal adhesion to plastic materials. Most fungal adhesion studies have focused on adhesion of the opportunistic pathogen *Candida albicans* to synthetic materials used for medical prostheses. In our earlier study [19], the adhesion rate of some fungal conidia was investigated on pPVC film and belt. *Aspergillus niger* and *Cladosporium herbarum* were found to adhere to pPVC more intensively than *Ulocladium atrum* or *Trichoderma viride*. Bacteria *Pseudomonas aeroginosa* and the yeast-like fungus *Aureobasidium pullulans* colonized pPVC during the first weeks of exposure. Nothing is known about the physiochemical factors controlling adhesion of fungi that colonize and deteriorate plastics within the environment. Fungi have considerable enzymatic capabilities. Webb et al. [33] detected high levels of the extracellular esterase, which was produced by fungi degrading pPVC plastic materials. Extracellular esterase production is hypothesized to aid in the colonization of pPVC through the hydrolysis of organic-ester plasticizers [2, 17, 22]. However, in this study extracellular esterase production was not investigated. Some fungi such as *Mortierella isabellina, Emericella nidulans* and *Trichoderma lignorum* became established on the pPVC much later than *A. pullulans, Aspergillus ruber, Paecilomyces varioti* and *Ulocladium atrum*. Therefore these fungi probably play a secondary role in the colonization of pPVC. They require metabolites of the other fungi or degrade bacterial cells whose number on pPVC samples decreases with exposure.

Bacterial concentration decreased after 56 weeks of exposure and was very low all the rest exposure period. Bacterial growth might be inhibited by desiccation, solar irradiation, or *in situ* acidification of the pPVC following phthalate degradation to phthalic acid or due to extracellular organic acid production by fungi. The present study suggested that high bacterial and yeast counts resulted from a small number of rapidly multiplying bacteria colonies. The CFU counts depended on how readily the microbial material on the pPVC breaks into individual propagules during the isolation procedure. For example, for the same amount of biomass, a colony of budding yeast *Rhodotorula rubra*, or filamentous *Geotrichum candidum* may yield much more CFUs than a spreading hyphal mycelium. Evidence supporting this hypothesis comes from the observation that only 2–7.6% of bacteria and yeast colonizing pPVC could grow on DOP agar. *Ulocladium atrum* grew well on pPVC and PVC powder agar, but showed only a faint clearing below the colony. This fungus could not grow and produce clear zones in DOP agar. Only a small part of the isolated fungi grew on DOP and DOA agar. It is possible that components of the pPVC formulation other than the plasticizers can support the growth of fungi on pPVC. Only one yeast species, *Rhodotorula rubra*, grew on pPVC and DOP agar. *Geotrichum candidum* did not grow on pPVC and DOP agar, but was common on pPVC samples in nature. It requires additional nutrients, *e.g.*, metabolites of other fungi. The same fungal species were isolated from the pPVC specimens and screened from the soil. Data presented in Table 3 show that the activity on pPVC additive-containing media did not differ among the fungi isolated from the surface of pPVC and from the soil.

Polyvinyl chloride today comes largely from petroleum-based feedstocks and has been manufactured at a relatively low cost [27]. However, the cost of these plastic materials increased significantly because they do not break down to any extent in the environment and must be recycled. Results of our investigation show that soil microorganisms can utilize pPVC plastic material additives, but they have difficulty using these materials in their metabolism. PVC plastic materials can be degraded in soil, but these processes are slow. Screened microorganisms can be used to accelerate PVC leaching and substrate transport processes.

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PLASTIFIKUOTŲ POLIVINILCHLORIDINIŲ MEDŽIAGŲ PAŽEIDIMAI AUGANT MIKROMICETAMS

S a n t r a u k a

Plastifikuotų polivinilchloridinių (pPVC) medžiagų struktūra pakinta, kai ant jų paviršiaus formuojasi augančių mikroorganizmų plėvelė. Tyrėme mikroorganizmų kolonijų formavimąsi ant pPVC pavyzdžių paviršiaus ilgalaikės ekspozicijos gamtinėmis sąlygomis metu. Plastifikuotų dioktilftalatu (DOF) ir dibutiladipinatu (DOA) polivinilchloridines medžiagas 8 metus eksponavome gamtinėmis sąlygomis. Tyrėme bakterijų, mielių ir mikromicetų populiacijas. Pagal morfologines savybes nustatėme augančių ir vyraujančių ant šių medžiagų mikroorganizmų rūšis. Pirmieji mikroorganizmai, formavę kolonijas ant pPVC pavyzdžių per pirmąsias 30–60 ekspozicijos savaites, buvo bakterija *Pseudomonas aeroginosa* ir mieliagrybis *Aureobasidium pullulans*. Kitų mikroorganizmų: mielių *Rhodotorulla rubra*, mieliagrybio *Geotrichum candidum* ir mikromicetų *Alternaria alternata*, *Cladosporium* spp., *Aspergillus* spp., *Penicillium* spp. ir *Ulocladium atrum* vystymasis ant pPVC medžiagų buvo nustatytas vėliau, po 60 eksponavimo savaičių. Po 50–60 savaičių gyvybingų bakterijų koncentracija ant eksponuojamų pPVC medžiagų buvo labai maža. Laboratorinėmis sąlygomis tyrėme išskirtų nuo pPVC medžiagų mikromicetų destrukcines savybes. Visi *Alternaria alternata, Aereobasidium pullulans, Cladosporium herbarum, Mortierella isabellina* ir *Aspergillus ruber* rūšių kamienai augo ant pPVC komponentų (PVC bei plastifikatorių) ir sumažino pPVC plėvelės, esančios vieninteliu anglies šaltiniu mikroorganizmams skystoje terpėje, svorį. Mielės grynoje kultūroje neaugo terpėse, kuriose vienintelis anglies šalinis buvo PVC milteliai arba plastifikatorius. Plastifikatorių (DOF) mielės utilizavo tik augdamos bakterijos, mielės ir mikromiceto komplekse.

Raktažodžiai: grybelių kolonizacija, plastifikuotos polivinilchloridinės medžiagos