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Growth Characteristics of Selected Fungi on Polyvinyl Chloride Film

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The objective of this study was to determine if plasticized polyvinyl chloride film would support the growth of any of nine species of fungi. The film was suspended in distilled water with no nutrients or with glucose or ammonium sulfate. Spores of each of the test species were inoculated into the suspension medium, and the mixture was incubated at 30°C for up to 18 weeks. Most species were found to be capable of utilizing the film for carbon or nitrogen when the other nutrient was supplied. Only two species, *Aspergillus fischeri* and *Paecilomyces* sp., were found to be capable of utilizing components of the film without added nutrients. Components of the polyvinyl chloride film were then incorporated into mineral salts medium to determine if these components could serve as carbon sources in the presence of ammonium nitrate. The only component found to be utilized by all the fungi as a carbon source was epoxidized oil, a plasticizer-stabilizer. Calcium-zinc stearate was an available carbon source for all except the *Penicillium* and *Verticillium* strains. The only other component utilized was a stearamide, which was metabolized solely by the *Aspergillus* sp. Only the stearamide contained enough nitrogen to serve as a primary source in the film. The compound, however, did not support growth of fungi in the presence of glucose. It was theorized that either the nitrogen of the stearamide was more readily available to the fungi in the whole film due to the presence of trace nutrients or the nitrogen was supplied by exogenous sources.

At least two conditions must be met before a mold species can utilize the components of and degrade a plastic film. (i) The mold must be on the film as a contaminant. Studies by Baribo et al. (1) and Sinell (18) with bacteria have shown that microorganisms on films number 1/1,000 to 20/1,000 cm^2 and that films are contaminated by these microorganisms during manufacture due to electrostatic charges.

(ii) The microorganism must be able to utilize components in or on polyvinyl chloride (PVC) film as nutrients. Studies have shown that plasticizers used to improve the flexibility of plastic films tend to be the major metabolites of contaminating microorganisms (13). For example, Brown (7) reports that plasticizers such as adipates, laurates, oleates, ricinoleates, sebacates, vegetable oils, and other fatty acid derivatives tend to be susceptible to attack, while phthalic and phosphoric acid derivatives have been found not to support fungal growth. Stahl and Pessin (19) have also found that the sebacates (with the exception of dimethyl sebacate) and ricinoleates support the growth of *Aspergillus versicolor* in shake-flask culture, while citrates, aconitates, phosphates, and phthalates are not utilized. Berk et al. (4) have confirmed previous findings as to susceptibility of these individual classes of plasticizers by testing the ability of 24 species of fungi to utilize 127 compounds as sole carbon sources. Other components of the film may also support the growth of fungi (8, 20). Aside from the PVC film components, environmental conditions such as temperature and moisture must be appropriate to allow growth of the fungi (5, 15).

Fungal growth on PVC film may have several detrimental consequences. For example, removal of the plasticizer has been shown to cause plastic film to become brittle, increase in tensile strength, and decrease in elongation (2). Berk (3) has reported that a PVC film which supported the growth of a *Trichoderma* sp. on mineral salts agar (MSA) had a 63% increase in tensile strength and a 67% loss in elongation when compared with an uninoculated control. The author concludes that the removal of the plasticizer, dibutyl sebacate, by the fungus contributes to the increase in tensile strength. Booth and Robb (6) show that PVC film plasticized with diisooctyl esters of adipic, sebacic, and azelaic acids loses weight and becomes stiff upon exposure to *Pseudomonas* spp. and *Brevibacterium* spp. in soil.

Fungal growth on a PVC film may also result in possible penetration of a manufactured package (11, 16) or production of toxic end products which could contaminate the packaged contents. Staining of the packaging material has also been shown to be a problem (9, 21). First reports of discoloration were made by Girard and Koda (10), who identified a *Penicillium* species which produces a pinkish-red pigment on films. Yeager (21) has shown that the microorganism *Streptomyces rubrireticuli* causes pink discoloration of vinyls. The discoloration results from the production of two prodiginine pigments (9). Tirpak (20) later found species of *Penicillium* and *Fusarium* which stain vinyl films orange and yellow.

The objectives of the present study were (i) to survey nine species of fungi for their growth capabilities on a PVC film with and without exogenous nutrients and (ii) to determine what components served as nutrient sources if the fungi were able to grow on the film.

MATERIALS AND METHODS

Fungi. *Aspergillus fischeri*, *Aspergillus niger*, *Cladosporium* sp., *Fusarium* sp., *Paecilomyces* sp., *Penicillium* sp., *Penicillium citrinum*, *Trichoderma* sp., and a *Verticillium* sp. were provided by Travenol Laboratories, Morton Grove, Ill. All of the fungal species were originally isolated as contaminants on PVC film. The cultures were received on potato dextrose agar (PDA) plates except for *A. fischeri* and the *Penicillium* sp., which were provided as spores. Stock cultures were stored at 4°C.

Preparation of spore suspensions. The inoculum was pre-

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pared by growing the fungi in glucose-salts-amino acids broth (17) at 30°C for 5 days, vortexing mycelial mats, and transferring each suspension to PDA plates. Plates were incubated at 30°C until sporulation occurred as determined by microscopic examination. Spore suspensions of each organism were prepared by adding 10 to 15 ml of aqueous 0.005% Triton X-100 to each plate and by loosening the spores with a glass rod. The spore suspensions were filtered through sterile nylon filters (25- to 54-nm pore size) and centrifuged at 12,000 × *g* for 8 min; the supernatant was decanted, and the spores were resuspended in sterile 0.005% Triton X-100. This process was continued until microscopic examination revealed a clean spore crop free of vegetative debris. Clean spores were suspended in 0.9% saline and checked for purity by streaking Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Purified spore suspensions were adjusted to approximately 10^6 spores per ml with a microscopic counting chamber (Hauser Scientific, Blue Bell, Pa.) and then were stored at 4°C.

PVC film. Plasticized PVC film was provided by Travenol Laboratories as 0.318-mm-thick sheets in bundles within a plastic wrapper. In addition to the PVC resin, the film contained dioctyl phthalate (plasticizer), epoxidized oil (plasticizer-stabilizer), calcium-zinc stearate (heat stabilizer), and a stearamide (lubricant). The film was stored at room temperature and was not treated with a cleaning solution before analysis.

Growth of fungi on PVC film. Plasticized PVC film was cut aseptically into strips (approximately 15 by 80 mm). Film to be heat treated was layered onto Whatman no. 4 filter paper with sterile forceps, covered with foil, and autoclaved at 121°C for 18 min. The purpose of the heat treatment was to determine if heat increased the susceptibility of the film to fungal attack. The heated and unheated PVC films were aseptically transferred to sterile culture tubes that had been twice cleaned in a strong base solution (200 g of KOH, 50 g of Na_2HPO_4, 5 g of Na_2H_2-EDTA, 1 liter of distilled water) and a strong acid (3 N HCl) to ensure that no residues were present (12).

To the culture tubes were added 9.9 ml of sterile 5% (wt/vol) glucose as the sole carbon source, 1% (wt/vol) ammonium sulfate as a sole nitrogen source, or distilled water containing no added nutrients. Mold spores were inoculated into the tubes to obtain a final level of 10^4 spores per tube. Controls consisted of inoculated tubes without PVC film.

Tubes were incubated 18 weeks at 30°C to obtain sufficient growth. Mold growth was evaluated every 2 weeks with the following approximate scale: 0, no growth; 10, very slight growth (<25% of film covered); 20, slight growth (25 to 50% of film covered); 30, moderate growth (50 to 75% of film covered); 40, abundant growth (>75% of film covered). Growth in controls was evaluated by comparison with the test samples containing PVC.

PVC component utilization. Mineral salts agar was used as the basal medium for determining the ability of the fungi to utilize plasticizers and related organic compounds as carbon and nitrogen sources. The medium as reported by Berk et al. (4) consisted of 1.0 g of NH_4NO_3, 0.005 g of NaCl, 0.002 g of $FeSO_4 \cdot 7H_2O$, 0.002 g of $ZnSO_4 \cdot 7H_2O$, 0.7 g of K_2HPO_4, 0.7 g of KH_2PO_4, 0.7 g of $MgSO_4 \cdot 7H_2O$, 0.001 g of $MnSO_4 \cdot 7H_2O$, and 15 g of agar (Difco Laboratories, Detroit, Mich.) per liter of deionized water. Each film ingredient was incorporated into the MSA at a level of 0.5% to determine if fungi could utilize the compound as a carbon source. A nonionic silicone surfactant (Antifoam B; Fisher Scientific, Fair Lawn, N.J.) was incorporated at a level of 0.1% to obtain a homogeneous dispersion of the film components in the MSA.

The epoxidized oil (plasticizer-stabilizer) and stearamide (lubricant) were the only film components found to contain nitrogen with the Kjeldahl nitrogen method (12). Therefore, for possible utilization as a nitrogen source, the ammonium nitrate in the MSA was replaced with 0.5% epoxidized oil or 0.5% stearamide, while 0.5% glucose was used as a carbon source. The MSA containing the appropriate film ingredient was steamed and autoclaved for 18 min at 121°C.

Portions (approximately 30 ml) of dispersed medium (pH 6.2) were poured into 9-cm sterile petri plates and allowed to solidify at room temperature. Solidified agar plates were inoculated with a 3-mm loop in the center of the plates with a suspension containing 10^6 spores per ml. Controls consisted of inoculated PDA and MSA without the film ingredients. Plates were incubated in an inverted position for 28 days at 30°C. Results were reported as diameters of colony growth in millimeters.

Statistical analysis. In the tube study, fungal growth in the controls was subtracted from growth on the test film to determine possible utilization of the film as a nutrient source. Data were subjected to analysis of variance to detect significant differences in main effects and interactions, and if differences existed, sample means were separated by Duncan's multiple range test ($\alpha = 0.05$). Both growth studies were done in duplicate, and results were averaged.

RESULTS AND DISCUSSION

Growth of fungi on PVC film. Most fungal species tested grew when exogenously supplied with either glucose or ammonium sulfate (Table 1). Since no differences were found between the heated (121°C for 18 min) and unheated films ($P > 0.05$), the results were combined. In the presence of glucose, *A. fischeri*, *A. niger*, and *Paecilomyces* sp. all grew rapidly to cover nearly the entire surface of the piece of test film in the culture tube within 12 weeks of incubation at 30°C. *Fusarium* sp. was also found to grow well, covering >75% of the test film in 18 weeks at 30°C. *Cladosporium* sp., *P. citrinum*, *Penicillium* sp., and *Trichoderma* sp. grew slower, covering approximately 25 to 75% of the film surface in the 18-week incubation period. *Verticillium* sp. was found not to grow on the film in the presence of glucose.

When ammonium sulfate was present as a nitrogen source, *A. fischeri* and *Fusarium* sp. were the only two species capable of maximum growth on the film (>75% coverage) in 12 weeks (Table 1). *A. niger* was also efficient in utilizing the film components for growth, reaching >75% coverage in 18 weeks. *Cladosporium* sp., *Paecilomyces* sp., *Penicillium* sp., and *P. citrinum* were capable of 50 to 75% coverage of the film in 18 weeks at 30°C (Table 1). Again, *Verticillium* sp. did not show any growth.

In contrast to the results with exogenously supplied nutrients, only *A. fischeri* and *Paecilomyces* sp. grew on the film when no nutrients were supplied (Table 1). Therefore, these species were the only ones utilizing film components for both carbon and nitrogen sources.

In addition to mycelium production, *A. fischeri* and *Fusarium* sp. in the presence of glucose stained the film yellow and purple, respectively. Upon microscopic examination of the film, no spores were found in direct association with the stain. Therefore, the pigments evidently were soluble in the plasticizer, resulting in discoloration (8, 14). The production of pigments was dependent upon glucose, since no staining took place in the other treatments. Other researchers have

also found that the production of pigments by microorganisms is dependent upon the environmental conditions during growth (9, 10, 21).

Slight growth was observed for most fungi in the 5% glucose control tubes (Table 1). A majority of this growth was observed within the first 2 weeks of the study. The growth was attributed to small amounts of nitrogen dissolved in the distilled water used to suspend the film.

Growth of fungi on PVC film components. This study was done to determine the ability of the fungi to utilize the components of the PVC film as possible carbon sources. All of the fungal species grew on PDA, which served as the viability control (Table 2). MSA containing 0.1% $(NH_4)NO_3$ as the sole nitrogen source was used as the negative control and was found not to support fungal growth. With epoxidized oil as a carbon source, all of the fungal species showed growth within 2 weeks of incubation. Three species, *A. fischeri*, *Fusarium* sp., and *Trichoderma* sp., grew to the maximum 90 mm within the 2-week incubation time. After 4 weeks, *Paecilomyces* sp. and *P. citrinum* had also reached 90 mm in diameter. *Verticillium* sp. actually grew to a greater extent on the MSA plus epoxidized oil than it did on the PDA. These results indicated that all the species were able to efficiently utilize the epoxidized oil as a carbon source.

With the exception of *Penicillium* sp., *P. citrinum*, and *Verticillium* sp., all of the organisms were also able to utilize the calcium-zinc stearate as a carbon source within the 4-week incubation period (Table 2). Again, *A. fischeri*, *Fusarium* sp., *Paecilomyces* sp., and *Trichoderma* sp. grew to the maximum 90 mm in 2 weeks. The three species which could not grow with the stearate were apparently not able to adapt enzymatically and utilize that component for carbon even after 28 days of incubation.

Only *A. fischeri* and *A. niger* were able to utilize the

TABLE 1. Mean growth level of fungi on PVC film in culture tubes[a]

Organism and time postinoculation (wk)	Growth level[b] in:					
	5% Glucose		1% Ammonium sulfate		No nutrient	
	Film	Control	Film	Control	Film	Control
A. fischeri						
6	30	15	20	10	10	0
12	40	20	40	10	30	0
18	40	20	40	10	30	0
A. niger						
6	27	10	20	0	0	0
12	40	10	35	0	0	0
18	40	20	40	0	0	0
Cladosporium sp.						
6	30	10	30	0	0	0
12	30	10	30	0	0	0
18	30	10	30	0	0	0
Fusarium sp.						
6	30	10	40	0	0	0
12	30	10	40	0	0	0
18	40	10	40	0	0	0
Paecilomyces sp.						
6	25	10	17	10	0	0
12	40	20	30	10	15	0
18	40	20	30	10	17	0
Penicillium sp.						
6	20	10	10	0	0	0
12	30	10	27	0	0	0
18	30	10	30	0	0	0
P. citrinum						
6	20	10	20	0	0	0
12	20	10	30	0	0	0
18	20	10	30	0	0	0
Trichoderma sp.						
6	7	10	0	0	0	0
12	10	15	10	0	0	0
18	20	15	20	0	0	0
Verticillium sp.[c]						
6	0	0	0	0	0	0
12	0	0	0	0	0	0
18	0	0	0	0	0	0

[a] Mean of two heated and two unheated samples; 10,000 spores per tube.
[b] 0, no growth; 10, <25% covered; 20, 25 to 50% covered; 30, 50 to 75% covered; 40, >75% covered.
[c] No growth in positive control.

TABLE 2. Ability of fungi to utilize components of PVC film as carbon sources at 30°C[a]

Organism and wk postinoculation	Mean colony diam (mm)[b] of film component:			
	PDA	Epoxidized oil	Calcium-zinc stearate	Stearamide
A. fischeri				
2	90	90	90	90
4	90	90	90	90
A. niger				
2	80	69	64	39
4	90	85	81	77
Cladosporium sp.				
2	33	22	26	0
4	69	32	55	0
Fusarium sp.				
2	72	90	90	0
4	90	90	90	0
Paecilomyces sp.				
2	90	71	90	0
4	90	90	90	0
Penicillium sp.				
2	90	21	0	0
4	90	74	0	0
P. citrinum				
2	90	65	0	0
4	90	90	0	0
Trichoderma sp.				
2	90	90	90	0
4	90	90	90	0
Verticillium sp.				
2	11	48	0	0
4	25	73	0	0

[a] MSA, dioctyl phthalate, and PVC produced no growth of the listed fungal species over the 4-week testing period.
[b] Maximum, 90 mm.

stearamide component of the PVC film as a carbon source. None of the other species showed growth in the presence of this component after 4 weeks of incubation. Utilization of several components of the film by the fungus *A. fischeri* gave a possible indication of why it was able to grow on the whole film without added nutrients. The plasticizer, dioctyl phthalate, and the PVC resin were found not to serve as carbon sources for any of the fungal species tested.

As was stated previously, the only film components which contained nitrogen were the epoxidized oil and the stearamide. Therefore, to determine whether the fungi could use the film components as nitrogen sources, 0.5% (wt/vol) epoxidized oil or stearamide was incorporated into MSA containing 0.5% (wt/vol) glucose as a carbon source. Results indicated that the fungi were not able to utilize either component of the film as a nitrogen source, since no difference was detected in growth levels on the control and test plates. Growth of fungi on the PVC film strongly suggested that the film could be utilized as a nitrogen source. While these compounds were not found to be utilized individually for nitrogen in the ingredient study, there still existed the possibility that the whole film provided a trace nutrient to allow utilization of the nitrogen. Nitrogen could also have been introduced from other sources, such as water, trace contamination of the medium ingredients, diffusion from the air, or a combination of these factors.

In conclusion, whole PVC film was found to be susceptible to fungal attack. The composition of the film, microorganisms present, and environmental conditions were shown to be determinants of growth on the film. Different fungal strains were found to have various requirements for exogenous nutrients. Only *A. fischeri* and a *Paecilomyces* sp. could grow without added nutrients. The components of the film most susceptible to attack were the epoxidized oil and the stearate.

LITERATURE CITED

1. **Baribo, L. E., J. S. Avens, and R. D. O'Neill.** 1966. Effect of electrostatic charge on the contamination of plastic food containers by airborne bacterial spores. Appl. Microbiol. **14:** 905–913.
2. **Bejuki, W. M.** 1966. Microbiological degradation, p. 716–725. *In* Encyclopedia of polymer science and technology, vol. 4. Interscience Publishers, Inc., New York.
3. **Berk, S.** 1950. Effect of fungus growth on the tensile strength of polyvinyl chloride films plasticized with three plasticizers. ASTM Bull. **168:**53–55.
4. **Berk, S. B., H. Ebert, and L. Teitell.** 1957. Utilization of plasticizers and related organic compounds by fungi. Ind. Eng. Chem. **49:**1115–1124.
5. **Block, S. S.** 1953. Humidity requirements for mold growth. Appl. Microbiol. **1:**287–293.
6. **Booth, G. H., and J. A. Robb.** 1968. Bacterial degradation of plasticized PVC: effect on some physical properties. J. Appl. Chem. **18:**194–197.
7. **Brown, A. E.** 1946. The problem of fungal growth on synthetic resins, plastics, and plasticizers. Mod. Plast. **23:**189–195.
8. **Eisenschiml, R., and W. H. Bauer.** 1968. Microbial degradation of plasticized vinyl films. Adv. Chem. Ser. **85:**250–271.
9. **Gerber, N. N., and D. P. Stahly.** 1975. Prodiginine (Prodigiosin-like) pigments from *Streptoverticillium rubrireticuli*, an organism that causes pink staining of polyvinyl chloride. Appl. Microbiol. **30:**807–810.
10. **Girard, T. A., and C. F. Koda.** 1959. Pink discoloration of vinyls. Mod. Plast. **36:**148.
11. **Hartman, M. W., J. J. Powers, and D. E. Pratt.** 1963. Bacterial permeability of selected food packaging films. Food Technol. **17:**1172–1174.
12. **Horwitz, W. (ed.).** 1980. Official methods of analysis, 13th ed. Association of Official Analytical Chemists, Washington, D.C.
13. **Klausmeier, R. E., and W. A. Jones.** 1961. Microbial degradation of plasticizers. Dev. Ind. Microbiol. **2:**47–53.
14. **Lasman, H. R., and J. P. Scullin.** 1977. Miscellaneous modifying additives, p. 801–845. *In* L. I. Nass (ed.), Encyclopedia of PVC, vol. 2. Marcel Dekker, Inc., New York.
15. **Mislivec, P. B.** 1979. Specific requirements and physical factors affecting fungal growth and multiplication, p. 20–28. *In* M. E. Rhodes (ed.), Food mycology. G. K. Hall & Co., Boston.
16. **Ronsivalli, L. J., J. B. Bernsteins, and B. L. Tinker.** 1966. Method for determining the bacterial permeability of plastic films. Food Technol. **20:**1074–1075.
17. **Shih, C. N., and E. H. Marth.** 1973. Aflatoxin production by *Aspergillus parasiticus* when incubated in the presence of different gases. J. Milk Food Technol. **36:**421–425.
18. **Sinell, H. J.** 1980. Packaging, p. 193–204. *In* Microbial ecology of foods, vol. 1. International Commission on the Microbiological Specifications for Foods. Academic Press, Inc., New York.
19. **Stahl, W. H., and H. Pessen.** 1953. The microbiological degradation of plasticizers. I. Growth on esters and alcohols. Appl. Microbiol. **1:**30–35.
20. **Tirpak, G.** 1970. Microbial degradation of plasticized PVC. SPE J. **26:**26–30.
21. **Yeager, C. C.** 1962. Pink staining in polyvinyl chloride. Plast. World **20:**14–15.