Fungistatic Effects of Sub-Atmospheric Pressures

To a great extent, the economic survival of the fresh produce industry depends on an intelligent use of chemicals, packaging, handling, and storage procedures that will extend shelf-life and maintain quality. It is known that sub-atmospheric pressure inhibits the ripening of many kinds of fruits such as banana, tomato, avocado, mango, sweet cherry, lime, guava, and apricot[1,2]. However, the effect of sub-atmospheric pressures on the growth of fungi which cause fruit deterioration has never been studied.

The purpose of this experiment reported here was to determine the effects of sub-atmospheric pressures on the growth of fungi associated with the deterioration of fruits during storage.

Five strains of fungi, *Penicillium expansum, Rhizopus nigricans, Aspergillus niger, Botrytis alli,* and *Alternaria* sp. were tested in this experiment. Fungi were cultivated on Potato Dextrose Agar (Difco) at pH 5.6 in petri dishes. Average sporulation and per cent mycelial coverage were rated on petri dishes inoculated at the center. For measuring the time of growth, suspended spores were dispersed on the agar plates. After inoculation, petri dishes were stored under different degree of atmospheric pressures: 646 mm Hg (control, atmospheric pressure at Utah State University, Logan, Utah, USA), 471 mm Hg, 278 mm Hg, and 102 mm Hg. These pressures were maintained by an oil sealed pump in connection with Matheson-49 vacuum regulators. To evaluate the effect of low oxygen partial pressure on fungi, some inoculated petri dishes were placed under controlled atmosphere of 2.7% oxygen and 97.3% nitrogen dispensed from high pressure cylinders. The oxygen partial pressure of this controlled atmosphere is the same as the sub-atmospheric pressure at 102 mm Hg. The temperature and the relative humidity was maintained at 21°C and 90 to 95% by moistening the sweeping air or gas with a humidifier. 40 petri dishes (8 of each fungus) were used in each treatment. 10 petri dishes inoculated with a given fungus were designated as controls. The incubation time was 8 days for *Penicillium expansum,* 5 days for *Rhizopus nigricans,* and 6 days for *Aspergillus niger, Botrytis alli,* and *Alternaria* sp. Sporulation ratings were recorded on the basis of 1 as slight and 10 as very profuse. Mycelial growth was measured by per cent coverage of the agar. Time for appearance of growth was measured when spores started to germinate.

Sub-atmospheric pressure at 471 mm Hg has little or no effect on the growth and sporulation of the fungi. The growth and sporulation of these fungi were significantly influenced at 278 mm Hg. Per cent mycelial coverage of these fungi was less than that of the control at this pressure. The time for appearance of growth was increased. Formation of spores was inhibited.

Sub-atmospheric pressure at 102 mm Hg retarded the growth and sporulation of the fungi. It is apparent that the lower the pressure applied the more the inhibition of growth and sporulation of fungi. For *Penicillium expansum,* per cent mycelial coverage was 80 when stored at 102 mm Hg. The time for appearance of growth was almost twice as long as that of the control. At the end of incubation, control *Penicillium expansum* showed uniform dark blue spores, whereas treated *Penicillium expansum* only scattered light blue spores indicating slower growth of the fungus when held at this pressure. The growth of *Rhizopus nigricans* was inhibited by 102 mm Hg. Per cent mycelial coverage was 75% of that of the control and the

[1] S. P. BURG and E. A. BURG, Science *153*, 314 (1966).
[2] M. T. WU and D. K. SALUNKHE, Utah Science, in press.

Effects of sub-atmospheric pressures and controlled atmosphere (low oxygen, but normal pressure) on mycelial spread, time for appearance of growth, and average sporulation rating of fungi

Fungi	Measurement	Control (646 mm Hg)	471 mm Hg	278 mm Hg	102 mm Hg	2.7% O_2 and 97.3% N_2 (646 mm Hg)
Penicillium expansum	Mycelial coverage (%)	100	100	85	85	95
	Time for appearance of growth (days)	2	2.5	3.5	3.5	2.5
	Average sporulation rating	8	7	4	3	6
Rhizopus nigricans	Mycelial coverage (%)	100	100	90	75	90
	Time for appearance of growth (days)	2	2	4	5	3
	Average sporulation rating	10	10	6	4	9
Aspergillus niger	Mycelial coverage (%)	100	90	75	65	85
	Time for appearance of growth (days)	3	3	4	5	3
	Average sporulation rating	10	8	5	2	8
Botrytis alli	Mycelial coverage (%)	100	100	90	60	90
	Time for appearance of growth (days)	2	2	3	4	2
	Average sporulation rating	8	8	6	4	7
Alternaria sp.	Mycelial coverage (%)	100	90	80	65	90
	Time for appearance of growth (days)	2	3	4	5	3
	Average sporulation rating	7	7	5	4	6

time for appearance of growth was two and a half times as long as that of the control. The spore formation was also retarded. 5 days after incubation, most of the sporangia in the treated fungi were still white in color, whereas those of the control fungi were already mature and black. The growth and spore formation of *Aspergillus niger, Botrytis Alli,* and *Alternaria* sp. were also retarded by 102 mm Hg. The time for appearance of growth was increased at this pressure. Controlled atmosphere of 2.7% oxygen and 97.3% nitrogen (646 mm Hg) has less effect on the growth and sporulation of the fungi when compared with those of subatmospheric pressure of 278 mm Hg and 102 mm Hg.

Sub-atmospheric pressure storage is a type of controlled atmosphere storage with emphasis on reducing the pressure exerted on the storage material. Controlled atmospere (CA) is the term used for the increased carbon dioxide and decreased oxygen as compared to normal atmosphere. The CA storage is employed commercially to lengthen shelf life of fresh produce by retarding respiratory metabolism. As far as fruit storage is concerned, subatmospheric pressure treatment not only reduces oxygen concentration but increases the diffusion of ethylene from the tissues of the fruit and consequently extend the storage life. A controlled atmosphere of high carbon dioxide and low oxygen is known to inhibit the growth and sporulation of certain fungi[3,4]. However, the subatmospheric pressure treatments employed in this study contain only trace amount of carbon dioxide. The oxygen partial pressure of the controlled atmosphere (2.7% oxygen) is equal to that of 102 mm Hg treatment, while the effect of controlled atmosphere (2.7% oxygen) on inhibition of growth and sporulation of fungi is less than that of 102 mm Hg treatment. This indicates that the inhibition of the fungi growth by sub-atmospheric pressure is due to not only lower oxygen concentration, but also lower pressure exerted on fungi. The inhibition of the growth of funigi by sub-atmospheric pressure seems to play a role in extending the storage life of fruits. The mechanism(s) of the inhibition of fungi growth by subatmospheric pressure is still not clear. However, from the point of view of practical application, it can be concluded that with proper combination of lower temperature, relative humidity, and gas composition, sub-atmospheric pressure should effectively control the fungi in storage of fresh produce and thus extend the storage life of perishable fruits and vegetable.

Zusammenfassung. Der Wuchs und die Sporenbildung der Pilze *Penicillium expansum, Rhizopus nigricans, Aspergillus niger, Botrytis alli* und *Alternaria* wurde durch den subatmosphärischen Druck gehemmt.

M. T. Wu and D. K. Salunkhe

Food Science Department, Utah State University, Logan (Utah 84321, USA), 3 January 1972.

[3] N. A. Littlefield, B. N. Wankier, D. K. Salunkhe and J. N. McGill, Appl. Microbiol. *14*, 579 (1966).
[4] W. C. Yackel, A. I. Nelson, L. S. Wei and M. P. Steinberg, Appl. Microbiol. *22*, 513 (1971).

Thermozymocidin New Antifungal Antibiotic From a Thermophilic Eumycete

During a research program on the antibiotic activity of thermophilic microorganisms from soil and compost[1-7], we have isolated a strain of eumycete (IPV F-433) with interesting antifungal activity. The antibiotic was named thermozymocidin and differentiated from already known antibiotics on the basis of the activity spectrum and its physical-chemical characteristics.

The strain has the following temperature requirements for growth: Minimum ~26°C, Optimum 40–45°C, Maximum ~53°C. After 3–5 days on potato-glucose-yeast extract agar at 43°C, the colonies are cottony, rather flat, with yellowish clear substrate mycelium and whitish aerial mycelium becoming ochraceous with age. The hyphae are separated, 2–15 μm in diameter. Conidia and sexual formations are not observed; reproduction seems to occur by fragments of hyphae. Therefore the strains i so far included in the group *Mycelia sterilia*[8,9]. Its main physiological characteristics are as follows: good growth at pH 5.5–8; proteolytic, amylolytic, lipolytic, milk clotting, ribonucleasic, antifungal activities present; positive utilization of glucose, fructose, galactose, xylose, maltose, saccharose, lactose, starch, inulin, glycerol, mannitol; good utilization of asparagine, peptone, urea, KNO_3, weak utilization of $(NH_4)_2SO_4$.

Fermentation conditions for the production of thermozymocidin in 5 l fermentors were: Medium composition: corn meal, 20 g; KH_2PO_4, 5 g; $MgSO_4 \cdot 7H_2O$, 0,5 g; tap water, 1 l; pH 7 after sterilization (1 atm. × 30′); inoculum: 10% of 48 h broth-culture; temperature: 43°C; aeration: 0.6/l/l/m; agitation 500 rpm; antifoam agent: silicone emulsion.

At the end of fermentation (80 h, pH 6.5) the mycelium is separated by centrifugation, washed with 0.2% NH_4OH and extracted with methanol, and the supernatant broth is charged into a column of Amberlite XAD-2 resin; the antibiotic is eluted with 80% metanol. The methanolic fractions are combined and concentrated to $^1/_5$ of the original volume. The precipitate, which is formed overnight at 5°C, is collected by filtration, washed with acetone and ethylacetate and crystallized from ethanol/water.

Thermozymocidin appears as a white microcrystalline substance running as single spot in TLC (Kieselgel G; eluent: isoamyloacetate 65, methanol 25, formic acid 5, water 5). It shows the following physical and chemical properties: mp 170–172°C (uncorrected); $[\alpha]_D^{25}$ + 4°

[1] R. Craveri and H. Pagani, Annal. Microbiol. *12*, 131 (1962).
[2] R. Craveri, C. Coronelli, H. Pagani and P. Sensi, Clin. Med. USA *71*, 511 (1964).
[3] C. Coronelli and R. Craveri, Br. Pat. Abstr. No 21259/64 (1964).
[4] R. Craveri, A. Craveri and A. Guicciardi, Annal. Microbiol. *17*, 1 (1967).
[5] A. Guicciardi, P. L. Manachini and R. Craveri, Atti XIV° Congr. Naz. Soc. Ital. Microb. Messina (1967).
[6] R. Craveri and F. Aragozzini, Atti XV° Congr. Naz. Soc. Ital. Microb., Saint Vincent (1969).
[7] F. Aragozzini, P. Toppino and B. Rindone, Annal. Microbiol. *20*, 44 (1970).
[8] C. J. Alexopoulus, *Introductory Mycology* (J. Wiley and Sons, New York 1962).
[9] D. G. Cooney, R. Emerson, *Thermophilic Fungi* (W. H. Freeman and Co., San Francisco 1964).