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.

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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°

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(C = 1, DMSO); UV: end absorption (methanol); IR (nujol): 3375, 3240, 3150, 1710, 1655, 1605, 1572, 1533, 1412 and 965 cm^{-1}; elemental analysis: calculated for $C_{21}H_{39}NO_6$: C 62.81%, H 9.79%, N 3.39%; found: C 62.64%, N 10.38%, N 3.48%; molecular weight 401 (MS).

Thermozymocidin is almost insoluble in water and in nonpolar solvents, sligthly soluble in lower alcohols, chloroform, pyridine, dimetylsulphoxide, soluble in N NaOH, N HCl, concentrated formic acid and acetic acid. Reaction with ninhydridin, dinitrophenylhydrazine, alkaline permanganate are positive. Specific test for sugars and polypeptides are negative. The antibiotic is stable on heating even in acids, alkalis and organic solvents (0.1% hydroalcoholic solutions retain full activity after 2 days at 50°C and pH 2–10).

Thermozymocidin is highly active against a great number of yeasts and filamentous fungi. MIC is equivalent to 0.03 µg/ml for *Kloeckera apiculata*, 0.1 µg/ml for *Saccharomyces cerevisiae* and *Cryptococcus neoformans*, 1 µg/ml for *Penicillium notatum*, 10 µg/ml for *Aspergillus oryzae* and *Trichophyton mentagrophytes*. It does not show antibiotic activity against gram + and gram − bacteria.

The activity in vitro seems not to be affected by 10% horse serum. LD_{50} of thermozymocidin in mice is 7.5 mg/kg by i.p. injection[10].

Riassunto. È stato isolato un nuovo antibiotico, denominato termozimocidina, attivo contro lieviti e funghi filamentosi, prodotto in coltura sommersa da un eumicete termofilo. Vengono presentate le principali proprietà chimiche, fisiche e biologiche che ne permettono la sua caratterizzazione.

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[10] This research was supported by Società Italiana Resine S.I.R (s.p.a.), Milano.

Virologie: sur la spécificite du facteur d'interférence de *Pseudomonas aeruginosa*

Les interférons des organismes supérieurs assurent une certaine protection de l'espèce productrice contre la plupart des virus susceptibles de l'attaquer mais sont dépourvus, dans la majorité des cas, d'effet protecteur notable vis-à-vis d'espèces hétérologues. L'étude de ces substances a utilisé principalement l'interaction entre virus et eucaryotes, bien que les procaryotes et, en particulier, les entérobactéries soient également le siège de phénomènes d'interférence et qu'une substance de type interféron ait été décrite chez *Pseudomonas aeruginosa*[1].

Parmi les obstacles qui s'opposent encore à l'utilisation thérapeutique de l'interféron, plusieurs sont liés à cette spécificité; il nous a dès lors semblé intéressant de décrire quelques observations relatives à la spécificité d'un facteur d'interférence viral d'origine bactérienne.

Souches: *Pseudomonas aeruginosa* ATCC 12055 et ATCC 12175, *Shigella flexneri* ATCC 12661, *Staphylococcus aureus* Twort Institut Pasteur 6451. Bactériophage de *P. aeruginosa* ATCC 12055 B_3, Bactériophage de *P. aeruginosa* ATCC 12175 B_2, Bactériophage de *S. flexneri* ATCC 12661 B, Bactériophage de *St. aureus* Twort Institut Pasteur. Les souches hôtes sont conservées à l'état lyophile, les bactériophages sont conservés à +5°C en suspension dans les filtrats de cultures lysées.

Milieu de culture A: Acide lactique: 2,4 g/l; chlorure de sodium: 5; Phosphate diammonique: 1; Phosphate monopotassique: 1; Sulfate de magnésium heptahydraté: 0,2; Hydrolysat de caséine: 1. B: Extrait de bœuf Difco: 3 g/l; Bactopeptone Difco: 5; Agar-agar Difco: 15.

Culture de *P. aeruginosa* ATCC 12055: On ensemence 100 cm^3 de milieu A par 2×10^9 bactéries et cultive en fiole de 250 cm^3 à 37°C pendant 3 h. On obtient 2×10^{10} bacries en phase exponentielle ($\mu = 0{,}11$).

Multiplication du phage B_3 sur *P. aeruginosa:* On ensemence 100 cm^3 de milieu A par 2×10^9 bactéries et 1×10^8 phages et cultive à 30°C pendant 15 h puis centrifuge 45 min à 9000 *g* et filtre le surnageant sur millipore HA. On obtient une suspension privée d'éléments bactériens contenant environ 1×10^{10} particules infectantes par cm^3.

La culture de *P. aeruginosa* sur milieu B et l'obtention de plaques d'infection par le phage B_3 sont effectuées selon DICKINSON[2]. Dans ces conditions, le nombre moyen de particules libérées par bactérie infectée (burst size) pour une multiplicité d'infection inférieure à 1 est de 55 à 75 et le rendement global (step size) de 35 à 60.

Phage B_3 inactivé: Le traitement par les ultraviolets a été effectué dans les conditions décrites pour les phages de *E. coli*[3]. L'irradiation de 20 cm^3 de suspension à $1{,}5 \times 10^{10}$ phages/cm^3 en boîte de Petri (diamètre 100 mm) à 30 cm d'une lampe germicide Westinghouse ($\lambda_{max.} = 2650$ Å) conduit en 6 mn (3.10^3 ergs/mm^2) à un taux de survivance asymptotique de 2×10^3 phages/cm^3.

Facteur d'interférence: A 250 cm^3 de suspension de phages atténués on ajoute 50 cm^3 de culture de *P. aeruginosa* et laisse en contact 1 h à 57°C. On centrifuge 1 h à 9000 *g*, le culot est remis en suspension dans 50 cm^3 de milieu A, puis incubé 2 h à 37°C. On centrifuge 1 h à

Bactérie	Phage	Rendement moyen en particules virales	
		Témoin	+ Facteur d'interférence de *P. aeruginosa*/B_3
P. aeruginosa ATCC 12055	12055 B_3	29	4
P. aeruginosa ATCC 12175	12175 B_2	39	31
Shigella flexneri	12661 B	29	21
St. aureus Twort	Tw.	34	42

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