tant soit des spores de *C. graminicola* soit des cellules de *Sp. roseus*. Les spores fongiques et les levures âgées respectivement de 7 et 3 jours sont en contact direct avec le milieu de culture de *C. hordei*. Périodiquement, les organismes testés sont retirés à l'aide de la feuille de cellophane support. Le tout est soumis au lavage par centrifugation pendant 10 min à 4000 tours/min (centrifugeuse Martin Christ, type UJ1S) dans 50 ml d'eau stérile. Les microorganismes ainsi traités sont ensuite étalés sur milieu nutritif gélosé et après 12 h d'incubation leur survie est déterminée au microscope par le pourcentage de germination des spores de *C. graminicola* ou par celui de bourgeonnement de cellules de *Sp. roseus*. L'observation des propagules restant sur le milieu de culture de *C. hordei* ne révèle aucune germination ou bourgeonnement de leur part. L'absence ultérieure sur ce même milieu de toute forme de croissance montre que ces propagules sont bien tuées.

Les résultats consignés dans le Tableau montrent que *C. graminicola* et *Sp. roseus* sont en grande partie tués après 3 h seulement de contact avec le milieu de croissance de *C. hordei*. Après 9 h d'incubation, aucune cellule n'est plus viable. A ce moment, l'observation au microscope optique de *Sp. roseus* et de *C. graminicola* ne montre aucune différence entre une cellule saine et une cellule tuée. Mais après 48 h de traitement, on peut constater que toutes les cellules de *Sp. roseus* et les spores de *C. graminicola* sont en partie lysées avec dissolution de leur cytoplasme. Cette dissolution est déjà très nette chez la plupart d'entre elles au bout de 72 h tandis que leur parois restent intactes (Figures 2 et 3). Ces observations confirment donc que l'antibiotique formé par *C. hordei* a effectivement provoqué, chez les microorganismes testés, la mort suivie de la lyse de leurs cellules[2].

Summary. A new species of yeast, *Candida hordei* De Miranda and Diem, is able to produce an antibiotic which kills and lyses diverse microorganisms.

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Cytokinin-Like Activity in Extracts from Culture Filtrates of *Pseudomonas savastanoi*

Olive knot, caused by the bacterium *Pseudomonas savastanoi* (E. F. Sm.) Stevens, is characterized by the formation of galls on young stems, leaves and other organs of the olive. Similar outgrowths are found on a narrow range of other species including oleander[1] and privet[2]. Gall development involves establishment of the bacteria within lysogenous cavities, followed by hypertrophy, hyperplasia and vascular differentiation of the surrounding cells[3]. The hypertrophy can be induced on oleander by treating it with culture filtrates from *P. savastanoi* or indole-3-acetic acid (IAA)[3]. The bacterium itself has been shown actively to synthesize indole-3-acetamide, IAA and its lysine conjugate[4–6]. Thus, the hypertrophied cells in the gall may result from auxin production by the bacteria. Substances which could cause hyperplasia have not been examined, but based on their well-known and characteristic stimulation of cell division, it has been suggested that cytokinins may be involved[3]. We report here on the production of some substance(s) having cytokinin-like activity by *P. savastanoi*.

A culture of *P. savastanoi* (NCPPB 639) was grown with agitation in Woolley's medium[7] for various periods of time at 25 °C. The cells were then removed by centrifugation and filtration (Millipore, 0.45 µm). After concentrating 2-fold at 40 °C under reduced pressure, the filtrate was adjusted to pH 7.8 with 1 *N* KOH and extracted 5 times with 1/5 volumes ethyl acetate. Excess dry Na_2SO_4 was added to the combined organic extract to remove the residual water. The extract was then filtered and evaporated to dryness at 40 °C in vacuo. The residue was redissolved in ethanol, filtered and again evaporated to dryness.

In the beginning of the work, this residue was simply redissolved in a minimal volume of 0.1 *N* NaOH and diluted with water before use. Later on it was redissolved in ethanol and spotted on TLC plates (Merck, Silica Gel 60 F-254, 0.25 mm) which were developed with chloroform:methanol (9:1). Under UV-light (254 and 366 nm) 11 bands could be distinguished. Each was scraped off separately and its contents eluted with ethanol. This was filtered and dried in vacuo. The residue was then redissolved in 0.1 *N* NaOH and diluted with water.

All preparations were tested for cytokinin-like activity using the growth response of tobacco callus and retention of chlorophyll by senescing oat leaves criteria. The techniques and medium (RM 1964) of Linsmaier and Skoog[8] were used for the tobacco callus assay. For each treatment 3 flasks, each containing 3 pieces of tobacco (cv. Wisconsin 307) callus, were used. The preparations were filter sterilized (Millipore, 0.45 µm) and incorporated into the medium just before it gelled. The medium contained no plant hormones initially when a crude ethyl acetate extract from a 7-day culture was tested, but later

Table I. Growth promotion of tobacco callus by ethyl acetate extracts from cultures of *P. savastanoi* of different ages

Treatment	Fresh wt. (g)[a]
3-day culture	0.91
7-day culture	1.27
10-day culture	2.39
12-day culture	2.25
complete medium[b]	2.92
– IAA	0.48
– kinetin	0.54

[a] Average of 9 callus pieces. Culture filtrate equivalent to 10 l per 1 medium. [b] The same controls apply to the data in Table II.

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IAA (2 mg/l) was added when similar extracts from cultures of different ages or the chromatographic bands were tested. After 4 weeks incubation at 24 °C, the fresh weight of callus in each treatment was determined. The effect of the preparations on the retention of chlorophyll by oat leaves cv. Criolla was tested using the method of THIMANN and SACHS[9]. Five 15 µl droplets (equivalent to 15 ml of culture filtrate) were used for each preparation.

If either IAA or kinetin was omitted from the control medium, there was little growth and the tissues soon turned brown. However, the crude ethyl acetate extract supported good growth in the absence of added plant hormones. Roots but not buds commonly formed, suggesting that the concentration of auxins and their ratio to cytokinin-like substances in the extract was high. The best growth was obtained with the 10-day culture filtrate (Table I). At this time, viability counts in the culture had begun to decline, after rising exponentially for 5 days and remaining in the stationary phase for another 4 days. Over the 12-day incubation period, the pH of the medium slowly rose from 6.6 to 7.8. Several of the chromatographic bands with Rf similar to known cytokinins, i.e., 6-(γ,γ-dimethylallylamino) purine and its ribonucleoside, also had cytokinin-like activity (Table II). In the oat leaf chlorophyll retention bioassay, the same bands were the most active.

Table II. Growth promotion of tobacco callus by individual bands from thin-layer chromatography of an extract from a 7-day culture of *P. savastanoi*

Band	Fresh wt. (g) [a]	Rf
1	0.88	0
2	0.44	0.05
3	0.65	0.09
4	1.08	0.17
5	2.86	0.29
6	0.83	0.38
7	0.99	0.41
8	0.52	0.55
9	0.79	0.63
10	0.56	0.74
11	0.51	0.80

[a] Average of 9 callus pieces. Culture filtrate equivalent to 12 l per l medium.

Table III. Growth promotion of olive callus by an ethyl acetate extract from a 7-day culture of *P. savastanoi*

Treatment	Fresh wt. (g) [a]
complete medium	0.70
IAA + kinetin + extract	0.98
NAA + extract	0.82
IAA + extract	1.28
kinetin + extract	1.01
extract	0.55

[a] Average of 9 callus pieces. Culture filtrate equivalent of 10 l per l medium.

The effect of the crude ethyl acetate extract on the growth of olive (cv. Cima di Mola) callus was also tested. Wood tissue from 1-year-old branches was grown in modified RM 1964 containing: (mg/l) IAA 2; α-naphthalene acetic acid (NAA) 1; kinetin 1; and ascorbic acid 1. On media lacking auxin or containing < 0.5 mg/l kinetin the callus soon turned brown and died. But, as with tobacco callus, the ethyl acetate extract supported good growth of olive callus in the absence of kinetin (Table III).

These results show that some substance(s) with cytokinin-like activity is synthesized by *P. savastanoi*. Perhaps it is also produced in developing galls where it might act in concert with auxins. However, the presence in galls of elevated levels of plant hormones, and their source, remain to be shown. Further work to elucidate these points and to identify the substance(s) in the extract having cytokinin-like activity is in progress.

Riassunto. Filtrati colturali parzialmente purificati del batterio *Pseudomonas savastanoi* hanno mostrato attività di tipo citocininico quando sono stati esaminati mediante sistemi di saggio biologico consistenti nella risposta di calli di tabacco e di olivo e nella ritenzione della clorofilla da parte di foglie senescenti di avena.

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Nitrogenous Excretory Products of Tobacco Hornworm, *Manduca sexta* (L.)

It has been shown by many workers that ammonia is the main nitrogenous excretory product in aquatic and semiaquatic insects[1], while in case of terrestial insects uric acid has been reported to be the main excretory product[2]. Purines other than uric acid are rarely found in insect excreta. Hypoxanthine and xanthine present in the excreta of *Melophagus ovinus*[3], *Drosophila melanogaster*[4] and *Galleria mellonella*[5,6] seem to reflect pecularities of purine metabolism. However, in case of cotton stainer, *Dystercus fasciatus*, the main nitrogenous excretory product was not uric acid but allantoin[7]. Urea, also has been found as a minor nitrogenous product in many insects[2].

Tobacco hornworm, *Manduca sexta*, was chosen for studying the various nitrogenous waste products because of its large size due to which sufficient quantities of excreta could be collected at short time intervals. The

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