dark. Strain 8M2 was found to produce Sclerotia in the dark over a wider range of temperature than the parent strain (20–33°C) and even a few in the light at 25°C. Outside these temperatures, however, only Conidia appeared, although their production was far less abundant than in strains 8 and 8M1 under similar conditions.

Cultures of the parent strain 8, and the variants 8M1 and 8M2 were also grown in the light and dark at 25°C on agar media with varying concentrations of glucose and mycological peptone. Under conditions of low glucose and peptone concentrations all three strains produced some Conidia in either the light or dark, but no Sclerotia. High concentrations of glucose and peptone were conducive to the production by all three strains of Conidia in the light and of Sclerotia in the dark; however, 8M2 still produced far fewer Conidia in the light than 8 or 8M1, and 8M1 produced only a few Sclerotia in the dark, compared to their abundant production under these conditions by 8 and 8M2.

Thus by varying simple cultural conditions it is possible to induce the parent strain to produce colonies with only Conidia, or Conidia and Sclerotia, or only Sclerotia; furthermore, variants have been easily selected in which the ability to produce principally either Conidia or Sclerotia has predominated. It appears that this fungus could provide a valuable tool for morphogenetic studies.

Zusammenfassung. *Aspergillus japonicus* bildet je nach den Belichtungs-, Temperatur- und Ernährungsbedingungen, unter denen er wächst, entweder nur Konidien oder nur Sklerotien oder beide zusammen.

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Fungi on the Surface of Legume Root Nodules and Phosphate Solubilization

While the importance of fungi on the root surface of plants has been recognized[1–3], the presence of fungi such as *Cephalosporium* sp., *Alternaria* sp., *Aspergillus* sp., and *Penicillium* sp. on the surface of root nodules of some legumes, and the antibiotic activity of some of them towards rhizobia, has recently been reported[4]. When *Cephalosporium* sp. and *Alternaria* sp. were screened for antibiotic activity towards phosphate-dissolving bacteria, it was found that the culture filtrate of *Cephalosporium* sp. inhibited the growth of four species of phosphate-dissolving bacteria on solid agar medium. As a natural corollary to this finding, the extent to which the fungus affected the phosphate-dissolving ability of these bacteria was determined by allowing the bacteria to interact individually with *Cephalosporium* sp. on a liquid substrate containing 5 g of Ca_3PO_4/l[5]. The solubilized

Table I. Effect of *Cephalosporium* sp. on phosphate-dissolving ability of bacteria

Treatment	mg P_2O_5 solubilized/ 50 ml medium (average of 4 replicates)	mg P_2O_5 solubilized over control	% P_2O_5[a] solubilized
Non-inoculated medium (control)	11.69	–	–
Bacillus circulans	18.58	6.89	6.70
B. circulans + *Cephalosporium* sp.	15.47[b]	3.78	3.68
Escherichia freundii	20.49	8.80	8.56
E. freundii + *Cephalosporium* sp.	18.45[b]	6.76	6.58
Bacillus megatherium var. *phosphaticum* (isolated from phosphobacterin, a bacterial fertiliser from USSR)	19.24	7.55	7.34
B. megatherium var. *phosphaticum* + *Cephalosporium* sp.	18.31	6.62	6.44
B. megatherium (Indian strain)	17.92	6.23	6.06
B. megatherium (Ind.) + *Cephalosporium* sp.	18.12	6.43	6.25
Cephalosporium sp.	18.32	6.63	6.45

[a] Figures obtained after deducting the amount of phosphorus solubilized due to autoclaving in the non-inoculated control medium.
[b] Significant inhibition. S.E. 0.708; C.D. at 5% level is 2.04.

Table II. Phosphate-dissolving ability of fungi on the surface of legume root nodules

Fungi	mg P_2O_5 solubilized/ 50 ml medium (average of 4 replicates)	mg P_2O_5 solubilized over control	% P_2O_5[a] solubilized
Non-inoculated medium (control)	11.92	–	–
Cephalosporium sp.	18.22	6.30	6.14
Alternaria sp.	18.98	7.06	6.88
Aspergillus sp.	18.67	6.75	6.58
Penicillium sp.	74.50	68.58	61.01

[a] Figures obtained after deducting the amount of phosphorus solubilized due to autoclaving in the non-inoculated control medium. S.E. 1.09; C.D. at 5% level is 3.28.

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phosphorus was estimated in terms of P_2O_5 by the vanadomolybdate method[6] in a Klett photoelectric colorimeter using 420 mμ filter. The results (Table I) showed that significant inhibition of phosphate-dissolving activity had occurred with regard to two of the four bacteria tested in the present study. However, interestingly enough, it was found that *Cephalosporium* sp. alone (in control series) had released as much phosphorus as the individual bacteria did. Some soil fungi are known to solubilize phosphates[7]. Nevertheless, it was considered important to extend the study to determine the phosphate-dissolving ability of the fungi occurring on the surface of nodules. The results of such a study (Table II) confirmed not only the ability of *Cephalosporium* sp. to solubilize phosphate but also indicated that all the four fungi tested had the same property. Noteworthy was the fact that *Penicillium* sp. released the maximum amount of phosphorus, amounting to nearly four times that of individual bacteria. Since the number and density of nodules are greatly stimulated by phosphorus[8], the results of the present study indicate the role of nodular surface fungi as probable agents in the mobilization of phosphates in situ from the soil into the nodules[9].

Zusammenfassung. Es wird nachgewiesen, dass an den Wurzelknöllchen von Leguminosen Schimmelpilze vorkommen, die Calciumphosphat abbauen und offenbar die Bildung der Wurzelknötchen selber fördern.

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Studies on the Kinetic of Alkaline Phosphatase Reaction in Ehrlich Ascites Tumour Cells with the Use of Interference Microscopy

The use of interference microscopy in enzyme histochemistry was presented by Davies et al.[1], Barter et al.[2], and Casselman[3], who estimated quantitatively alkaline phosphatase activity in kidney and intestine brush border. The method is based on the measurement of the increase of the optical path difference (OPD) during incubation of the tissue in Gomori's medium for alkaline phosphatase. The enzymatic activity can be expressed as an increase of OPD per unit of time, which reflects the precipitation rate of calcium phosphate due to enzymatic action.

In the present work this method was applied to smears of cells with a low alkaline phosphatase activity. The Ehrlich ascites tumour cells were taken from mice 5 days after inoculation. The smears were made on cover slides 5 × 4.5 cm and fixed for 4 h in cold acetone. The incubation and measurements were carried out in the multipurpose Rose's chamber[4]. The chamber with the smears on its upper cover slide was filled with an incubation medium of the following composition: sodium β-glycerophosphate $0.02M$ (0.2–$0.002M$), $CaCl_2$ $0.04M$, $MgCl_2$ $0.005M$, in $0.05M$ barbiturate buffer pH 9.2 (8.8–9.6). OPD measurements were performed with the MPI interference microscope[5,6]. Ten cells chosen at random were measured at 15 min intervals. The incubation lasted 45 min, because within this period the OPD increase was proportional to the incubation time.

Because of a lower phosphatase activity in Ehrlich ascites tumour cells than in brush border, the error in measurements is relatively higher. The limitation of the error was obtained by OPD measurement of 10 cells in one field of view and getting eventually the mean value of the OPD increase per cell per 15 min. This value was taken as representing the enzymatic activity of a chosen group of cells in a particular incubation medium. After 45 min incubation, calcium phosphate was removed from the cells with barbiturate buffer at pH 6.8, the chamber was filled with a new incubation medium and the reaction was repeated. OPD of the same cells was measured again. It was found that incubation repeated 5 times did not lower enzymatic activity. In order to establish the effect of hydrogen ion concentration on enzymatic activity, the same cells were measured in media at different pH. Incubation was repeated on the same cells 4 or 5 times and the first and the last incubations were performed at the same pH to find out whether the enzymatic activity was lowered during the procedure or not. The results are presented in Figure 1. At pH 9.2 the enzymatic activity

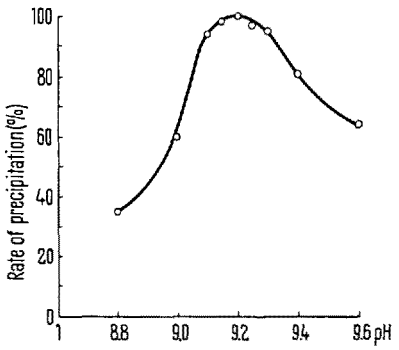

Fig. 1

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