mental period, 25% of plants died. The plants which were grown at Ni 21 and Ni 28 levels had also developed slight chlorosis with necrotic lesions on the leaves, petiol and stem. Such lesions were more in tomato plants grown at Ni 28 than those at Ni 21 level. The stem of these plants were slightly bent at one side. The disease symptoms were very mild mosaic with severe chlorosis of young leaves at Ni 28 and Ni 35 level, but at Ni 21 level the disease symptoms consisted of mild chlorosis with mosaic.

The results given in the Table show that levels of nickel which increased vegetative growth also increased the potato virus X concentration (up to Ni 24). Excess of Ni caused stunting of the plants. Increase in level of Ni caused a proportionate stunting of the plants. Its effect on virus concentration, however, was not proportionate. It has also been observed that the nitrogen and free amino acid contents were higher in healthy tomato leaves than in diseased at each treatment. The higher content of nitrogen and amino acid were noted in healthy tomato plant leaf grown at Ni 14. Twelve amino acids viz. arginine, aspartic acid, glycine, glutamic acid, alanine, threonine, methionine, valine, prolin, tryptophan, isoleucine and leucine were observed in healthy and diseased tomato leaves. The concentration of each amino acid varied in the healthy and diseased leaves growing at the same level, but the number remained the same[7].

Zusammenfassung. Der Effekt von Nickel (Ni) auf die Vermehrung des Kartoffelvirus in der Tomatenpflanze (Lycopersicum esculentum Mill cv. Marglobe) wurde untersucht. In Abhängigkeit vom Nickel-Spiegel nahm sowohl das vegetative Wachstum der Pflanze, der Virus, wie auch der Gesamtgehalt an Stickstoff und freien Aminosäuren zu.

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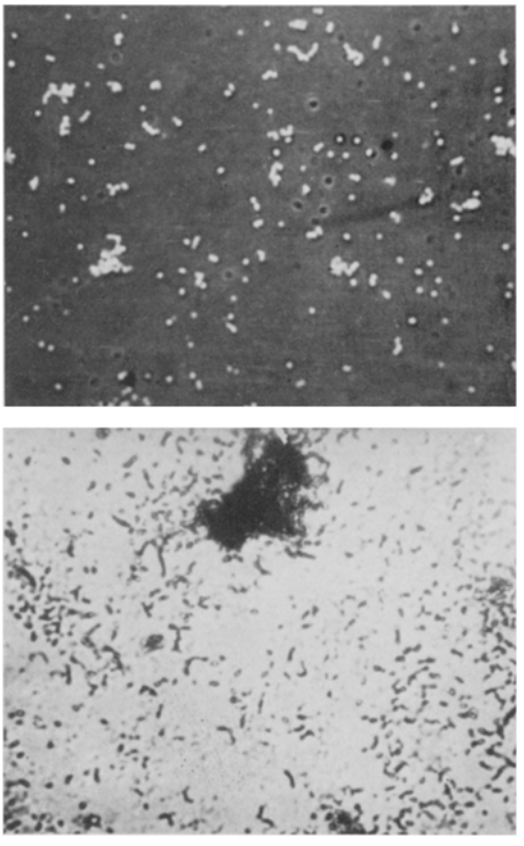
Endosymbiotic Microorganisms in *Cletus signatus* Walker (Coreidae: Heteroptera)

Symbiosis between insects and microorganisms is well known[1]. The bug *Cletus signatus* Walker harbours its bacterial symbiotes in the mycetome, which is associated with the last section of the midgut, the epithelial cells of which evaginate to form 2 rows of caeca[2]. Studies dealt with here were conducted to discover various characteristics of symbiotic microorganisms of *C. signatus*, their mode of transmission to progeny, elimination leading to aposymbiotic insects and their probable functions.

Symbiotes were coccoid rod-shaped bacteria both in culture media and tissue (Figures A and B), gram-positive, capsulated, spore-forming and motile. They hydrolysed starch, gelatin and casein and utilized sucrose, glucose and mannose without producing gas, but did not utilize xylose, arabinose and lactose. Citrate test, reduction of nitrates, production of ammonia from peptone, Voges Proskauer test, methyl red test, methylene blue reduction were positive, while indole test and lecithinase tests were negative. They fixed nitrogen in JENSEN's[3] nitrogen-free medium (5.6 mg/g sugar in 14 days). They were fatal to guinea-pigs in hypodermal injection of a heavy dose. On the basis of above characteristics the bacteria were identified as very closely related to *Bacillus cereus* Frankland, and Frankland[4] with certain differences, and were tentatively named *Bacillus cereus* var. *signatus*. Serological tests conducted confirmed that the cultured bacterium was similar to those found in various tissues.

Transmission of bacteria was found to occur through the ovaries, since the smears of ovaries, eggs and haemolymph showed the presence of bacterial symbiotes. The pathway of transmission is thus from mycetone to haemolymph and then to ovaries and developing oocytes. Symbiotes were also present in the smears of haemolymph of male bugs, malpighian tubes of both male and female bugs, and testes, although in much less numbers. The cause of such a distribution is not known at present. BUCHNER's[1] generalization that transmission in heteropterons having mycetones in the intestine is always external does not hold true in this case.

Cultured symbiotes were found autotrophic to all essential vitamins, when reared on a vitamin-free medium.



Photomicrographs showing a) cultured symbiotes; b) symbiotes in smear of mycetomic tissue; ×500.

[1] P. BUCHNER, *Endosymbiosis of Animals with Plant Microorganisms*, engl. edn. (Wiley Interscience, New York 1965), p. 909.
[2] M. K. NARULA, Doctoral Thesis, Indian Agricultural Research Institute, New Delhi–12, India (1969).
[3] H. L. JENSEN, Proc. Soc. appl. Bact. *14*, 89 (1951).
[4] R. S. BREED, E. G. D. MURRAY and N. R. SMITH, *Bergey's Manual of determinative Bacteriology*, 7th edn. (1957), p. 1094.

Quantitatively they synthesized 0.08 µg pantothenic acid and 0.18 µg niacin/ml of culture medium in 48 h. They also synthesized 0.9 mg ascorbic acid/g of mannose in 3 h. This amount was reduced to 0.1 mg/g mannose, when mycetomal tissue homogenates were treated with 1% kanamycin. PIERRE[5] reported such a synthesis by the symbiotes of *Leucophaea maderae* (F). However the utility of such a synthesis to insects could not be demonstrated, as neither a holidic diet for the bug could be formulated nor could the insect be made aposymbiotic.

Attempts to produce aposymbiotic bugs by treating the insects with various concentrations of streptomycin, chloramphenicol, tetracyclines, penicillins, kanamycin and sulphadiazine, either injected with glass capillary needles or by feeding the bugs on soaked seeds and on dipped twigs of the host plant, were unsuccessful. Mixture with dimethyl sulphoxide was of no value. Centrifugation of eggs (18,000 rpm for 20 min, 10,000 rpm for 30 min) did not yield any result. Lysozyme could not inhibit the multiplication of bacteria in culture media. Haemolymph taken out aseptically from kanamycin-treated bugs (600 µg/g body weight), 4 h after injection, did not produce any inhibition zones to plated cultured symbiotes, showing degradation of the antibiotic. The bacterial symbiotes were not killed by dipping the mycetomal tissue for 1 h in tubes of 1% kanamycin solution or 2% ledermycin, and turbidity appeared on inoculation in nutrient broth.

Cultured symbiotes were found to degrade 29 µg of DDT. 820 µg of parathion and 18 µg of carbaryl, when definite quantities of these insecticides in acetone solution were incubated with symbiotes in nutrient broth for 6 h. Such degradation by the cultured symbiotes of apple maggot, was also studied by MALLORY and MATASMURA[6]. Full details of these findings will be published elsewhere[7].

Zusammenfassung. Es wurden symbiontische Bakterien aus der Wanze *Cletus signatus* Walker isoliert, in vitro kultiviert und als neue Var. *signatus* von *Bacillus cereus* bestimmt.

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[5] L. L. PIERRE, Nature, Lond. *193*, 904 (1962).
[6] G. MALLORY BOUSCH and F. MATASMURA, J. econ. Ent. *60*, 918 (1967).
[7] Thanks are due to N. C. PANT, A. SEN, K. N. MEHROTRA, P. SARUP and T. S. RAMAN all from Indian Agricultural Research Institute, New Delhi-12 (India), for their help.

Ribosomal Proteins from the Liver of the South American Rattlesnake, *Crotalus durissus terrificus*

The structure of eukaryotic ribosomes has been widely investigated in the last few years[1]. However, little is known on this subject in Reptilia[2]. This paper reports studies on the ribosomes of the reptile *Crotalus durissus terrificus*, with special emphasis on the ribosomal proteins.

Material and methods. South American rattlesnakes of both sexes (200–300 g body weight) were used in all experiments. Ribosomes and ribosomal subunits were obtained from the liver as described in a previous paper[2]. Only preparations with $A_{260\,nm}/A_{235\,nm}$ and $A_{260\,nm}/A_{280\,nm}$ ratios of about 1.50 and 1.85, respectively, were used[3]. Protein and RNA contents of the ribosomes were measured as reported by FRIEDMAN et al.[4]. The sedimentation coefficient of the monosomes was determined by sucrose-density-gradient centrifugation under the conditions described previously[2]. Rat liver ribosomes, isolated according to MOLDAVE and SKOGERSON[5], were used as a marker. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of the ribosomal proteins was carried out by the procedure of BICKLE and TRAUT[6]. The buffer for electrophoresis contained 0.1*M* sodium phosphate, pH 7.2, and 0.1% sodium dodecyl sulphate. Gels were stained[6] with Comassie brilliant blue and scanned at 600 nm in a Beckman model Acta III spectrophotometer. The molecular weights of the ribosomal proteins were estimated as a function of their relative mobilities[7], using bovine serum albumin, ovalbumin, chymotrypsinogen and cytochrome c as standards.

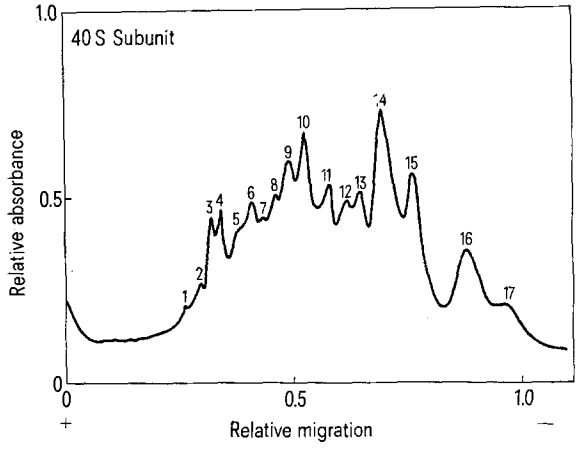

Fig. 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of ribosomal proteins from the 40 S subunit. Electrophoretic analysis was carried out on a 10% gel at 8 mA per tube at room temperature until the bromophenol blue band had just reached the end of the gel.

[1] B. E. H. MADEN, in *Progress in Biophysics and Molecular Biology* (Eds. J. A. V. BUTTLER and D. NOBLE; Pergamon Press, New York 1971), vol. 22, p. 127.
[2] E. M. B. RODRIGUES and F. L. DE LUCCA, Experientia *29*, 37 (1973).
[3] T. E. MARTIN, F. S. ROLLESTON, R. B. LOW and I. G. WOLL, J. molec. Biol. *43*, 135 (1969).
[4] D. I. FRIEDMAN, B. POLLARA and E. D. GRAY, J. molec. Biol. *22*, 53 (1966).
[5] K. MOLDAVE and L. SKOGERSON, in *Methods in Enzymology* (Eds. L. GROSMANN and K. MOLDAVE; Academic Press, New York 1967), vol. 12, part A, p. 478.
[6] T. A. BICKLE and R. R. TRAUT, J. biol. Chem. *246*, 6828 (1971).
[7] K. WEBER and M. OSBORN, J. biol. Chem. *244*, 4406 (1969).