Nematologica 15 (1969) : 619-620. E. J. Brill, Leiden

SHORT COMMUNICATIONS

E. J. BUECHER, E. L. HANSEN & T. GOTTFRIED[1]): *Yeast ribosomes as a source of growth factor for nematodes.* .

There is a current interest in the study of nematodes in axenic (pure) culture. One aspect of these studies is the purification and identification of the proteinaceous growth factor added to the defined medium for maintenance of these pure cultures (Hansen, Buecher & Yarwood, 1964). The activation of nematode growth factor isolated from liver has been interpreted as changes in molecular structure of the soluble protein (Sayre, Fishler, Humphreys & Jayko, 1968). This postulate appears to be erroneous since treatments used for activation not only cause a portion of the protein to precipitate but, in fact, the biological activity resides in the precipitate rather than in the soluble portion of the protein (Buecher, Perez-Mendez & Hansen, 1969).

The nature of growth factor was re-examined when fractions of freshly prepared yeast extract were found to support nematode growth and reproduction (Buecher & Hansen, 1969). The most active fractions were those which easily sedimented and which contained the greatest amount of RNA, suggesting that biological activity might be associated with ribosomes (Buecher, Hansen & Gottfried, 1970).

Two ribosomal preparations were made from yeast by the method of Lucas, Schuurs & Simpson (1964); one was from a 24 hr harvest of *Saccharomyces cerevisiae,* the other from baker's yeast cake (Fleischmann's). Thirty g of washed, frozen cells were ground at 4° C with 60 g of levigated alumina and suspended in a solution containing 5×10^{-2} M Tris buffer, pH 7.6, and 5×10^{-3} M magnesium chloride (iris-Mg buffer). After centrifugation of the suspension at 10,000 *X g* for 15 min, and of the resulting supernatant fluid at 30,000 *Xg* for 20 min, the ribosomes were sedimented at 25,000 rev/min for 4 hr in a Spinco SW 25.1 rotor.

Washed ribosomes from yeast cake were prepared by twice resuspending the ribosomal pellet in a volume of Tris-Mg buffer equal to the original suspension volume, centrifuging at 10,000 X g for 10 min and centrifuging the resulting supernatant at 25,000 rev/min for 4 hr in the Spinco SW 25.1 rotor.

Biological activity in supporting nematode maturation was determined by adding the ribosomes at 60 µg protein/ml to chemically defined basal medium (Buecher, Hansen & Yarwood, 1966). In the assay (Lower, Hansen & Yarwood, 1966), three newly hatched *Caenorhabditis briggsae* larvae were inoculated into each of two tubes containing 0.25 ml of the test medium, and the time for maturation was determined. Both washed and unwashed ribosomal preparations were highly active if first aggregated by frozen storage or by prefreezing the complete medium prior to inoculation; nematode maturation times were 3.5 to 3.8 days. Pronase-treated ribosomes and ribosomal proteins extracted with acetic acid (Hardy, Kurland, Voynow & Mora, 1969) were biologically inactive.

The present hypothesis as to the nature of nematode growth factor is that it is a protein(s) which is made available to the nematode in a particulate form without excessive denaturation. Both partially denatured globulins from liver (Buecher *et al.* 1969) and aggregated ribosomes provide proteins in a particulate form. Other proteins which are of a particulate nature are under study ac possible nematode growth factors.

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1) Clinical Pharmacology Research Institute, 2030 Haste Street, Berkeley, California 94704, U.S.A

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George C. MARTIN[1]): *Survival and infectivity of eggs and larvae of* Meloidogyne javanica *after ingestion by a rodent.*

Martin (1968) summarized the published work on the survival of eggs and larvae of plant parasitic nematodes which had been ingested by warm blooded animals, and drew attention to the lack of information on the infectivity of such survivors. He recorded the survival and infectivity of *Meloidogyne arenaria* (Neal) after ingestion by a bovine. The present study was undertaken to ascertain whether or not eggs and larvae of *M. javanica* (Treub) are capable of infecting ad reproducing in a known host plant after passing through the gut of the common mole rat, *Cryptomys hottentotus* Lesson.

A juvenile common mole rat, trapped from a naturally occurring population in the Eastern Highlands of Rhodesia, was maintained in a cage containing a 10 cm depth of fine wood chips and shavings. Each morning a potato tuber containing numerous *M. javanica* egg masses, was introduced into the cage. In the late afternoon, the rodent, which had fed on the tuber, was cleaned of adhering potato debris and placed on a screen in a glass desiccator containing a little water. Faeces voided overnight by the rodent fell through the mesh of the screen into the water. Each morning the mole rat was returned to the cage and the faeces, usually four to six pellets, were surface washed and mashed in water before mixing with moist heat sterilized soil. This procedure was continued for 7 days, after which time, tomato seedlings reared in sterilized soil were transplanted into the faeces-soil mixture. To serve as controls, tomato seedlings were transplanted into heat sterilized soil without the addition of mole rat excreta.

Direct examination of faecal pellets taken 4 days after the rodent was fed with infected tubers revealed numbers of living eggs and larvae of a *Meloidogyne sp.*

Ten weeks after exposure to infection the tomato roots possessed numerous galls of *M. javanica,* and these galls contained mature females with large well filled egg masses. No infection was found in the roots of control tomato plants.

Mole rats are found in *many* parts of the world and cause damage to crops. In the Eastern Highlands of Rhodesia, the common mole rat causes appreciable damage in apple and peach orchards and at the Rhodes Inyanga Experiment Station, has caused the decline or death of more than 40% of the fruit trees in experimental orchards, thus jeopardizing the statistical validity of observations.

The mole rat lives most of its life underground migrating considerable distance in search of its food which consists mainly of the underground portions of plants. Consequently, the chances of ingestion of plant endoparasitic nematodes are high, and the conditions prevailing below soil level would enhance the survival of the nematodes and their infection of host plant roots.

These results indicate that many eggs, or larvae, or both, of *M. javanica* are capable of infection and reproducing in host plants after passing through the digestive tract of the common mole rat *C. hottentotus* and the natural behaviour pattern of the mole rat contributes to the spread of plant nematode infections.

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1) Plant Protection Branch, Ministry of Agriculture, Salisbury, Rhodesia.