

ons. In the second type (2%) two or three radicles each enclosed by a cupule emerge and grow normally (figure 12). But only the earlier emerged embryo develops into a plant. Very rarely two seedlings emerge and survive. In the third type (1%) plumule emerges when radicle is 1-1.5 cm long. It grows faster producing miniature leaves while the radicle remained 3 cm long (figure 13).

Schmidt<sup>1</sup> observed no sclerenchyma in the pericarp except the macrosclereides of epidermis. But isolated patches of fibres and brachysclereids have been observed in the ground tissue of the pericarp in the current investigation. Also, protoxylem lacunae observed by Rost and Simper<sup>2</sup> could not be found in the present material. It is probable that they might have mistaken enlarged protoxylem vessel elements for protoxylem lacunae. A significant observation of the present investigation is the presence of vasculature in seed coat, which is a primitive feature found in Gymnosperms and rarely in Angiosperms. Roth<sup>3</sup> recognised coleorhiza as a part of suspensor or hypocotyle. The hypocotyledonary cupule partially enclosing the radicle is hence comparable to coleorhiza. The cupule is absent elsewhere in Dicotyledons. The abnormal germination of the embryo after breaking the cotyledons is probably due to the elongation of suspensor pushing embryo deep into the seed. In this process the orientation of embryo in relation to the polarity of the seed is disturbed resulting in abnormal placement and hence germination.

The authors are thankful to Prof. K. S. Rao for encouragement and facilities.

4 December 1981

1. Schmidt, R., In *La Jojoba, Memorias de la II Conferencia Internacional Sobre*, Baja California, Mexico, 1976, p. 143.
2. Rost, T. L. and Simper, A. D., In *La Jojoba, Memorias de la II Conferencia Internacional Sobre*, Baja California, Mexico, 1976, p. 135.
3. Roth, J., *Flora*, 1957, 144, 163.

### LYTIC PHAGES FOR *BACILLUS THURINGIENSIS* IN THE LOCALITY AROUND CALCUTTA

NIRMAL CHANDRA SOM, B. B. GHOSH AND M. K. MAJUMDAR

Department of Physiology, Calcutta University, Calcutta 700 009, India.

\*Central Drugs Laboratory, 3, Kyd Street, Calcutta 700 016, India.

THE use of *Bacillus thuringiensis* as an insect pathogen is widely accepted<sup>1</sup>. There are many reports on the

presence of phage of *B. thuringiensis*<sup>2-6</sup>. As phage infection may transform *B. thuringiensis* which is apparently non-pathogenic to mammalian pathogenic variety<sup>2</sup>, we tried to survey the locality around Calcutta for the presence of the phage. But due to the presence of some spreading type chloroform resistant sporing bacteria in the locality, we were unable to determine phage titer by conventional method. In the present communication we report the phage population in the locality around Calcutta by using a tetracycline and streptomycin resistant strain (developed in this laboratory) of *B. thuringiensis* ATCC 13366.

A strain *B. thuringiensis* TcSm resistant to 100 µg of streptomycin and 20 µg of tetracycline, and pathogenic to *Bombyx mori* developed in this laboratory from *B. thuringiensis* ATCC 13366 was used in the present work. Samples including soils from garden field, river/canals and municipal dumping ground were collected at random. Besides, water and mud from the river and the canals were also taken as samples. In 50 ml sterile stoppered tube, 20 g of soil/mud were brought to 30 ml level by adding sterile water. The tube was vigorously shaken for 30 min and 15 ml suspension were centrifuged at 2000 r.p.m. for 10 min. From the resulting supernatant, 10 ml were treated with 0.5 ml chloroform, 200 µg tetracycline and 1000 µg of streptomycin. The above preparation in 7.5 ml (such high volume was necessary as the phage count was low) portions were mixed with 0.5 ml of *B. thuringiensis* TcSm spore suspension and 2.0 ml of molten and temperate agar medium-I<sup>7</sup> (4 times concentrated and contained 4% agar). This was distributed as seed layer over agar plates containing 25 ml of medium-I supplemented with tetracycline and streptomycin in the above stated concentrations. Plates were incubated and the plaque count was made after 16 hr.

For the determination of phage titer altogether 108 soil samples collected from the locality around Calcutta were examined. The result (table 1) shows that the highest titer was found in the river/canal side soil or water or mud and a good number of samples were phage-positive in the above mentioned site. In all samples the plaques were round and clear with sharp margin. But the diameter was variable. By noting the diameter, three typical plaques were purified by the conventional method. They were designated as P-1, P-2 and P-3. Although their plaque sizes were found variable under different cultural conditions, the relative plaque size was constant.

For cross infectivity studies with these three phages, the bacteria used were *Bacillus subtilis* NCTC 8236, *B. subtilis* ATCC 6633, *B. cereus* NCTC 10320, *B. cereus* mycoides sub sp. ATCC 11778, *B. pumilus* NCTC 8241 and 50 isolated sporing soil *Bacillus*. Besides these, *B. thuringiensis* ATCC 13366 and its mutants (developed in this laboratory): NSm (resistant to 3000



TABLE I

Determination of *B. thuringiensis* phage titer in some soil samples.

Isolation site	No. of Samples screened	No. of Samples containing phage	No. of phage in 100 g or ml positive samples
Garden soil	32	6	20-40
River/canal side soil or water or mud	32	18	50-200
Field soil	34	4	20-40
Municipal dumping ground	10	—	—

$\mu\text{g/ml}$  of streptomycin), Tc(d) (resistant to 75  $\mu\text{g/ml}$  of tetracycline), Bnc (resistant to 20  $\mu\text{g/ml}$  of bacitracin) and TcSm (resistant to 20  $\mu\text{g/ml}$  of tetracycline and 100  $\mu\text{g/ml}$  of streptomycin) were used. Bacterial lawn in M-1 medium was made, using the spore inocula of all the strains and each plate was inoculated at three sites with isolated phages P-1, P-2 and P-3. Infection of the bacteria was recorded noting the lysis at the inoculated spots.

The study shows that phages P-1, P-2 and P-3 could lyse the cells of *B. thuringiensis* ATCC 13366 and its mutant strains NSm, Tc(d), Bnc and TcSm but ineffective to all other *Bacillus* strains studied.

18 December 1981

- Burges, H. D. and Hussey, N. W., *Microbial control of insects and mites*, Academic press, London, New York, 1971, 67.
- Yoder, P. E. and Nelson, E. L. J., *Insect Pathol.*, 1960, 2, 198.
- Van Tassel, L. and Yousten, A. A., *Can. J. Microbiol.*, 1976, 22, 583.
- Skvortsova, M. M., Burtseva, L. I., Shashkina, N. I., Robertus, L. A., Fillippova, E. A. and Zhuravetskaya, N. I., *Izv. sib. Acad. Nauk, SSSR Ser. Biol.*, 1976, Nauk 49.
- Chapman, H. M. and Norris, J. R., *J. Appl. Bacteriol.*, 1966, 29, 529.
- Vasantharajan, V. N. and Munirathnamma, N., *Curr. Sci.* 1980, 49, 248.
- Prescott, S. C. and Dunn, C. G., *Ind. Microbiol.*, 3rd ed., McGraw Hill, 1952, p. 762.

## DISCOVERY OF ENDOTHYRIID FORAMINIFERS FROM THE BEDDED MALDEOTA PHOSPHORITE, GARHWAL HIMALAYA

PRABHA KALIA

Department of Geology, University of Delhi, Delhi 110 007, India.

ENDOTHYRIID foraminifers are being recorded from the rock phosphates of Mussoorie-Dehradun Sector, Garhwal Himalaya. The occurrence of endothyriids from the Transitional Limestone unit, intervening between the Upper Krol and the Phosphorite horizon, from Maldeota, Dehradun has been earlier reported<sup>1,2</sup>. The present samples yielding foraminifers were collected from the Phosphorite (Surkhet) mine, Maldeota, Dehradun. The samples were collected from the phosphorite overlying the Krol-Limestone (Sample No. 1a), from the Chert-Phosphorite (Sample No. 1), from the level in phosphorite rich in chert (Sample No. 2) and from the Chert-main ore contact (Sample No. 3). The samples (Nos. 1-3) have yielded relatively few foraminiferal remains. The sample of phosphorite sampled near the adit one (No. 4) has yielded a rich and diverse assemblage of endothyriid foraminifera. The main ore body (Nos. 4-6) contains rich assemblages of foraminifera. Sample (Nos. 4 and 5) contain mainly *Endothyra* spp., *Nodosinella* spp., *Paraendothyra* sp., *Globivalvulina bulloides* and *Tetrataxis conica*. In Sample No. 6 taken from the upper middle part of the ore body, a few members of the family Tournayallidae also appear.

The foraminiferal assemblage recorded from Phosphorite includes, *Endothyra* spp. (figures 1-3) *Nodosinella digitata* Brady, 1876, (figure 4), *N. Nodosariformis* (Cushman and Waters), 1828, (figure 5), *Tetrataxis conica* Ehrenberg, 1854, (figures 8, 9, 11, 12), *Paraendothyra* sp. (figure 10) and *Globivalvulina bulloides* Brady, 1875, (figures 2, 13). The members of the family Tournayallidae are commonly present in the upper-middle part of the ore body and are represented by *Tournayella* sp. (figure 7) and *Glo-mospiroides* sp. (figure 6). This assemblage is characteristic of Carboniferous-Permian age<sup>3</sup>.

The discovery of endothyriid foraminifers from the phosphorites of Garhwal Himalaya is significant for the geology of the region since, these foraminifers are reliable indices of the geological age. The occurrence is significant because only the endothyriid group of foraminifers is present along with skeletal blue green algae, preserved in phosphorite. Such an association indicates restricted basin with low salinity<sup>4</sup>.