

Singh⁸ has tested a number of bacteria as food for free-living amoebae. On the basis of utilization of bacteria as food for amoebae he classified bacteria into four groups: (1) Species readily and completely eaten; (2) slowly and completely eaten; (3) those that were partially consumed and (4) an entirely inedible group. In a large number of bacterial species any particular character such as gram staining, motility, presence of proteolytic ferment, slime production among other aspects could not be correlated with their edibility or inedibility by amoebae. However, amoebae failed to grow in presence of bacteria producing toxic pigments or metabolites. In the present study similar results have been obtained when amoebae were cultured on different strains of mycobacteria. No correlation could be observed between strains that were eaten up and those that were not eaten up by the amoebae.

The authors are thankful to Dr. Nitya Nand, Director, Central Drug Research Institute, for his encouragement and interest in this line of work. Thanks are also due to Shri S. K. Chakraborty and Miss Reeta Srivastava for their technical assistance.

Central Drug Research
Institute,
Lucknow 226 001, India.
August 26, 1977.

B. N. KRISHNA PRASAD,
S. K. GUPTA.

1. Carter, R. F., *Trans. Roy. Soc. Trop. Med. Hyg.*, 1972, 66, 193.
2. Dwivedi, J. M. and Singh, C. M., *Indian J. Microbiol.*, 1965, 5, 31.
3. Wang, S. S. and Feldman, H. A., *The New England J. Med.*, 1967, 277, 1174.
4. Armstrong, J. A. and Periera, M. S., *Brit. Med. J.*, 1967, 1, 212.
5. Skocil, V., Cerva, L. and Serbus, C., *J. Hyg. Epidem. Microbiol., Immunol.*, 1970, 14, 61.
6. —, —, — and Nejeldo, V., *Csika Epidem. Microbiol. Immunol.*, 1970, 5, 240.
7. Proca-Ciobanu, M. and Jonescu, M. D., *Arch. Roum. Path. Exp. Microbiol.*, 1975, 34, 329.
8. Singh, B. N., *Pathogenic and Non-pathogenic Amoebae*. Publishers: The Macmillan Press Ltd., London and Basingstoke, 1975, p. 4.

EFFECT OF IRRADIATED MEDIUM ON GROWTH AND SYNTHESIS OF MACROMOLECULES IN GERMINATING SPORES OF *B. SUBTILIS*

THE effect of irradiation has been studied in a variety of biological test systems ranging from bacteriophages to human cells which were cultured on irradiated media¹. Irradiated bacterial medium has been shown to induce auxotrophic mutations and also retardation of growth²⁻⁵. Frey and Pollard⁶

observed that the irradiated medium caused a cessation of DNA synthesis in *E. coli*. Contrary to the inhibitory effects, some investigators found stimulatory effects of irradiation on the germination of seeds, plants and bacterial growth⁷⁻⁹. The stimulatory effects of irradiation largely depended on irradiation dose and duration of treatment. This communication deals with the effect of gamma irradiated medium on growth and on synthesis of protein and nucleic acids in germinating spores of *B. subtilis*.

The *B. subtilis* spores were prepared in yeast extract-peptone (Y.E.P.) medium as described earlier¹⁰. The preliminary experiments on the growth of *B. subtilis* spores in Y.E.P. medium showed that the replication began after 4 hrs of incubation at 37°C, as evidenced by the increase in the titer of the spore concentration. The yeast extract-peptone medium was irradiated at 100 Kr using the gamma source.

About 500 ml of spore culture (containing about 10⁶ spores/ml) was centrifuged at 5000 rpm and the pellet was divided into two equal halves and each was suspended in 200 ml of freshly irradiated and unirradiated media. The cultures were incubated at 37°C and stirred with a magnetic stirrer. Aliquots of 0.5 ml were withdrawn from both the cultures at various time intervals (0 to 8 hrs) and spore/cell concentrations were determined by titration on Y.E.P. agar medium plates¹¹.

The percentage of growth increase in irradiated and control cultures is presented in Table I. It is evident that irradiated medium at 100 Kr stimulated the growth of *B. subtilis* spores. Dharkar⁸ observed stimulation of growth of several types of bacteria grown on medium containing the extract from irradiated potatoes. It was stated that stimulation of growth might occur due to a greater availability of amino acids and sugars because of radiolytic breakdown of proteins and starch or carbohydrates contained in the medium¹.

TABLE I

Growth of *B. subtilis* spores in unirradiated and 100 Kr irradiated Y.E.P. medium

Hours of incubation	Control medium titre/ml	% increase	Irradiated medium titre/ml	% increase
0	35.0 × 10 ⁶	0.0	38.4 × 10 ⁶	0.0
4	36.0 × 10 ⁶	2.9	164.0 × 10 ⁶	327.0
6	52.0 × 10 ⁶	48.6	220.0 × 10 ⁶	472.9
8	76.0 × 10 ⁶	117.1	344.0 × 10 ⁶	795.8

The measurements of protein, RNA and DNA were carried out in the above experiment by withdrawing samples of 10 ml from irradiated and control cultures at 0 and 8 hrs. The samples were washed twice with saline and the protein, RNA and DNA were extracted¹². The protein estimation was done by the method of Lowry *et al.*¹³; and RNA and DNA were estimated by orcinol¹⁴ and by diphenylamine¹⁵ methods respectively. The contents of protein in irradiated medium culture were greater than controls (Table II) suggesting the stimulatory effect of irradiated medium on protein synthesis. Similarly, there was a stimulation in RNA and DNA synthesis in 100 Kr medium culture. Increase in RNA and DNA contents in *Penicillium expansum* irradiated with 50 Kr was reported by Chou *et al.*¹².

TABLE II

The measurements of protein, RNA and DNA of germinating *B. subtilis* spores cultured in 100 Kr irradiated and control Y.E.P. medium

Content	Hours after incubation	Control medium mg/10 ml culture	% increase	Irradiated medium (mg/100 ml culture)	% increase
Protein	0	10.840	0.0	11.000	0.0
	8	13.000	19.6	16.500	50.0
RNA	0	0.190	0.0	0.200	0.0
	8	0.399	110.0	0.649	224.5
DNA	0	0.130	0.0	0.140	0.0
	8	0.301	131.5	0.719	413.6

Y.E.P. medium contained 1% yeast extract, 0.8% peptone and 0.5% sodium chloride.

Thus, it may be concluded that irradiated Y.E.P. medium stimulated the growth and synthesis of macromolecules as compared to the controls.

Department of Microbiology,
Osmania University,
Hyderabad 400 007 (A.P.),
September 17, 1977.

H. POLASA.
SUNEETHA RAJE.
L. GEETHA.
S. P. RAO.

- Bhavnani, D. and Polasa, H., *Ind. J. Hered.*, 1973, 5, 19.
- Frey, H. E. and Pollard, E. C., *Rad. Res.*, 1966, 28, 668.
- Chopra, V. L., Natarajan, A. T. and Swaminathan, M. S., *Rad. Bot.*, 1963, 3, 1.
- Govilla, O. P., Rao, C. H. and Iyer, R. D., In *Plant Tissue and Organ Culture—Symp. Int. Soc. Plant. Morph. India*, ed. P. Maheswari and N. S. Rangaswami, 1963.
- Dharkar, S. D., *Food Irrad. BARC (Trombay)*, 1969, p. 27.
- Raghavan, D. and Polasa, H., *The Ind. J. Hered.*, 1974, 6, 109.
- Clowes, R. C. and Hayes, W. (eds.), *Experiments in Microbial Genetics*, Blackwell Scientific Publ., Oxford, 1968.
- Chou, T. W., Salunkhe, D. K. and Singh, B., *Rad. Bot.*, 1971, 11, 329.
- Lowry, O. H., Rosebrough, H. J., Farr, A. L. and Randall, R. L., *J. Biol. Chem.*, 1951, 193, 265.
- Pessey, M., *Bull. Soc. Chem. Biol.*, 1950, 32, 701.
- Burton, K., *Biochem. J.*, 1956, 62, 315.

AN INTERESTING OBSERVATION ON A COMPSOPOGON GROWING AT ALLAHABAD

OUT of 11 species of the genus *Compsopogon* 5 species viz., *C. coeruleus* (Belbis) Montagne, *C. hookerii*, *C. iyengarii* Krishnamurthy, *C. indicus* Das and *C. aeruginosus* (J. Ag.) Kuetzing have been reported from India¹⁻⁴. The present note records an interesting observation on a form growing at Allahabad which is not on record for any taxon of the genus *Compsopogon*. The alga was found growing in an uncommon habitat, i.e., outlet of a continuously flowing water channel issuing from a tube-well at Allahabad. The alga grows in this habitat all round the year growing epiphytically on filaments of *Cladophora* sp. and also on bricks of the water channel and mud. Externally the thalli are olive blue green or dark blue green in colour. The thalli are coarse and profusely branched, up to 42 cm long and 2 cm broad, and are in the form of a floating mass of filaments in slow moving water. Older filaments became brittle and nodulated. The diameter of filaments at constrictions varies between 300 to 700 μ . Branches are numerous and they arise at angles from 30° to 70° with respect to the main axis. The main axis is multiseriate and normally up to 800 μ thick.

Many mature thalli show twisting and curling of the branches. At the region of twisting or curling such a filament develops a small swelling which later grows into a large discoid vegetative structure (Figs. 1 and 2). One to three such structures may occur in

- Keshavan, P. C. and Swaminathan, M. S., *Rad. Bot.*, 1971, 11, 253.
- Blank, I. H. and Arnold, W. J., *Bact.*, 1935, 30, 21.
- Stone, W. S., Wyss, O. and Haas, F., *Proc. Natl. Acad. Sci. (U.S.)*, 1947, 33, 59.
- Chopra, V. L., *Mut. Res.*, 1969, 8, 25.