Wolf[11] has stated that Lymphocystis is a unique, benign, giant cell disease of world-wide distribution in freshwater and marine teleosts of relatively advanced evolutionary status. Various theories have been presented for Lymphocystis by several workers[1,2,7-9,12]. However, Walker[5] and more recently Walker and Weissenberg[6] demonstrated that these bodies were filled with virus particles when viewed under the electron microscope.

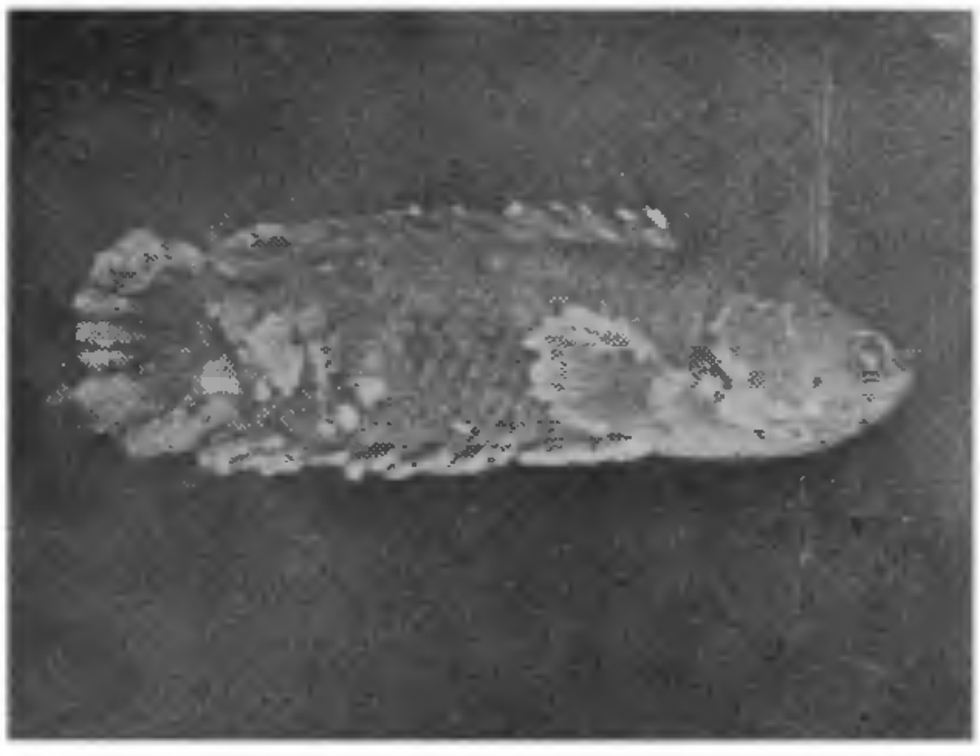

FIG. 1. Lymphocystis growth on the fins and body of *Anabas testudineus*.

There is no earlier report available on the occurrence of this disease in *Anabas testudineus*. Its occurrence has, however, been reported in other species[3,4,7,10]. The disease is known to be highly infectious but there is little information about the mode of its transmission.

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1. Awerinzew, S., *Zool. Anz.*, 1907, 31, 881.
2. Sandeman, G., *Ann. Rept. Scott. Fish Board*, 1892, 11, 391.
3. Shelton, R. G. J. and Wilson, K. W., *Aquaculture*, 1973, 2, 395.
4. Van Duijn, Jr. C., *Disease of Fishes*, Water life, Dorset House, London, 1956, p. 112.
5. Walker, R., *Virology*, 1962, 18, 503.
6. — and Weissenberg, R., *Ann. N.Y. Acad. Sci.*, 1965, 126, 375.
7. Weissenberg, R., *Sitzungsber. Kgl. Preuss. Acad. Wiss. Sitz. Physik. Mathem.*, Jg., 1914, p. 792.
8. Weissenberg, R., *Biol. Bull.*, 1939, 76, 251.
9. —, *Zoologica*, 1939, 24, 245.
10. —, *Ibid.*, 1945, 30, 169.
11. Wolf, K., *Symp. Zool. Soc. Lond.*, 1972, 30, 305.
12. Woodcock, H. M., *Rep. Lancs.*, Sea-Fish. Labs., 1904, 12, 63.

STUDIES ON PROTEASE PRODUCTION IN *PENICILLIUM JAVANICUM* VAN BEYMA

Penicillium javanicum Van Beyma P×26 elaborates a proteolytic enzyme. The optimum production of which could be induced with 10 days incubation at 25° C. Presence of glucose in the medium completely inhibits enzyme production.

Although the strain *Penicillium javanicum* displays a variety of biosynthetic activity and produces antibiotics[7], pigments[2], fat[5], etc., no studies have yet been done with regard to the abilities of the organism to elaborate proteolytic enzyme.

To find out a suitable nutritional environment conducive to steady accumulation of the enzyme the strain *P. Javanicum*, P×26 was grown in Czapek–Dox[1] medium where glucose was replaced by 3% wheat bran (WB), 1% wheat flour (WF), 3% rice bran (RB) and 0·1% Bovine Serum Albumin (BSA) separately. Thirty ml of each medium was taken in 100 ml capacity Erlenmeyer flask and was inoculated with conidial suspension having a concentration of $12{\cdot}0 \times 10^6$ (approx.) and incubated at 25° C for 10 days in the case of BSA and 5 days in the case of others, before the respective mycelia were collected. Ten replicates for each medium were considered.

Wheat bran–Czapek–Dox medium (WB–CD) was found to be the most suitable for protease production and was selected for further study. The flasks containing inoculum were kept at different temperatures, 15°, 20°, 25°, 30° and 35° C and the enzyme assay was done on the 5th day. Optimum incubation period of 10 days was determined by observing the accumulation of enzyme on 5th, 10th, 15th, 20th and 25th day at 25° C.

The effect of glucose on the production of enzyme was studied by adding glucose at different concentrations (1, 5, 10, 15 and 20%) to WB-CD medium and noting the result on the 5th and 10th days. The initial pH of the medium was adjusted to 6·5. One flask without glucose was taken as the control.

The enzyme reaction was carried out in 1 ml of 1·5% milk casein in 0·15 M phosphate buffer at pH 7·3 with 1 ml of culture filtrate and 1 ml ethylene—diamine tetraacetic acid (0·458 g EDTA dissolved in 100 ml of water) at 30° C for half an hour. The reaction was stopped by the addition of 1·0 ml of 20% TCA. Precipitate was then removed by filtration. After filtration, 0·6 ml of the filtrate was taken in a tube and 4 ml of protein reagent added to it. After 10

minutes. 0·4 ml Folin reagent was added to the mixture which was left for 30 minutes at room temperature. Colour of the enzyme assay mixture was read with a Bausch and Lomb Spectronic 20 at 540 nm against water blank. All the readings were corrected with reference to the values of blanks prepared by adding TCA at 0 minute in the reaction mixture containing enzyme solution, milk casein and EDTA. Unit activity of alkaline protease is expressed as the amount of enzyme that yields a change in optical density at 540 nm equivalent to 1 μg of Bovine Serum albumin per 30 min at 30° C.

Of all the substrates tried wheat bran was found most suitable for protease production (Table I). Variation in proteinase production with fermentation medium has also been reported in other fungi[3,6,8]. Wang and Hesseltine[11] reported in a more or less similar situation that proteolytic activity of *Rhizopus* was higher in wheat flour medium than in other media.

TABLE I

Effect of substrate on mycelial growth and enzyme production

Substrate	Mycelial wt. (mg)	Unit production of enzyme
Wheat bran	250·00	14·5
Wheat flour	350·00	10·2
Rice bran	150·25	5·0
Bovine Serum Albumin	100·15	..

The difference in the behaviour of the organism was ascribed as partially due to difference in the C/N ratio of the medium. It was also found by the same authors that proteolytic activity of *R. oligosporus* reached its maximum at 72-96 hours of incubation. Similar result was reported with other organisms[3,8,10] although with *Mucor pusillus*[10] the incubation temperature for optimum production was 35°C. However, the incubation time requried for optimum production of enzyme in *P. javanicum* is rather long extending 16 to days at 25° C (Figs. 1 and 2).

Epstein and Beckwith[4] reported that formation of inducive enzyme is often strongly inhibited when the bacteria have alternative energy source such as glucose. Sing *et al.*[9] showed that protease production in an *Aspergillus niger* mutant is inhibited in the presence of gluscose in the medium. A similar situation observed by Somkuti and Babel[10] in *Mucor pusillus* was explained as due to accumulation of organic acid in the medium with subsequent lowering of pH. But in the present investigation this does not appear to be the reason as the pH was acidic (4·5–5·0) on the 5th day and increased to 8·5 on the 10th day while there was no change of proteolytic acitvity.

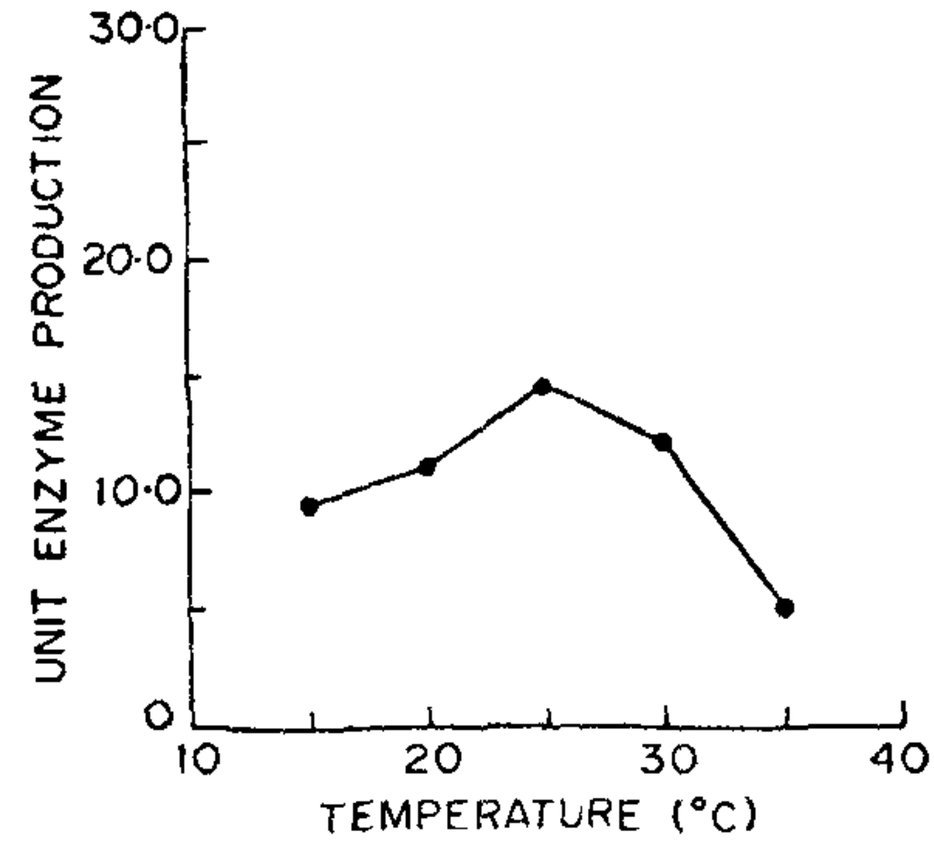

FIG. 1 Effect of incubation period on protease production in *P. javanicum*.

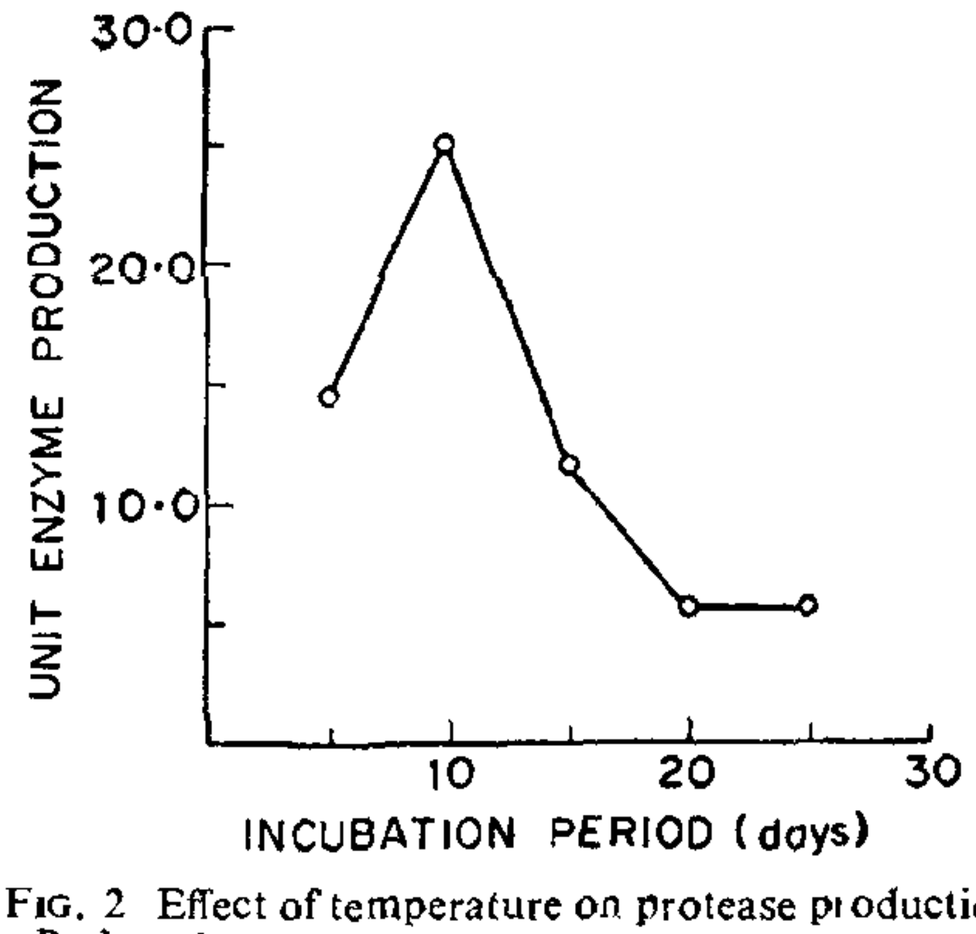

FIG. 2 Effect of temperature on protease production in *P. javanicum*.

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1. Basu, S. N., Bose, R. G. and Bhattacharya, J. P, *J. Scient. Ind. Res. (India)*, 1955, **14 C**, 46.
2. Bhattacharya, M. and Chaudhuri, K. L., *Proc.* 60th *Ind. Sci. Congr. III*, 1973, No. 140, p. 343.
3. Dworschack, R. G., Koepsell, H. J. and Lagoda, A. A., *Arch. Biochem. Biophys.*, 1952, **41**, 48.
4. Epstein, W. and Beckwith, J. R., *Ann. Rev. Biochem.*, 1968, **37**, 411.

5. Gray, W. D., *The Relation of Fungi to Human Affairs*, Henry Holt and Company, Inc., New York, 1959.
6. Johnson, A. G. and Martin, S. M., *Agr, Biol. Chem.*, 1964, 28, 734.
7. Mukherjee, M. and Chaudhuri, K. L., *Sci. and Cult.*, 1975, **41**, 548.
8. Oleniacz, S. W. and Pisano, M. A., *Appl. Microbiol.*, 1968, **16**, 96.
9. Sing, D. P., Sing, R. and Vyas, S. R., *Indian J. Microbiol.*, 1975, **15** 109.
10. Somukuti, G. A. and Babel, F. J., *Appl. Microbiol.*, 1967, **15**, 1309.
11. Wang, H. W. A. L. and Hesseltine, C. W., *Can. J. Microbiol.*, 1965, **11**, 727.

PASSION FLOWER MOSAIC VIRUS DISEASE A NEW RECORD FOR INDIA

THE genus Passiflora consists of over 300 species of herbaceous and woody climbers, occasionally shrubs or small trees. *Passiflora caerulea, P. foetida, P. edulis, P. alba, P. alatocaerulea, P. trifasciata, P. van volexmi, P. holosericea, P. laurifolia, P. decaisneana, P. murucuia, P. quadrangularis, P. racemosa* and *P. raddiana* are commonly cultivated in India. Passion flower (*P. caerulea*) plants growing in the University campus were found to be affected by virus-like disease with 70–80% incidence.

The symptoms observed on young foliage of diseased plants were dark green mosaic mottling, puckering or blistering, malformation and distortion of leaves. On older leaves the symptoms of chlorotic ring spots and mottling was evident, no malformation or distortion of the older leaves was observed. (Fig. 1). In the case of severe infection the plants were considerably stunted.

FIG. 1. Symptoms of passion flower mosaic virus disease on *Passiflora caerulea*.

Transmission studies by sap inoculation and budding were undertaken to prove the viral nature of the disease in question. Sap inoculation was done by macerating the leaves of passion flower displaying typical symptoms of moaic with 0·05 M phosphate buffer (pH 7·5) by pestle and mortar. The sap was inoculated on the young leaves of *Gomphrena globosa* and *Chenopodium amaranticolor* by cotton swab method. Necrotic local lesions were produced on the leaves of *G. globosa* after about 15 days of inoculation while chlorotic local lesions were developed on *C. amaranticolor* after 8 days of inoculation. This disease was also transmitted by budding and approach grafting. In about 25–30 days after budding the leaves of new sprouts arising from the budding of *P. edulis f. flavicarpa* exhibited typical symptoms of mosaic (Fig. 2). The seedlings of *P. edulis f. flavicarpa* for graft transmission purpose were grown from healthy seeds in earthen pots containing steam sterilized soil with compost mixture. In the case of approach grafting, the typical symptoms of mosaic developed after 30–35 days.

FIG. 2. Symptoms of passion flower mosaic on *Passiflora edulis f. flavicarpa* budding.

Besides mechanical and graft transmission this disease was also transmitted by an aphid vector. *Aphis gossypii*, Glover was found naturally colonizing on *P. caerulea* vines. Apterus forms of *A. gossypii* from naturally infected plants when transferred to seedlings of *P. caerulea* grown from healthy cuttings in glass-house, transmitted the disease readily.

The mosaic disease under study resembles *Passiflora* mosaic virus reported from Malaysia by Ong Ching Ang and Tig Wen Ponh[3]; Passiflora ring spots reported from Ivory coast by De Wijs[2] and Passiflora virus diseases reported from Kenya by Bakker[1] in symptomatology and transmission by aphid vector and grafting. However, it differs from passion flower yellow vein mosaic virus disease reported from India by Wilson and Satyaraj[4] in symptomatology and transmission properties. The reaction of *C. amaranticolor* and *G. globosa* to the present virus is