Indian Journal of Ecology 5: 253-255, 1978

ESTIMATION OF CELLULOLYTIC AND PHOSPHORUS-SOLUBLIZING ACTIVITY OF THERMOPHILIC *NOCARDIA* SPP. AND *STREPTOMYCES ALBUS*

Microbial enzymes find a mani-fold application in the fields of industry, medicine and agriculture; for instance, the proteolytic enzymes are used in the treatment of necrotic wounds and ulcers ; cellulolytic organisms degrade organic matter rich in cellulose and this property is being exploited for the production of single-cell protein from cellulosic wastes. Phosphorus-solublizing capacity is also beneficial to the plants, as they can pick up this element in the solublized form and this way micro-organisms help to maintain phosphorus balance in the soil. Keeping in view these considerations and the tropical climate of India, which is more conducive to the growth of thermophilic actinomycetes, the work has been undertaken on the relatively little-explored field of thermophilic actinomycetes. (Mizusawa *et al.*, 1964, 66; Desai and Dhalla, 1969; Fergus, 1964).

In the present investigation, enzymatic analysis was carried out with *Streptomyces albus* (a chromogenic variety from the soil), *Nocardia* spp. (from bagasse) and two more *Nocardia* spp. (from manure). These organisms had been isolated from the sources mentioned above, using glucose-yeast extract agar and starch-yeast extract agar and incubating the plates at 50°C in a humidified incubator for 5—7 days. An addition of 0.3 ml of one per cent streptomycin sulphate solution was made at the time of pouring the medium to suppress bacterial growth.

Cellulolytic activity was determined by growing the culture in a medium containing cellulose 1.5%, potassium dihydrogen phosphate 0.2%, ammonium sulphate 0.1%, urea (separately cold-sterilized and added at the time of inoculation) 0.03%, magnesium sulphate 0.03% and zinc sulphate 1.2 ppm and the pH was adjusted to 6.8—7.0

The sterilized flasks containing the above medium were inoculated with the test culture in triplicate and incubated at 50°C for 7 days and the filtrate was analysed for CMCase activity by using the method of Miller *et al.* (1960).

Proteolytic activity was determined by inoculating the test cultures into a medium containing casein 1.5%,dipotassium hydrogen orthophosphate 0.15%,sodium metaphosphate 0.15%. glucose 0.4%, ammonium sulphate 0.15%, calcium chloride 0.2%, and the pH was adjusted to 7.2. The sterilized and inoculated flasks were incubated at 50°C for 12 days and the broth was filtered and analysed for proteolytic activity, using the method of Ouchi (1962).

Phosphorus solublization was assessed by growing the cultures in a basal medium containing glucose 1%. peptone 0.5% and sodium chloride 0.3% with the addition of phosphorus sources (calcium phosphate, phytin and lecithin)

made at a rate of 50 mg/50 ml of the medium, and the pH was adjusted to 7.0. The sterilized and inoculated flasks were incubated at 50 C for 12 days and then filterate was used to assess phosphorus solublization by using the method of Koeing and Johnson (1942).

All the isolates showed cellulolytic, proteolytic and phosphorus-solublizing activity. Carboxy methyl cellulase (CMCase) production varied between 0.30 and 0.40 units/ml, as presented in Table 1. One of the *Nocardia* species from manure showed the maximum CMCase (0.45 units/ml) in comparison with the *Nocardia* species isolated from bagasse which showed only 0.30 units/ml. Proteolytic activity ranged from 0.033—0.063 units/ml and with a slight change in pH towards the alkaline side, probably because of the liberation of nitrogenous products. Desai and Dhalla (1969) made a similar finding when they observed that caseinolytic enzymes were active even in the alkaline range. The isolate with a poor production of proteolytic enzymes would have less accessibility to proteinaceous substrates such as casein, and would be degrading it to a smaller extent in the environment.

Table 1. Production of carboxy methyl cellulase (CMCase) and protease by thermophilic *Nocardia* spp. and *Streptomyces albus*

Isolate	Source of isolation	CMCase units/ml	pH	Protease units/ml	pH
Nocardia sp.	Manure	0.34	7.4	0.042	7.4
Nocardia sp.	Manure	0.45	7.4	0.033	7.4
Nocardia sp.	Bagasse	0.30	7.5	0.063	7.5
Streptomyces albus	Soil	0.37	7.4	0.048	7.6

Phosphorus solublization, which ranged from 4.5 to 13.0 mg/50 mg Phosphorus source added (Table 2), provided a common observation with all the test cultures that an inorganic source, e.g. calcium phosphate, was solublized more than the organic form. Again in the organic form, phytin was solublized more than to lecithin and this observation suggests that in these actinomycetes, phytase production was more than lecithinase.

So far, the reports available on solublization are restricted mainly to bacteria and fungi (Sen and Paul, 1957; Vidhyasekran *et al.*, 1973), but much information is not available on the solublization of phosphorus by thermophilic actinomycetes.

Table 2. Rhosphorus solublization by thermophilic *Nocardia* spp. and *Streptomyces albus*

Isolate	Source of isolation	mg P_2 05 solublized/50 mg P source in 50 ml of the medium		
		Calcium phosphate	Phytin	Lecithin
Nocardia sp.	Manure	7.0	6.5	5.3
Nocardia sp.	Manure	6.5	6.0	4.5
Nocardia sp.	Bagasse	13.0	12.0	11.5
Streptomyces albus	Soil	10.0	10.0	8.5

The foregoing discussion suggests that this group of microorganisms has the ability to play a vital role in degrading organic substrates, especially when the temperature rises during degradation, as in composting. Secondly, better variants of this group of organisms can be isolated and screened for industrial purposes. In this way, these activities, which play an important role in the ecosystem, can be exploited with certain limitations and improvements for various purposes, such as those of industrial and medicinal importance.

Department of Microbiology
Punjab Agricultural University
Ludhiana

Vijay K. Sharma
D.S. Chahal

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