AUXIN PRODUCTION POTENTIALITY OF NITROGEN FIXERS ISOLATED FROM THE PHYLLOSPHERE OF CROP PLANTS

EXISTENCE of an indigenous nitrogen fixing bacterial flora on the leaf surfaces of a large number of tropical plants discovered by Ruinen[1,2], led to investigate the frequency of such population in a number of crop plants[3,4]. While investigating the various properties of the phyllosphere organisms it is noticed that many of the isolates are not only capable of fixing nitrogen *in vitro* but are also producing antimicrobial substances[5] and plant growth regulators like indole acetic acid (IAA). It has been reported that both epiphytic bacteria and fungi are capable of synthesising a variety of growth-promoting substances either on the root surfaces or on buds or leaves[6,7]. Various species of *Azotobacter* are potent synthesisers of plant growth substances[3–10]. Even epiphyllous yeasts have been reported to produce IAA[11]. Libbert *et al.*[12] and Yakushkina and Tarasenko[13] also reported formation of hormones by some epiphytic microorganisms. Auxins and their precursors are synthesised by many resident fungal population on the leaf surfaces[14]. It is now unequivocally established that many bacteria and fungi are potential phytohormone producers[15], particularly when tryptophan is available to these organisms. Nitrogen fixing organisms are quite versatile in this respect as they liberate various amino acids, including tryptophan, into their surroundings.

In the present communication the IAA producing capacities of different nitrogen fixing bacterial isolates obtained from the phyllosphere of various crops, as influenced by varying temperatures, change of sugar in the medium and aeration, are reported.

The organisms were isolated in Burk's agar medium from seven common crop plants like potato, tomato, rice, sugarcane, jute, wheat and mustard. Excepting in rice, sugarcane and jute only one type of nitrogen fixer could be isolated for which a single strain was selected from each of the other crops, *i.e.*, potato, tomato, wheat and mustard. While in these three crops at least two types were isolated and studied. Inoculum was prepared by growing the organisms in Burk's broth for 24 hours. To measure IAA production capacity a test medium developed by Libbert and Risch[15] was used. This medium (20 ml), taken in 100 ml flasks, were inoculated with 1 ml of the 24 hour old culture and incubated at 30° C, unless otherwise stated. For each strain different sets of flasks were incubated both on a rotary shaker (90 cycles/min) and in still condition. To find out the effect of temperature on IAA production, the different batches of flasks were incubated at 25°, 30°, 35° and 40° C. The effect of two sugars glucose and sucrose was studied after the addition of the respective sugar at 2% concentration in the medium.

For IAA analysis in the medium, cells were separated by centrifugation of 24 hours old culture at 5000 g for 30 min in Sorval cold centrifuge at 5° C. One part of the supernatant fluid was mixed with two parts of Salkowaski reagent[16] in a test-tube and blended thoroughly with the aid of a Vortex mixer for two minutes. After one hour the intensity of the developing red colour was measured in a Klett Summerson Colorimeter using a green filter. The amount of IAA produced was known after comparing this reading with a standard curve.

IAA is produced by a variety of organisms growing on leaf surfaces which include fungi and bacteria. Nitrogen fixing bacteria like *Azotobacter* is a potent producer of this auxin[3] which incidentally is also isolated from leaf surfaces[14,13]. The organisms isolated from crop plants and studied here are nitrogen fixing organisms but not all of them are *Azotobacter* species. Earlier IAA-producing organisms other than nitrogen fixers, were reported from maize, oat[11,9] and pea[15] but were not reported from such crops as reported now. Sugars bear differential influence on IAA production and it was noted curiously that sucrose supported better production of this important plant auxin, while many of the strains were totally incapable of synthesising IAA in glucose medium (Table I). Another

TABLE I

Amount of IAA produced (ug/ml) in medium with and without shaking
(*Data represent an average of five determinations*)

Organism	Medium with sucrose		Medium with glucose	
	Still culture	Shake culture	Still culture	Shake culture
Potato	1·3	2·2	1·0	1·4
Tomato	2·5	3·2	1·9	2·6
Rice (REN_2)	4·0	8·1	1·4	3·9
Rice (PEN_2)	2·0	3·1	0·0	0·0
Sugarcane (SCN_1)	3·5	5·1	0·0	0·0
Sugarcane (SCN_2)	5·0	6·7	0·0	0·0
Jute (JN_1)	1·0	1·5	0·8	1·2
Jute (JN_2)	1·4	2·2	0·0	0·0
Wheat	1·8	3·0	1·0	2·6
Mustard	4·1	5·2	0·0	0·0

major factor is temperature. In the fields the plants are exposed to varied temperature fluctuations. This temperature dependance of IAA production by the phyllosphere organisms have not been reported earlier excepting that by some fungi[14]. Here the objective was to determine the temperature congenial for IAA production. The organisms can grow equally well within a temperature range of 25° to 35° C but IAA production remains restricted uniformly almost around 30° C in all organisms which of course is much lower in case of fungi[14]. A sharp inhibition was noticed both at 25° and 40° C (Table II). It becomes obvious therefore that in tropical countries phyllosphere bacteria play a major role in at least supplying this plant hormone to the supporting hosts as the field temperature is higher. The auxin is produced at specific time when the temperature is around 30° C. In cold climates the leaf surface fungi on the other hand may be helpful agents in supplying auxin to the inhabiting plants because of their lower temperature dependance for IAA production.

TABLE II

Effect of temperature on IAA production (expressed in μg/ml) by the bacterial isolates from crop plants (Data represent an average of five determinations from each isolate obtained from each crop)

Isolated from	Temperature			
	25° C	30° C	35° C	40° C
Potato	0·3	1·4	0·9	0·6
Tomato	0·2	2·5	1·6	1·3
Rice (REN_2)	0·4	4·0	1·5	0·8
Rice (PEN_2)	0·2	2·0	1·2	0·8
Sugarcane (SCN_1)	0·3	3·3	1·6	1·0
Sugarcane (SCN_2)	0·4	4·8	2·6	1·6
Jute (JN_1)	0·1	1·2	0·8	0·3
Jute (JN_2)	0·2	1·4	1·2	6·5
Wheat	0·0	2·0	1·3	0·8
Mustard	0·3	4·3	2·3	1·7

The resident nitrogen fixers may remain handicapped in fixing enormous nitrogen because the process itself occurs at low oxygen tension; but, auxin production is accelerated with enhanced aeration. From Table I it is evident that aeration in shake cultures is congenial for IAA production by the phyllosphere organisms.

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Microbiology Laboratory,
Department of Botany,
University College of Science,
35, Ballygunge Circular Road,
Calcutta 700 019,
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MAHUYA BANERJEE.
A. K. CHANDRA.

1. Ruinen, J., *Nature*, 1956, **177**, 220.
2. —, *Plant and Soil*, 1965, **22**, 375.
3. Shende, S. T., Balasundaram, V. R., Bhurat, M. C. and Sen, A., *Indian J. Agric. Sci.*, 1968, **38**, 298.
4. Bhurat, M. C. and Sen, A., *Ibid.*, 1968, **38**, 319.
5. Chandra, A. K., Pati, B. R. and Gupta, S., *Abstract, The Fifth International Conference on Global Impacts of Applied Microbiology*, Bangkok, 1977, p. 52.
6. Chandramohan, D. and Mahadevan, A., *Planta*, 1968, **81**, 201.
7. Katznelson, H. and Cole, S. E., *Canad. J. Microbiol.*, 1965, **11**, 733.
8. Brown, M. E. and Burtingham, S. K., *J. Gen. Microbiol.*, 1968, **53**, 135.
9. Azcon, R. and Barea, J. M., *Plant and Soil*, 1975, **43**, 609.
10. Barea, J. M., Navarro, E. and Monto, Ya, E., *J. Appl. Bact.*, 1976, **40**, 129.
11. CaDien, H. C. R., *Hebb. Seaness. Acad. Sci. Sea D Sci. Nature*, 1971, **272**, 941.
12. Libbert, E., Wichner, S., Schiewer, U., Risch, H. and Kaiser, W., *Planta*. 1966, **68**, 327.
13. Yakushkina, N. I. and Tarasenko, A. A., *Biol. Nauk.*, 1971, **14**, 74.
14. Buckley, N. G. and Pugh, G. J. F., *Nature (Lond.)*, 1971, **231**, 332.
15. Libbert, E. and Risch, H., *Physiol. Plant.*, 1969, **22**, 51.
16. Gordon, S. A. and Webber, R. P., *Plant Physiol. Lancaster*, 1951, **26**, 192.
17. Iswaran, V., Sen, A. and Apte, R., *Plant and Soil*, 1973, **39**, 461.
18. Ruinen, J., In *The Biology of Nitrogen Fixation*, ed. A. Quispel, Elsevier Publishing Co., 1974.
19. Libbert, E. and Mantenffel, R., *Physiol. Plant.*, 1970, **23**, 93.