SUITABILITY OF CERTAIN CARBON SOURCES FOR THE ENRICHMENT OF NITROGEN-FIXING BACTERIA FROM SALINE ALKALI SOILS

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SINCE Beijerinck[1] (1901) discovered the aerobic nitrogen-fixing bacterium called *Azotobacter*, a large number of carbon sources have been reported to serve as the energy source and building blocks for the species[2-5] (Skinner,[2] Bryan,[3] Fedrov[4] and Jensen[5]). This communication reports results of an investigation designed to find out which of the several carbon sources serve as efficient substrates for the isolation of *Azotobacter* and other species of aerobic nitrogen-fixing bacteria from saline alkali soils.

A wide variety of carbon sources as formate, acetate, succinate, citrate, benzoate, salicylate, alcohol, mannitol, sorbitol, glucose and sucrose were tested for their suitability for the enrichment of the aerobic nitrogen-fixing species. The saline alkali soils used as inocula in these experiments were obtained from the soil-testing laboratories of the Punjab Agricultural University. Some were also collected from the Regional Agricultural Station, Gurgaon, Haryana. The soil samples were stored at room temperature (30° C) in glass bottles prior to examination, which was done wherever possible soon after collection and storage.

Ashby's medium,[6] suitably modified with the substitution of the various carbon sources listed above, was used in this study as it proved to be efficient in most experiments conducted with normal soils. The pH of the medium was maintained at 7·0 for all the soils and irrespective of their pH except in few cases when the mannitol medium pH was adjusted correspondingly to the soils used as inocula with a view to see if the pH adjustments of the medium could lead to different results.

Enrichments in 100 ml of the liquid medium with various carbon sources were set in 250 ml. Erlenmeyers flasks inoculated with different soils of pH ranging from 8·0 to as high as 11·2 on a rotary shaker (28° C) for 3-4 days. A loopful of the liquid enrichments was streaked on the corresponding solid medium after 2-3 successive transfers were made through the liquid medium. By repeated streaking on the solid medium pure cultures of the isolates were made. These were subsequently maintained on Ashby's mannitol agar slants.

In as much as sucrose has been reported to be the most suitable source for the enrichment of *Azotobacter* by Darzniek[7] and Babak,[8] a comparison of the results was considered desirable to be made with sucrose on one hand and mannitol on the other.

The cultures were identified by the procedures detailed in the *Bergy's Manual*[9] and the capacity of each of the cultures to fix nitrogen was estimated using uniformly mannitol as the carbon source. The initial and residual mannitol in the cultures before and after growth was determined by Burton's[10] procedure (Table III).

TABLE I

Relationship between the enrichment of aerobic nitrogen-fixing bacteria and the carbon source

Carbon source	pH of the soil sample			pH of the enrichment medium
	8·0	9·0	9·8	
Formate ..	—	—	—	7·0
Acetate ..	—	—	—	7·0
Succinate ..	—	*	*	7·0
Citrate ..	—	*	*	7·0
Benzoate ..	—	—	*	7·0
Salicylate ..	—	*	—	7·0
Alcohol ..	*A. chroococcum*	—	*A. chroococcum*	7·0
Mannitol ..	*	—	*Derxia* sp.	7·0
" ..	*	*	do.	9·8
Sorbitol ..	*	—	*A. chroococcum*	7·0
Glucose ..	*	*A. chroococcum*	do.	7·0

* = Represents unidentified.
— = not enriched.

From the results reported in Table I, it is clear that only alcohol, mannitol, sorbitol and glucose serve as suitable sources for the enrichment of both *Azotobacter* and *Derxia* species. Whereas nine species of *Azotobacter chroococcum* could be isolated from saline alkali soils, only two strains of *Derxia* appeared; interestingly, as many as 22 other bacteria also grew in the nitrogen-deficient medium but in as much as none of them were found capable of fixing nitrogen, they are not considered important in this connection and were not as such taken for identification.

It is interesting to observe that in one soil of pH 9·0 glucose succeeded in enriching the

Azotobacter species whereas alcohol failed to do so. Equally interesting was the observation that only from one soil that sorbitol could seek out the nitrogen fixer. Mannitol, on the other hand, not only brought out both the *Derxia* species, but proved to be superior to sucrose in the enrichments of *Azotobacter* as well (Table II). Curiously, sucrose provided better enrichment conditions for the isolation of a solitary species of *Azotobacter* from a highly alkaline soil of pH 10·2.

TABLE II

Effect of sucrose and mannitol on the enrichment of aerobic nitrogen-fixing bacteria

Carbon source	pH of the soil	Isolate	pH of the enrichment medium
Sucrose ..	8·6	*	7·0
,, ..	8·9	*	7·0
,, ..	9·5	*	7·0
,, ..	9·8	*	7·0
,, ..	10·2	*A. chroococcum*	7·0
,, ..	11·1	*	7·0
,, ..	11·2	*	7·0
Mannitol ..	9·7	*A. ehroococcum*	7·0
,, ..	9·4	,,	7·0
,, ..	9·6	*	7·0
,, ..	9·8	*	7·0
,, ..	9·8	*A. chroococcum*	7·0
,, ..	9·8	*	7·0

* = Represents unidentified.

TABLE III

Nitrogen-fixing abilities of different isolates at their optimum pH for growth in Ashby's mannitol medium at 28° C

Sl. No.	Name of the organism	pH (optimum)	Nitrogen fixed (mg)	Residual sugar (mg)
1	*Derxia* sp. ..	8·0	8·4	0
2	,, ..	8·0	12·5	0
3	*A. chroococcum* ..	8·0	2·8	63
4	,, ..	8·0	4·2	27
5	,, ..	8·0	5·6	45
6	,, ..	8·0	4·2	27
7	,, ..	8·0	5·6	9
8	,, ..	7·0	4·2	0
9	,, ..	8·0	3·5	36
10	,, ..	8·0	4·2	45
11	,, ..	8·0	5·6	18

Derxia represents an interesting genus of nitrogen-fixing bacteria. Jensen *et al.*[11] reported on the nitrogen-fixing capacity of *D. gummosa* from West Bengal soils of pH 6·5. Its isolation from any other soils has not been so far reported. The organism isolated by the authors resembles *D. gummosa* in every respect except that it has not only a higher optimum pH (Fig. 1) but has a wider vigorous growth range of pH 6 to 10 as compared to that of the former which ranges from 5·5 to 9·0. Both the *Derxia* species reported here could fix appreciable amounts of nitrogen at their optimum pH of 8·0. It needs to be pointed in this connection that the very same species got enriched when the mannitol medium was adjusted at pH 7·0 or 9·8, the pH of the soil wherefrom the strains were isolated.

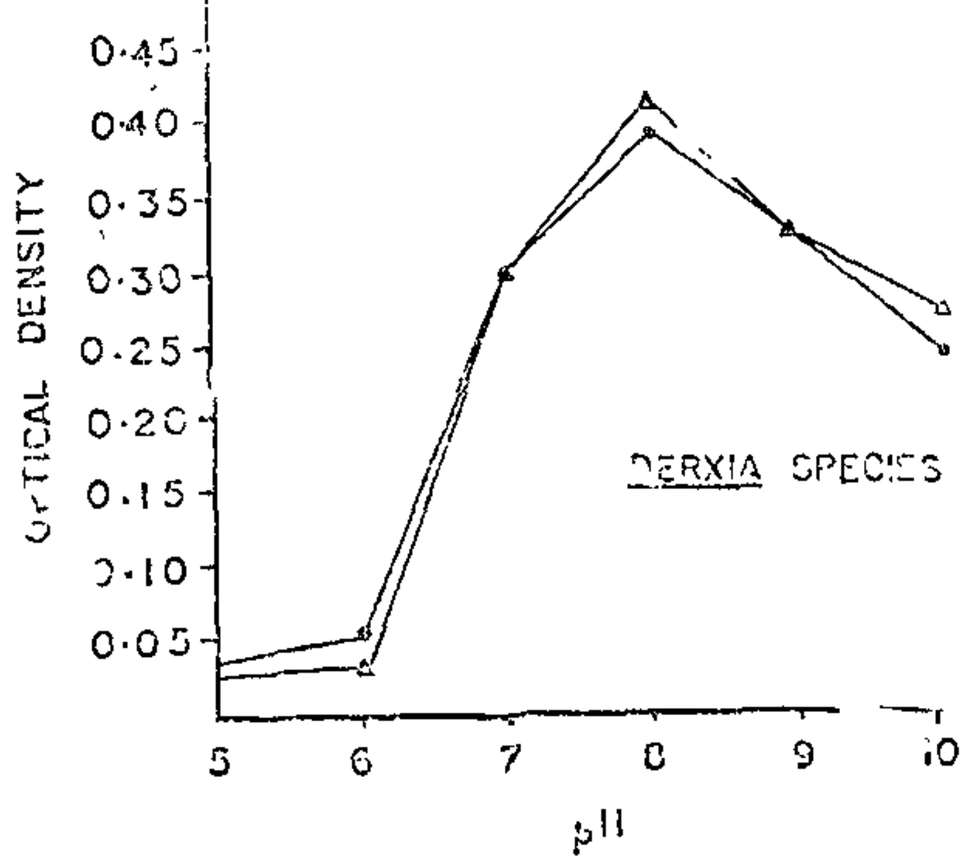

FIG. 1. Effect of pH on the growth of various isolates of *Derxia* species.

To conclude, it may be stated that both *Azotobacter* and *Derxia* species seem to thrive in saline alkali soils and mannitol appears to be the substrate of choice for their enrichments. It may be desirable to have the mannitol broths adjusted to neutrality and/or corresponding to the pH of the soils selected for isolation.

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