Potato plug : Growth excellent, wrinkled ; mycelium pale reddish brown ; aerial mycelium poor, white with pale rose patches ; plug turned to deep blackish brown.

Nutrien agar : Growth good ; reverse yellowish brown to brown (14–F–9) ; aerial mycelium scant white ; deep brown soluble pigment.

Yeast extract-malt extract agar ISP : Growth good ; reverse brown (12–H–10) ; aerial mycelium good, light dull pink (4–D–1) ; light brown soluble pigment.

The following biochemical reactions were positive : melanin, H_2S, tyrosinase, amylolytic activity, nitrate reduction, milk coagulation with acidic reaction, acid from glucose, catalase formation, gelatin liquefaction, casein hydrolysis, cellulolytic activity (slight) and sucrose inversion. The following reactions were negative : Indole production from tryptone, Loeffler's serum liquefaction, blood haemolysis and milk peptonization.

Carbon utilisation : *Excellent to good growth* : arabinose, fructose, glucose, galactose, inulin, inositol, maltose, mannitol, mannose, lactose, rhamnose, starch, sorbitol, sucrose, salicin and xylose. *Moderate to poor growth* : cellulose, glycerol, raffinose, sodium acetate, sodium citrate and sodium succinate. *Doubtful growth* : Dulcitol, digitonin and sorbose.

No growth : paraffin. Although the isolate broadly possesses poor antibiotic activity, the extracellular metabolite showed good activity on *P. vulgaris* and *S. typhosa* and the intracellular metabolite showed good activity on coagulase positive *Staph. aureus.*

Discussion

A detailed survey of the literature indicated that our isolate is related to *S. griscoroseus*[3] and *S. roseochromogenes*[4,5] and authentic cultures of these two were obtained and comparative studies made. The data show that *S. vijaensis* more closely resembles *S. griseoroseus* than *S. roseochromogenes.* However, even *S. griseoroseus,* although similar, differs from our isolate in (1) tyrosinase negative, (2) positive peptonization of milk and (3) no utilisation of raffinose, sorbitol, inositol and inulin.

In view of the above, our isolate is considered a new species and designated *S. vijaensis* sp. nov., as its origin in Vijayawada.

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Fermentation Technology, Laboratory,
Department of Pharmaceutical Sciences, Andhra University,
Waltair (A.P.), India, *June* 29, 1976.

K. SAMBAMURTHY.
P. ELLAIAH.

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NITROGEN FIXATION BY *AZOTOBACTER* OCCURRING IN THE RHIZOSPHERE OF C_4 PLANT SPECIES

THE non-symbiotic nitrogen fixing bacterium, *Azotobacter,* is receiving greater recognition among scientists[1,2]. The rank and luxuriant growth of certain tropical grasses like *Paspalum notatum, Digitaria decumbens* Hall., *Cynodon dactylon* Pers. and *Zea mays* L. is believed to be due to the association of active and efficient *Azotobacter* sp. in their root system[3]. Such plant species strangely enough exhibit an alternative but more efficient mechanism of carbon dioxide fixation called "C_4 pathway" (C_4-dicarboxylic acid pathway)[4] quite distinct from the universally functioning Calvin's pathway. In the present communication the N-fixing efficiency of several isolates of *Azotobacter* obtained from C_3 and C_4 plant species is compared.

From the rhizosphere of three species of C_4 plants (Table I) and from three C_3 plant species (Table II) *Azotobacter* cultures were isolated following standard microbiological techniques[5] and they were maintained on Waksman No. 77 agar medium. The N-fixing ability of the isolates was determined following the procedures detailed by Rangaswami and Subba Raja[6]. The total nitrogen in the broth after 10 days of growth of the organism under shake culture conditions was determined in a micro-Kjeldhal unit[7]. The respiratory activity of the isolates was measured in a Warburg respirometer[8]. The growth-promoting efficiency of *Azotobacter* isolates was tested on three rice varieties following the paper roll towel seed germination technique[9]. The results are presented in Tables I to III.

TABLE I
Nitrogen fixing efficiency of Azotobacter *isolates*

Source of *Azotobacter*	Isolate No.	mg of nitrogen fixed/g of mannite
C_3 Plants:		
Oryza sativa L.	AB. 1	16·80
	AB. 2	18·00
	AB. 3	18·60
	AB. 4	11·50
	AB. 5	12·30
Gossypium arboreum L.	GA. 1	7·64
Eleusine coracana Gaertn.	EC. 1	8·93
	EC. 2	8·33
Capsicum annuum L.	CA. 1	9·52
C_4 Plants:		
Amaranthus viridis L.	Am. 1	19·60
	Am. 2	22·40
	Am. 3	19·80
Brachiaria mutica Stapf.	Bm. 1	12·50
	Bm. 2	22·40
Cyperus rotundus L.	Cyp. 1	22·40
	Cyp. 2	14·00
	Cyp. 3	25·20
	Cyp. 4	19·60

TABLE II
Respiratory activity of Azotobacter

Isolate No.	Respiratory activity* (μ moles of O_2 consumed/g of cell/hr)
AB. 2	249
AB. 3	68
Am. 1	260
Am. 2	229
Am. 3	102
Bm. 1	246
Bm. 2	110
Cyp. 1	139
Cyp. 2	186
Cyp. 3	622
Cyp. 4	485

* Data represent average of two estimations.

TABLE III
Effect of seed inoculation with Azotobacter *on three rice varieties*

Source of *Azotobacter*	Vaigai		IR. 20		Bhavani	
	Plumule (mm)	Radicle (mm)	Plumule (mm)	Radicle (mm)	Plumule (mm)	Radicle (mm)
1. C_3 Isolates*	16·2	42·3	6·5	25·7	12·3	30·5
2. C_4 Isolates*	26·1	51·7	7·0	30·0	14·8	34·3
3. Water control	15·6	40·6	4·0	19·7	8·6	27·7

Data represent average of 20 seedlings measured five days after germination.
* Average values for nine isolates.

The data clearly reveal that many of the *Azotobacter* isolates from the C_4 plants have proved to be very efficient in N-fixation as compared to the isolates from the C_3 plants like rice, cotton, chillies and ragi. The isolate from *Cyperus rotundus* L. has fixed the maximum quantity of nitrogen and the minimum was by GA. 1 from cotton. Admittedly, a few *Azotobacter* isolates like AB. 1, AB. 2 and AB. 3 have exhibited greater N-fixing ability than the isolates, Bm. 1 and Cyp. 2, obtained from C_4 plants. Among the isolates, Cyp. 4 has recorded the maximum respiratory activity and the least by AB. 3. The isolate, AB. 2, though from a C_3 plant species, exhibited a high respiratory activity comparable to that of C_4 isolates. It is quite reasonable that as suggested by Hill *et al.*[10], the "oxygenlabile nitrogenase" is conferred protection through the exceedingly higher respiratory activity.

The data on the response of three rice varieties to seed inoculation with *Azotobacter* are revealing. The isolates from the C_4 plants in general promoted the growth of rice seedlings much more than the isolates from the C_3 plant species. This observation is amply supported by the recent work of Iswaran and Sen[11] who inoculated the rice seedlings of the variety, Jaya with a culture of *Azotobacter* isolated from the roots of *Cyperus rotundus* L. and

obtained increased grain yields. The increased growth of rice seedlings observed in the present study may be due to the cumulative effects of both N-fixation and the synthesis of growth-promoting substances[12] and this area of investigation is receiving our attention. It may therefore be generalised that the *Azotobacter* isolates from the C_4 plant species are not only more efficient, but also exhibit more growth-promoting effect than the isolates from the C_3 plants.

Tamil Nadu Agricultural University,
Coimbatore 641 003,
July 29, 1976.

D. PURUSHOTHAMAN.
C. KASIRAJAN.
G. RANGASWAMI.

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INHIBITION OF INDUCTION OF STREPTOZOTOCIN DIABETES BY MANNOHEPTULOSE

STREPTOZOTOCIN (SZN), derived from *Streptomyces achromogenes,* induces diabetes by destruction of the beta cells of pancreatic islets[12]. Chemically, it is 2-deoxy, -2- (3-methyl-nitrosoureido) -D- glucopyranose[9]. Its diabetogenic action is prevented by 2-deoxyglucose (2DG)[6] which is an inhibitor of insulin release[10] and of glucose metabolism[13]. This, and some other observations[2,5], suggest that the betacytotoxic action of SZN is related to insulin release or glucose metabolism. Against this possibility is an observation[6] that SZN diabetes was not prevented by mannoheptulose (MH), a more potent inhibitor of insulin release[3] and glucose metabolism[4]. In view of the theoretical importance of this negative finding, we have investigated again the interaction between MH and SZN.

The diabetogenic action of a single injection of SZN (gift from Upjohn Co.) at different time intervals after a dose of MH (from Sigma Chemicals Co.) was determined, comparing the glucose tolerance of the treated male albino mice (20 to 25 g) with that of untreated controls and of mice receiving the same dose of SZN alone. Glucose tolerance was estimated as the area (GTA) under the glucose tolerance curve, after a glucose load of 3 g/kg after 16 hours of fasting. Blood samples collected from the orbital sinus at 0, 60 and 120 min after glucose injection were analysed for reducing sugar[1]. The mean control GTA was 228 mg hr/100 ml. ($\pm$ 19, S.D., in 24 mice). The increase (*i.e.*, Δ GTA) over this value, on the fifth day after treatment was a measure of the resultant diabetes and was calculated as percentage (% Δ GTA) of the mean control GTA. SZN (in citrate buffer at 5° C, pH 4·5), MH (in isotonic saline at 5° C) and glucose (in water) were given intraperitoneally in volumes of 0·01 ml./g body weight.

Mice which received SZN alone (Group A, Table I) or along with MH (Group B) became equally diabetic. But when MH preceded SZN with increasing time intervals (Group C, D, E), there was progressive reduction in the effect of SZN. With an interval of 60 min (Group E), diabetes was completely prevented. At 75 min (Group F), MH became altogether ineffective. Thus the protective action of MH against SZN was very much time dependent and the optimal time for MH administration was 60 min before SZN. A slightly lower dose of MH (125 mg/kg, Group G) was ineffective against the same dose of SZN (125 mg/kg). Thus the minimal dose of MH effective against 125 mg/kg SZN was 150 mg/kg. This dose was equally effective against a more severely diabetogenic dose of SZN (175 mg/kg, Groups H and I).

The present study establishes that MH can prevent the diabetogenic action of SZN. The preferential uptake of SZN by mouse beta cells is related to the glucose moiety in this nitrosourea[11]. The entry of MH into mouse beta cells is also by a glucose sensitive transport mechanism[8]. But the protective action of MH against SZN is not by competition for a common transport mechanism, because the protection is offered not when the circulating MH : SZN ratio would have been the highest (Group B), but 60 min later (Group I), by which time the