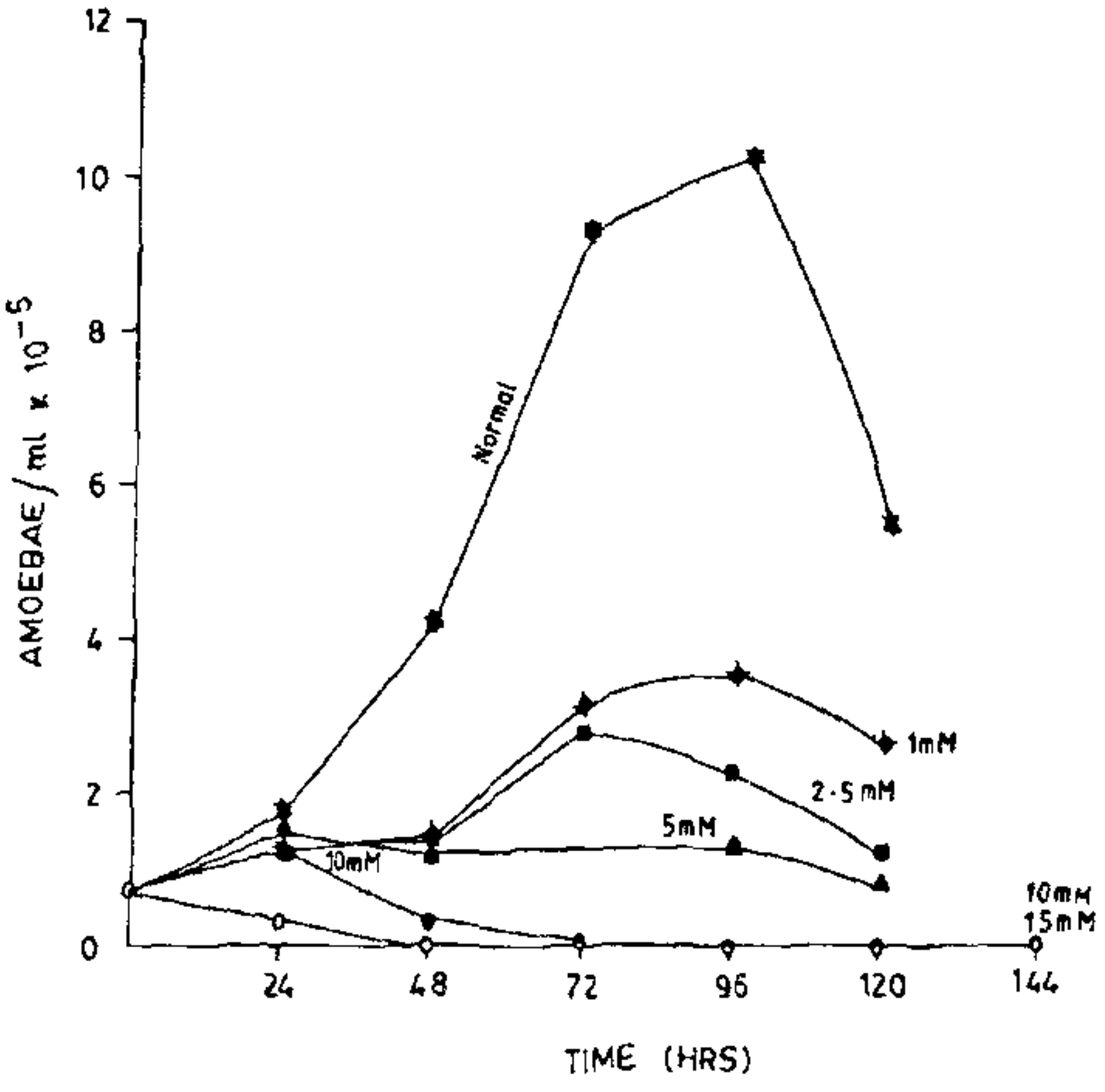

Figure 1. Effect of methylglyoxalbisguanylhydrazone on growth of *E. histolytica.*

Table 1 *Effect of α-methylornithine on the growth of E. histolytica.*

Conc. methyl-ornithine (mM)	Amoebae/ml × 10^{-5}			
	24 hr	48 hr	72 hr	96 hr
1.0	+	++	+++	4.95
2.5	+	++	+++	4.95
5.0	+	++	+++	4.20
Control	1.55	2.00	6.94	7.35

amoebic growth by α-methylornithine may be due to the relative insensitivity of amoebic ornithine decarboxylase to this inhibitor or due to the presence of alternate pathway for putrescine biosynthesis. Gillin *et al*[16] observed that growth of *E. histolytica* could not be inhibited by 20 mM DFMO. Ornithine decarboxylase of *E. histolytica* may thus differ from the enzyme from other protozoal systems which are highly sensitive to α-methylornithine. On the other hand, inhibition of amoebic growth by MGBG indicates the presence of a S-adenosylmethionine decarboxylase that is sensitive to this compound. The inhibition of growth of *E. histolytica* by synefungin[15] may also be directed at this site, and analogs of these inhibitors may serve as alternative chemotherapeutic agents.

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DINITROGEN FIXATION IN PEARL MILLET

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PEARL millet is an important grain and fodder crop grown in northern India during summer, generally with low levels of nitrogen fertilization. It is reported that this crop harbours a variety of nitrogen-fixing bacteria in its rhizosphere and rhizoplane[1,2] which may provide a substantial part of its nitrogen requirement. An attempt was, therefore, made to determine the extent of nitrogen fixation in this crop and

subsequently, to isolate and characterize some of the organisms involved in nitrogen fixation.

Two varieties of pearl millet namely HS1 and HC4 grown on the University farm during the summer and without any added nitrogen fertilization were selected for this study. *In situ* nitrogen fixation in these varieties was determined by the soil cores acetylene reducation assay (ARA)[3] at 8, 12 and 16 hr at the flowering stage.

The most probable number (MPN)[4] of nitrogen-fixing bacteria associated with the rhizoplane was determined by suspending 5 g of the soil free root mass in 45 ml of normal sterile saline for 30 min under shaking, after which, appropriate dilutions of the aqueous extract were transferred to 5 ml Dobereiner's nitrogen free semi-solid medium[5] and incubated for 3 days at 30°C. To estimate the MPN of nitrogen-fixing bacteria within the roots, 1 g of the washed root biomass was processed as described earlier[6].

ARA in the tubes was determined gas-chromatographically using a Nucon 5500 gas chromatograph[6]. To isolate the nitrogen-fixing bacteria, the growth from the tubes which were ARA-positive was streaked on solid agar medium of the same composition and incubated at 30°C for 3 days, after which colonies were selected based on morphology. The isolates were purified and the selected isolates were characterized by standard procedures[7]. The usefulness of selected isolates to support plant growth was tested by seed bacterization in pot culture experiments. MPN counts for rhizosphere and rhizoplane were determined. Root ARA was estimated by taking unwashed roots in air tight containers and incubation for 24 hr at 30°C under 10% acetylene atmosphere.

When soil root cores were assayed at the flowering stage for ARA, considerable activity was detected. The ARA level in HS1 and HC4 varieties was 21 and 5 at 8:00, 48 and 15 at 12:00 and 16 and 5 nmol C_2H_4/hr/plant at 16:00, respectively.

Thus, the extent of ARA varied both with the time of the day as well as with the variety and the variety HS1 had a higher level of ARA than HC4.

The MPN of nitrogen-fixing bacteria in the rhizosphere and within the roots (root macerate) was, however, similar in both the varieties (33 and 28 in rhizosphere and 260 and 230 × 10^5 cells/g in HS1 and HC4, respectively).

From ARA-positive tubes, a total of 78 isolates (23 from HS1 and 55 from HC4) were selected. These cultures were purified and tested individually for their ARA in solid and semi-solid media after 3 days of growth at 30°C. The ARA of these cultures varied significantly (0.0 to 27.3 nmol C_2H_4/hr/tube). Among these two isolates namely 10 Baj, isolated from variety HS1 and 54 Baj, isolated from the HC4 showed the highest activity. These cultures were identified as species of *Pseudomonas* and *Klebsiella*, respectively.

To determine the optimum incubation time for the maximum ARA expression, these two cultures were incubated in Dobereiner's medium and assayed for ARA at intervals of 24 hr (table 1). It was found that maximal ARA was detected after 48 hr and the ARA was higher when cultured on solid medium than on semi-solid medium. This appears to be a major difference in the nitrogen-fixing activities of these bacteria compared to others reported earlier[5] wherein, the maximum expression of the ARA occurs under semi-solid growth conditions.

To test whether the ARA can further be improved by supplementation with yeast extract and vitamins, the basal medium was supplemented with these components at 0–2 g/l and with and without vitamins (described in Dobereiner's medium) and assayed for ARA. It was found that the addition of yeast extract alone increased the ARA level from 0 to 20.8 and 0 to 25.9 nmol C_2H_4/hr/tube of 10 Baj and 54 Baj, respectively. The addition of vitamins with yeast extract increased the level slightly; however, the effect of vitamins was more at lower levels of yeast extract. No ARA was detected without these constituents.

To determine the optimum level of malate, which is considered to be the best carbon source for associate diazotrophs, the malate concentrations in the medium were changed from 0 to 100 mM (table 2). A significant increase in ARA was detected upto a malate concentration of 50–100 mM.

Pearl millet is a crop generally grown with very little nitrogen fertilization. To determine the effect of low levels of nitrogen on the ARA activity of these cultures, the cultures were grown in a medium containing varying levels of ammonium chloride (table 3). No

Table 1 *Effect of incubation period on ARA of pearl millet isolates*

Incubation period (d)	ARA (nmol C_2H_4/hr/tube)			
	10 Baj		54 Baj	
	Solid	Semisolid	Solid	Semisolid
3	6.6	ND	2.9	ND
4	27.3	8.9	20.7	6.3
5	10 5	6 7	14.2	5.0
6	3.9	3.1	4.3	2.5

ND, not detectable.

Table 2 *Effect of sodium malate concentration on ARA of pearl millet isolates*

Sodium malate (mM)	ARA (nmol C_2H_4/hr/tube)	
	10 Baj	54 Baj
Basal medium (BM)	ND	ND
BM +1	1.3	2.9
BM +5	2.6	4.1
BM +10	14.4	10.5
BM +20	31.0	18.2
BM +50	42.4	21.1
BM +100	42.0	24.4

BM, Dobereiner's medium without malate; ND, not detectable.

Table 3 *Effect of ammonium chloride on ARA of pearl millet isolates*

Ammonium chloride (mM)	ARA (nmol C_2H_4/hr/tube)	
	10 Baj	54 Baj
Basal medium (BM)	17.4	16.8
BM +1	18.1	20.9
BM +2	23.8	22.5
BM +5	30.6	19.9
BM +10	35.9	19.5
BM +20	9.0	4.3
BM +50	ND	ND
BM +100	ND	ND

BM, Dobereiner's malate medium; ND, not detectable.

significant inhibition in ARA was noticed upto a concentration of 5–10 mM of ammonium chloride but higher concentrations suppressed ARA activity but not growth.

To determine the effectiveness of these bacteria, pot culture experiments were conducted using both the varieties under sterilized conditions (table 4). The data suggest that these bacteria establish well within the rhizoplane and the rhizosphere of both the varieties and the roots samples show detectable levels of ARA in the dry matter yield. Significant difference was observed between the treatments and the control, which is consistant with the reports of others with several cereal crops[2]. The increase in the biomass, however, need not be only due to nitrogen fixation but also due to other contributing factors such as the release of growth-promoting substances, increased root biomass etc. The ability to fix nitrogen under aerobic conditions and to support plant growth significantly, by these bacteria suggest the need for further work with these bacteria associated with this crop.

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Table 4 *Effect of bacterization on ARA and dry matter yield of pearl millet.*

Treatment	MPN ($\times 10^4$ cells/g root)				ARA (nmol C_2H_4/24hr, pot)		Dry matter yield			
	Rhizosphere		Rhizoplane				Root		Shoot	
	HS 1	HC 4	HS 1	HC 4	HS 1	HC4	HS 1	HC 4	HS 1	HC 4
10 Baj	175	145	189	285	1.2	6.3	333	483	820	740
54 Baj	129	129	154	162	2.2	8 6	353	570	883	883
10 Baj + 54 Baj	43	183	112	222	1 8	5.9	348	630	773	953
12 S (*Azospirillum*)	27	146	16	134	6.0	4.4	305	643	726	900
Control (uninoculated)	8	54	12	134	ND	1 2	203	453	580	683