cuttings during the initial period of root initiation due to its utilization for the formation of rhizocaline which may be considered as one of the steps leading to root initiation[16]. Hess[17] also found that the phenolic compound catechol reacts synergistically with IAA in root production. It is probable that the accumulation of high amounts of phenols in the buds and leaves in young cuttings might have combined with auxins (both endogenous and exogenous) resulting in the formation of root-forming substance (rhizocaline) and induced profuse rooting.

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ISOLATION OF *AZOSPIRILLUM* STRAINS RESISTANT TO HIGH SOIL TEMPERATURE

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In recent years the associative nitrogen-fixing bacterium, *Azospirillum* has received increasing attention as it is used as a biofertilizer for many crops[1,2]. The optimum temperature for its growth and nitrogen fixation varies from 30 to 35°C. However, during summer the soil temperatures often exceeds 50°C in many parts of India[3]. High soil temperatures have been found detrimental to nitrogen fixation by *Azospirillum*[4]. We report here the isolation of a few high temperature resistant strains in *Azospirillum*.

Sixteen locations were spotted in Tamil Nadu through a survey where the top soil (0–10 cm)

Table 1 *Nitrogen fixing ability and nitrogenase activity of Azospirillum isolates*

Azospirillum isolates	Nitrogen fixation*		Nitrogen activity**	
	30°C	50°C	30°C	50°C
AZ.HT.1	13.98	22 64	110.7	140.3
AZ.HT.2	10.87	33.51	68.7	76.4
AZ.HT.3	13.78	19.95	33.5	124.3
AZ.HT.4	16.47	16.70	65 4	84.7
AZ.HT.5	15.57	19.50	27.8	32.6
AZ.HT.6	10.42	17.37	19.4	28.7
AZ.HT.7	13 89	16.48	123 8	69.4
AZ.HT.8	10.42	16.48	15.4	43 8
AZ.HT.9	11.09	21.74	67.8	136.5
AZ.HT.10	13.90	15.58	164 4	132.4
AZ.HT.11	13.78	17.34	10.7	27.8
AZ.HT.12	13.78	20.17	10.7	14.2
AZ.HT.13	10.42	19.95	16 9	31.7
AZ.HT.14	12.10	15.17	183.6	233.6
AZ.HT.15	13.78	19.95	60.5	272.4
SP.7 (ATTC 29145)	18.60	4.36	322.0	11.7

Data represent mean of two determinations; *mg of nitrogen fixed per g of malic acid; **Expressed as nmol of ethylene formed per mg of protein per hour.

temperature recorded more than 50°C during the hot weather in 1986. From plants inclusive of certain weeds growing in those locations, roots were collected and from the root tissues 65 strains of *Azospirillum* were isolated following the enrichment technique of Day and Dobereiner[5] using N-free malic acid semisolid medium.

All the strains were grown in 3 ml of N-free malic acid broth containing 2 g of silica gel and 100 μg of glutamic acid per ml for two weeks at 50°C. The surviving isolates were streaked on yeast extract glucose medium. This screening yielded 15 isolates of high temperature-resistant (HTR) cultures. For comparison, a mesophilic strain of *Azospirillum brasilense* (SP.7 ATTC 29145) was also included.

The nitrogen-fixing ability of the isolates was studied following the method of Humphries[6] in a Tecator Kjeltech auto analyser. The nitrogenase activity of the isolates was also determined using a gas chromatograph fitted with a porapak column (100 mesh) and FI detector. The assay conditions were essentially the same as detailed by Bergersen[7] and Purushothaman *et al*[8]. Proteins were determined[9] and the results are presented in tables 1 and 2. We have recognized two promising isolates, AZ.HT.1 and AZ.HT.2 as high temperature-resistant ones. Along with the standard mesophilic isolate, SP.7 the growth characteristics were studied[10] in yeast extract broth at 30 and 50° C.

Though 65 isolates were obtained, only 15 of them showed high temperature resistance. It is not uncommon to find even mesophilic strains very often exhibiting tolerance to high temperatures. The results on the nitrogen-fixing ability of the isolates indicated that for the HTR strains, a higher incubation temperature was required. At 30° C they fixed only meagre amounts of nitrogen. However, the mesophilic strain, SP.7 could not fix nitrogen at high temperature. This indicates its true mesophilic nature. The data on the nitrogenase activity also reflected the same trend.

The growth curve of the isolates at two temperatures indicated interesting information. When grown at 30°C, AZ.HT 1 and 2 registered a long lag phase and the generation time was also high: 84 and 108 min. However, at 50°C the lag phase was very short and so was the generation time. Interestingly, the strain, SP.7 virtually could not grow at 50°C and exhibited a very long lag phase of 480 min with 420 min as the generation time.

The present study indicated that at elevated temperatures the normal strain of *Azospirillum* may not fix substantial amounts of nitrogen and the use of high temperature-resistant strains in *Azospirillum* would be more useful for crop productivity.

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Table 2 *Growth characteristics of Azospirillum isolates*

Azospirillum isolates	Lag phase (min)		Generation time (min)	
	30°C	50°C	30°C	50°C
AZ.HT.1	150	48	84	48
AZ.HT.2	240	60	108	36
SP 7 (ATTC 29145)	96	480	78	420

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