present discovery extends the upper limit of this genus upto Pinjor Formation of Upper Sivaliks.

The gyrogonites of this find are small with a conical apex and with about 8-10 convex convolutions. Though, in some of its morphological features it resembles with *Sphaerochara tiwarii* known from the Dhokpathan Formation of Middle Sivaliks, yet there are some significant differences which might warrant its assignation to a new species. Further research work on this find, which entails the working out of the detailed morphology and its exhaustive comparisons with other known species of *Sphaerochara*, is underway and results will be reported separately. The present charophyte fructifications are assigned here to *Sphaerochara* sp. (figures 1A, B, and C).

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GROWTH AND NITROGEN FIXATION BY BACTERIAL ISOLATES FROM BARLEY ROOTS.

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DINITROGEN fixation in cereals and grasses is highly variable[1]. The associative bacteria have been isolated mainly by culture techniques used for Azospirilla but no attempt has so far been made to see the type, number and features of nitrogen fixers isolated during various stages of plant growth and their behaviour under cultural conditions. The present studies were aimed at isolating the associative nitrogen-fixing bacteria of barley roots and to know the optimum conditions for nitrogen fixation.

Barley (*Hordeum vulgare*) Var. BG 25 was grown on the research farm of the university using normal agronomical practices except the application of inorganic nitrogen. Root samples were collected from different locations at 30, 60 and 90 days of the plant growth. To estimate the most probable number (MPN) of nitrogen-fixing bacteria in the rhizosphere, the adhering soil was removed by gently shaking and 5 samples of 5 g each were taken at each stage of the plant growth and transferred to 45 ml sterile normal saline in conical flasks. The flasks were kept on a rotary shaker for 1 hr to suspend the bacteria of the root surface. To estimate the MPN of nitrogen-fixing bacteria within the roots, macerated root samples were prepared as reported earlier[2]. MPN for nitrogen fixers was determined using Dobereiner's semisolid medium[3]. The acetylene reduction activity (ARA) was taken as the parameter for nitrogen fixation and was determined using a Nucon Gas chromatograph 5500. The cell protein was estimated by Lowry's method[4].

The suspension from the tubes showing positive ARA was streaked on Dobereiner's medium plates and a total of 150 isolates were initially picked up based on colony morphology. All the isolates were checked for ARA on solid as well as semisolid media Two isolates (2B and 3B) showing higher ARA and maximum frequency of occurrence were finally selected and identified[5]. The optimum temperature and the pH for nitrogen fixation were determined by selecting the temperature and the pH ranges between 28 and 45° C and 6 and 9.5, respectively. To know the most suitable carbon source, ribose, arabinose, glucose, mannose, mannitol (5 g/l) and malate, succinate, α-ketoglutarate (2 to 10 mM) were tested in the basal medium devoid of sodium malate. Nitrogen sources such as glutamate, glutamine, aspartate, asparagine,

TABLE 1

Effect of sugars on in vitro nitrogenase activity of barley isolates.

Sugars	Nitrogenase activity			
	Solid medium*		Semi solid medium†	
	2B	3B	2B	3B
Basal medium	16	19 3	41	93
Glucose	ND	9 4	69	39
Mannose	ND	1.3	46	133
Mannitol	ND	ND	48	ND
Ribbose	ND	ND	ND	ND
Arbinose	ND	ND	ND	ND

ND Not detectable; * nM C_2H_2 red/hr/mg protein; † nM C_2H_2 red/24 hr/tube.

histidine and methionine at 2 and 4 mM concentrations were tested for their effect on nitrogen fixation.

Barley is grown in Northern India during winter on marginal lands generally without inorganic fertilizer. It is likely that this crop may harbour a variety of effective nitrogen-fixing organisms in its root system which provides a part of its nitrogen requirement. The MPN of nitrogen-fixing bacteria was 7, 50 and 80 $\times 10^5$ cells/g fresh root weight in the root washings and 6, 70 and 171 $\times 10^5$ cells/g in macerated roots at 30, 60, 90 d of plant growth, respectively. Increased MPN with plant age may be due to multiplication of nitrogen fixers in the plant rhizosphere on the availability of more root exudates. These results suggest that nitrogen fixers are present in large numbers in plant roots and rhizosphere, till late stage of plant growth and may contribute a substantial portion of nitrogen requirement by the crop.

Two groups of bacteria with distinct cultural characteristics (flat, spreading colonies with irregular margine and fluorescence) having maximum frequency of occurrence and showing the maximum ARA represented by two isolates, 2B and 3B were identified as gram-negative rods and *Pseudomonas* sp.on the basis of various biochemical tests (catalase, oxidase, nitrate reductase, starch hydrolysis, malonate utilization, citrate utilization, indole, methyl red, Voges-Proskauer, arginine dihydrolase, gelatine liquefaction, H_2S production, urea hydrolysis, phenylalanine deaminase, gluconate utilization, Huge and Leifson, ornithin decarboxylase and lysine decarboxylase) which were negative for 2B except catalase, whereas 3B showed positive test with catalase, oxidase nitrate reducatase, gelatin liquefaction, H_2S production and Huge and Leifson. When tested for ARA, 2B and 3B isolates showed the maximum nitrogenase activity (17.5 and 21 nmol C_2H_2 red/h/mg cell protein (on solid medium) and 41.8 and 92.5 nmol C_2H_2 red/24hr/5ml (in semisolid medium) at 28° C, respectively. The pH optimum for ARA was 6.5 and 7 for 2B and 3B isolates, respectively. These results suggest that these bacteria exhibit ARA under variable oxygen tension, mesophilic temperature and neutral pH.

To determine the suitable carbon source and the concentration required for maximal nitrogenase expression, the two isolates were cultured in media containing various (TCA) cycle intermediates. The optimum concentration for nitrogenase activity of 2B isolate was 4 mM succinate (20.5 nmol C_2H_2 red/hr/mg cell protein and 45 nmol C_2H_2 red/24 hr/5 ml in solid and semi solid media, respectively) 8 mM malate (32.5 nmol C_2H_2 red/hr/mg cell protein and 101.9 nmol C_2H_2 red/24 hr/5 ml in solid and semi soild media) and 8 mM fumarate (19.2 nmol C_2H_2 red/hr/mg cell protein and 72.4 nmol C_2H_2 red/24 hr/5 ml in solid and semi solid media). It expressed

TABLE 2

In vitro nitrogenase activity of barley isolates influenced by different amino acids:

Amino acid	Conc. (mM)	Nitrogenase activity			
		Solid medium*		Semi solid medium†	
		2B	3B	2B	3B
Control	0	16	20	41	93
Glutamate	2	17	49	717	274
	4	44	8	596	69
Glutamine	2	2	14	61	8
	4	ND	ND	ND	ND
Aspartate	2	43	10	369	46
	4	8	3	282	12
Asparagine	2	8	ND	78	ND
	4	ND	ND	58	ND
Histidine	2	5	4	52	77
	4	ND	ND	ND	63
Methionine	2	50	ND	408	ND
	4	29	ND	232	ND

ND Not detectable; * nM C_2H_2 red/hr/mg cell protein; † nM C_2H_2 red/24 hr/tube.

activity with α-ketoglutarate only in semi solid medium (46.6 nmol C_2H_2 red/24 hr/5 ml). Isolate 3B showed highest nitrogenase activity (10, 41.2 and 180.7 nmol C_2H_2 red/hr/mg cell protein in solid medium and 158.3, 60.5 and 260.8 nmol C_2H_2 red/24 hr/5 ml in semi solid medium at 6.8 and 10 mM of succinate, fumarate and malate, respectively. These results indicate that nitrogen-fixing isolates from barley roots vary in their requirement for carbon compounds for nitrogen fixation. Hence, no generalization can be made with regard to the suitability of carbon compound for nitrogen fixation by associative bacteria. When sodium malate was replaced with sugars in the basal medium both the isolates grew satisfactorily except in the presence of ribose and arabinose where the growth was poor. Isolate 3B showed nitrogenase activity in the medium containing glucose, mannose in both semisolid and solid media (table 1). Pentoses were not suitable among the sugars tested for ARA under cultural conditions. The data show that the 2B and 3B isolates can utilize carbohydrates for nitrogen fixation but are less effective compared to TCA cycle intermediate compounds.

Among the amino acids tested at 2 mM and 4 mM concentrations, glutamate enhanced the expression of nitrogenase activity of 2B and 3B isolates over control in both the media (table 2). Aspartate and methionine were suitable only for 2B isolate. This suggests that glutamate and aspartate are actively involved in steps of nitrogen fixation by nitrogen-fixing organisms.

These studies suggest that barley roots are associated with a high concentration of nitrogen-fixing bacteria which remain active till flowering stage. These are mainly of two types, *Pseudomonas* sp and gram-negative rods. They need a mesophilic temperature, neutral pH, specific carbon and nitrogen compounds for optimal nitrogen fixation.

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A NOTE ON THE DISCOVERY OF UPPER PALAEOLITHIC CULTURE FROM THE CENTRAL NARMADA VALLEY, MADHYA PRADESH

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THE evidence of human activity in the Central Narmada Valley during the prehistoric times (from Lower Palaeolithic to Mesolithic) is supported by the yield of Stone Age tools from different sites, in the form of handaxes, cleavers, choppers, scrapers, blades, points, lunates, etc made of quartzite, chert, jasper and chalcedony. The area is rich, not only in lithic assemblages, but also in faunal remains and has yielded a large number of dental and osteological remains of extinct mammals and reptiles either in association with Stone Age tools or in close proximity to them[1].

The stratigraphy of the Central Narmada deposits is generally characterised by 5 to 10 m thick bed of sandy pebbly gravel overlain by yellow or red sandy silt of 15 to 20 m thickness. At some sections, however, the sandy pebbly gravel rests on 5 to 10 m thick red brown concretionary silt. The unexposed basal rock is either laterite or Deccan Trap. A lithostratigaphical column at Devakachar (about 10 km NW of Narsingpur) is shown in figure 1.

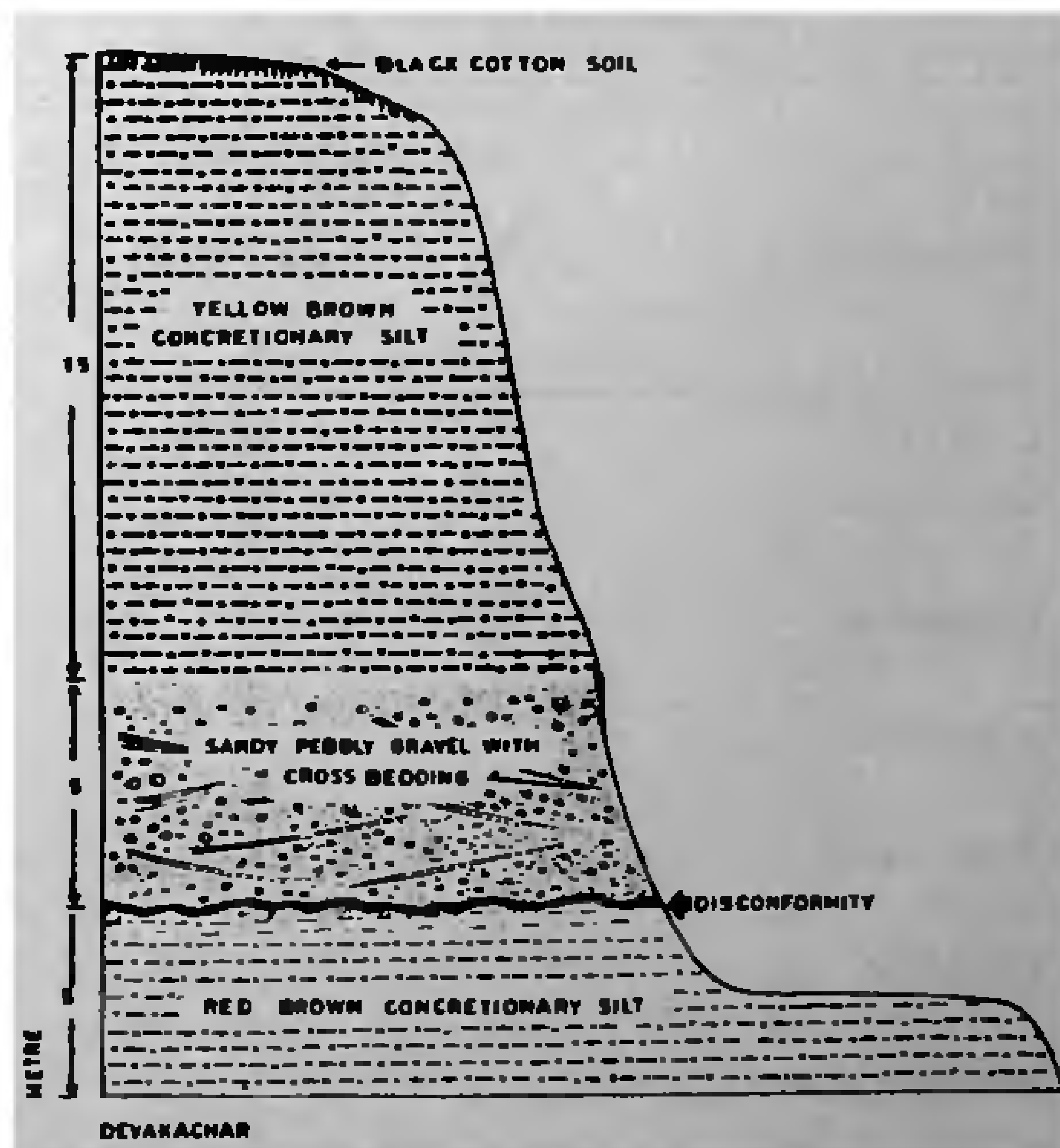

Figure 1. Lithostratigraphical column at Devakachar.