MORPHOLOGICAL AND CULTURAL CHARACTERISTICS OF SALT AND ALKALI TOLERANT STRAIN OF *AZOTOBACTER CHROOCOCCUM*

EARLIER studies on Azotobacter species revea'ed a positives significant coroelation between its population and the amount of aerobic non-symbiotic nitrogen fixation in calcareous soil (Rai *et al.*[5]) and a diminution in their number was noticed in the ca'careous saline a'kali soils (Rai *et al.*[4]). Detailed examination of these soils in respect of different species of Azotobacter led to the isolation of sa't and alkali tolerant strains of *Azotobacter chroococcum* (Iso'ates S_1 and S_2 from ca'careous saline alkali soil of Pusa and non-calcareous saline alkali soil of Riga respective'y). The soils of Pusa and Riga were sandy loam in texture having pH 9·2, 9·4; organic carbon 0·45, 0·30%; $CaCo_3$ 35·5, 0·85%; E.C. 4·8, 5·0 m. mhos/cm and exchangeable Na 25·6, 16·8% respective'y. The isolates mentioned above were characterised for their morphological and cultural features and their nitrogen fixing, capacity was determined in Ashby's mannite solution (Table I).

TABLE I

Morphological and Cultural characteristics of Azotobacter chroococcum (S_1) *and* (S_2)

	Azotobacter chroococcum (S_1)	*Azotobacter chroococcum* (S_2)
Shape	Rod	Rod
Size	2·0–3·0 × 3·0 = 5·0	2·0–3·0 × 3·0–5·0
Pigmentation	Brown	Brownish black
Optimum temperature	25°–28° C	25°–30° C
Optimum pH	7·0	7·0
*N fixed mg/g.c. :		
Without salt	11·6	9·6
With salt	11·3	9·2

* 100 ml Ashby's mannite solution at pH 9·0 with or without 0·2% NaCl.

S_1 and S_2 utilized glucose, sucrose and mannitol as carbon sources; no growth was noticed in peptone.

There was no marked difference in their shape, size, optimum pH, optimum temperature and utilization of glucose, sucrose or mannitol and growth in peptone. There was, however, a slight difference in their pigmentation, S_1 giving brown and S_2 brownish black pigment. The nitrogen fixing capacity of isolate S_1 (11·6 mg/g.c.) was higher than that of S_2 (9·6mg/g.c.). Generally Azotobacter fails to grow at pH 9·0 (Mishustin and Shil'nicova[3]) but it is remarkable that the strain S_2 of *Azotobacter chroococcum* has got the ability to grow even at pH 9·5.

Bacterization with Azotobacter cultures has been found to increase sugarcane yield in normal calcareous soils (Ahmad *et al.*[2] and Sahi *et al.*[6]). However, the inability of Azotobacter cultures to thrive well in saline alkali soils has limited their use in such soils. The cultures reported may be useful for enrichment of soil nitrogen specially in saline alkali soils.

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MELANOGENESIS IN *SOLANUM KHASIANUM* TISSUE CULTURES

THE mechanism of production of melanin, the brown or black pigment, by oxidative metabolism of tyrosine is well documented in the literature[1]. Melanins which impart colour to the skin of animals have also been reported from a number of higher plants, fungi and bacteria[2–6]. However, such reports are meagre in tissue cultures of higher plants. Chan and Staba[7] first of all observed the blackening of *Datura* tissue when tropane precursors were added to the medium. Later on, Khanna and Nag[8] further investigated the tissue of *D. metel* and reported that it was due to the deposition of melanin pigment produced by tyrosine-tyrosinase activity. The present communication reports melanin formation in callus tissues of *Solanum khasianum* Clarke.

Tissue cultures of *S. khasianum* were derived from whole seedlings and differentiated parts of the plant, *viz.*, shoot-apex, leaf, radicle, root excised from *in vitro*-grown plants as well as pericarp and placental tissues obtained from field-grown plants. The cultures were established on Murashige and Skoog's[9] medium (MS) supplemented with 2 mg/l each of 2, 4-dichlorophenoxyacetic acid (2, 4-D), 3-indoleacetic

acid (IAA), kinetin (Kn) and solidified with 7,000 mg/l agar. The tissues were maintained for a period of eight months by subculturing them every 5–7 weeks. Eight-month-old tissues were then transferred to MS medium supplemented with 250, 500 or 1,000 mg/l tyrosine. The tissues were tested for tyrosine-tyrosinase activity for a period of eight weeks. The cultures were grown at 27 ± 1° C, under diffused light of *ca.* 300 lux intensity. The relative humidity of the culture room was maintained at 70 ± 4%.

In order to detect tyrosine-tyrosinase activity, approximately 250 mg creamish, non-pigmented tissue(s) was taken separately into eleven vials. To the first vial (I), 4 ml of 0·1 M phosphate buffer (pH 6·8) was added which comprised the control. To the vials II, III, IV, V and VI were added 4 ml each of 0·1 M phosphate buffer containing 0·10, 0·20, 0·25, 0·50 and 1·0 mg/ml phenylalanine, respectively. All the vials with their contents were incubated at 4° C for 6–8 hr. The pigmented tissues were also treated with oxidizing agents, *e.g.*, hydrogen peroxide, potassium permanganate and potassium chlorate solutions.

The tissues grown on MS and MS + tyrosine media were harvested and extracted in 50% ethanol after every two months. The extracts as also the standard sample of tyrosine were applied separately to Whatman No. 1 chromatography paper-strips. Chromatograms were developed in *n*-butanol-acetic acid-water (250: 60: 250)[10]. Developed strips were air-dried, sprayed with 0·25% ninhydrin in distilled water, heated in an oven at 100° C for 5 min and the spots identified.

The tissues grown on MS medium first turned brown in 3–4 weeks and then black during following 7–8 weeks. The melanin saturated cells almost lost the capacity to divide. The callus tissue, therefore, had to be periodically transferred onto the fresh MS medium for its further growth after every 5–7 weeks. The callus tissues grown on MS + tyrosine medium started turning black in 24 hr and within a week the entire tissue as well as the medium turned black. The growth of the tissue was almost checked. It indicated a very high degree of tyrosinase activity in the tissues. The tissues in the control (I) and phenylalanine containing vials (VII–XI) remained unchanged, whereas the tissues in the tyrosine containing vials (II–VI) turned black. Under the present experimental conditions the phenylalanine was not converted into tyrosine. The maximum melanin production in the tissues was recorded at 0·25 mg/ml concentration of tyrosine. The dark brown or black pigment was bleached by oxidizing agents.

Paper chromatography showed the presence of tyrosine (R_f 0·4) in the tissues grown on MS and MS + tyrosine media for a period of 5–7 weeks. Beyond this period the black tissue gave rise to new tissue whose growth appeared to be of the same type as exhibited on MS medium devoid of tyrosine, *i.e.*, first creamish and then brownish. This implied the complete consumption of tyrosine from the medium during the initial culture period of 5–7 weeks. Thus, it can be concluded that callus cultures derived from different plant parts of *S. khasianum* produced melanin pigment on agar media.

Thanks are due to Dr. T. N. Khoshoo, Director, National Botanical Research Institute, Lucknow, for the facilities provided and Mr. M. S. Gorkha and Mr. J. P. Misra for technical assistance.

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December 18, 1978.

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AN APPARATUS AND METHODS OF STUDYING *IN VITRO* FEEDING OF BLOOD BY STRONGYLES OF HORSE AND THE MEASUREMENT OF THEIR PHARYNGEAL ACTIVITY

AN apparatus to study the *in vitro* feeding of *Ancylostoma caninum* in dogs has been described by Roche and Martin Torres[1]. In this study a simple modification of the above apparatus is described by means of which the *in vitro* feeding behaviour of the strongyles of the horse can be observed. An attempt has also been made to detect electrical impulses originating from the worms feeding on blood, and recording them graphically. This procedure could enable a continuous study of the pharyngeal activity of the worms for many hours and relate it to its feeding habits.