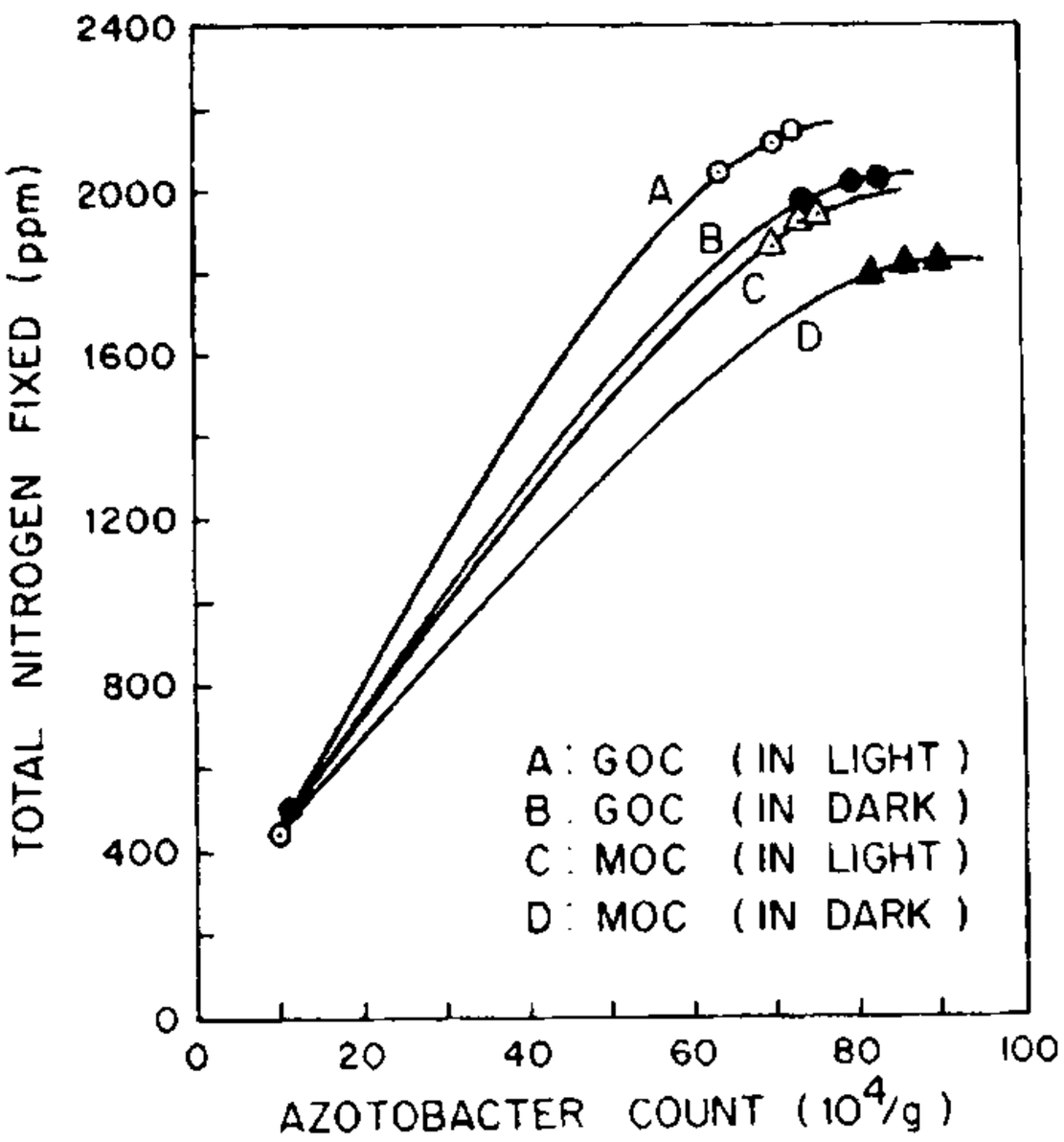

Figure 1. Increase in total nitrogen fixed with increase in *Azotobacter* count in the presence of oil cakes and mixed fertilizers in 180 days.

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EFFECT OF PYRIDINE DERIVATIVES ON ENCYSTMENT OF *ACANTHAMOEBA CULBERTSONI*

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MANY different physiological and environmental conditions trigger differentiation in amoebae[1,2]. Amino acids have been characterized as excystment agents for many species of amoebae but no unique and universal agent is known to promote encystment of different amoebae[1]. A medium containing magnesium chloride and taurine promoted very good encystment of *A. culbertsoni*; cyclic AMP and biogenic amines were also highly effective[3–5]. Srivastava and Shukla[6,7] observed that a purely inorganic medium containing sodium sulphate and magnesium sulphate, as well as several other organic effectors promoted very good encystment of this amoeba, while sugars, amino acids and organic acids exerted catabolite repression of encystation[8]. Several aliphatic and aromatic amines as well as pyridine failed to promote encystment; these compounds also caused lysis of amoebae[7]. The present communication reports the ability of certain hydroxypyridines and pyridine carboxylic acids to promote excellent excystment of *A. culbertsoni*.

An axenic culture of *A. culbertsoni* [(strain A-1) kindly provided by Dr B. N. Singh of this Institute] was maintained in the peptone medium of Kaushal and Shukla[9] containing 2% peptone, 0.5% sodium chloride, 1 mg/100 ml thiamine and 0.5 μg/100 ml cyanocobalamine (pH 7.0). The basal medium for testing the effect of encystment agents contained 0.086 M sodium chloride and 0.015 M magnesium chloride. The growth medium and the basal encystment medium (NM) of the above composition were sterilized at 15 lb/in^2 of steam for 20 min. Aqueous solutions of pyridine derivatives were adjusted to pH 7.0, sterilized separately and added to the medium to the desired concentration; volatile compounds were sterilized by filtration through Swinex filters containing 0.22 μm millipore filters. The cells of *A. culbertsoni* were grown with shaking (Rotary shaker, Emenvee Engineers, Poona) at 37 ± 1°C for 4 days, harvested by centrifugation (1000 g × 10 min) and washed with 150 mM sodium chloride. Freshly harvested amoebae (0.5 ml) of desired cell density were added to 4.5 ml of basal encystment medium supplemented with different compounds. The tubes were incubated with shaking at 28 ± 2°C, and examined periodically for cyst forma-

tion. The trophozoites, intermediate forms and cysts were counted using a haemocytometer (Erma, Brightline, Japan); the data reported in the table are mean values of four different counts. The cysts and amoebae were examined for viability by eosine staining (0.125 % aqueous solution); eosine stained dead cells in few seconds while live ones did not take up stain for 15–20 min. The viability of cysts was also confirmed by their excystment in growth medium[9]. Sterility of culture was tested by streaking on nutrient agar slants. All operations during growth and encystment were conducted under aseptic conditions.

Earlier studies[5,7] revealed that biogenic amines promote good encystation but aliphatic and aromatic amines and pyridine were ineffective; these agents also caused lysis of amoebae. Pyridine (1 mM) also caused lysis of amoebae even when added to complete encystment medium (containing NaCl, $MgCl_2$, taurine). 2-Methylpyridine (α-picoline) and 4-Methyl pyridine (γ-picoline) did not cause lysis but suppressed encystation yielding only 8 % and 9 % encystation respectively at 1 mM concentration. 3-Methylpyridine (β-picoline) on the other hand caused complete lysis of amoebae. Monohydroxypyridines and pyridine monocarboxylic acids, on the other hand, exhibited considerable encystment-promoting activity for *A. culbertsoni* (table 1). 2-Hydroxypyridine was most effective amongst monohydroxypyridines followed by 4-hydroxypyridine while 3-hydroxypyridine was only slightly effective. Pyridine monocarboxylic acids were more potent encystment agents. Isonicotinic acid as well as nicotinic acid at 10 mM concentration were more effective than 20 mM taurine; lower concentration (1 mM) also promoted good encystation. Nicotinic acid and isonicotinic acid also enhanced encystation when added to complete encystment medium containing taurine, and 77–80 % encystment was consistently obtained. 2-Hydroxypyridine, nicotinic acid and isonicotinic acid also appear to have protective action on amoebae, as higher total cell recovery was obtained, the overall yield of cysts was also higher (table 1). The cysts formed in the media containing these three pyridine compounds were mature cysts containing a double layered cyst wall, did not take up eosine and gave 90–95 % excystment in liquid growth medium[9].

The present studies therefore establish nicotinic acid and isonicotinic acid as effective agents for inducing encystment of *A. culbertsoni*. Pyridine carboxylic acids and their derivatives have been observed to exert various physiological effects including antimicrobial[10], hypocholesterimic action[11], phenotypic alteration in *Bordtella pertussis*[12], as well as altered jejunal secretion in response to enterotoxin[13]. The encystment enhancement of *A. culbertsoni* also appears to be a target for modulation by pyridine

Table 1 *Effect of pyridine derivatives on encystment of A. culbertsoni.*

Medium	Con. (mM)	Trophozoites	Intermediate forms	Cysts	Total	% Encystment
NM*		40	15	17	72	23.61
NM + taurine	20	19	7	45	71	63.38
NM + 2-hydroxypyridine	1	62	12	61	135	45.19
	10	62	29	42	133	31.58
NM + 3-hydroxypyridine	1	49	16	16	81	19.75
	10	18	21	29	68	42.65
NM + 4-hydroxypyridine	1	46	12	36	94	38.29
	10	41	21	38	100	38.00
NM + α-picolinic acid	1	29	31	30	80	37.50
	10	40	19	42	101	41.58
NM + nicotinic acid	1	34	19	49	102	48.04
	10	26	18	88	132	66.67
NM + isonicotinic acid	1	36	16	56	108	51.85
	10	30	16	105	151	69.63

* The basal encystment medium (NM) contained 86 mM NaCl and 20 mM $MgCl_2$ (pH 7.0). The data are mean values of 4 different counts corresponding to 10^{-4} ml volume.

derivatives. The molecular mechanism for action of pyridine compounds on encystation is not known but could be related to their effect on cAMP levels, as several effects of nicotinic acid are mediated through adenylate cyclase system[14,15].

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TRACE FOSSILS FROM PRECAMBRIAN ROCKS OF MEGHALAYA

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THE proterozoic metasediments forming the intracratonic rocks of Shillong Group occupy central and eastern parts of Meghalaya Plateau and comprise low grade metasediments representing essentially, an assemblage of rudaceous- arenaceous facies. These metasediments have been intruded by metadolerites and granites. The latter has been dated at 754 ± 25 M.Y[1].

The trace fossils *Chondrites* Sp (figure 1) have been found in the brown coloured phyllitic rocks of Shillong Group from the area around the village Raitong (25°46′:92°01′) in East Khasi Hill District of Meghalaya. The fossils are present within the lower sequences overlying the basal conglomerate horizon.

These trace fossils, sometimes referred to as fucoid, and described as "consisting of plant-like ramifying tunnel structures that neither cross each other nor anastomose but radiate around a central tube[2]"; are seen as ramifying burrows varying in length from

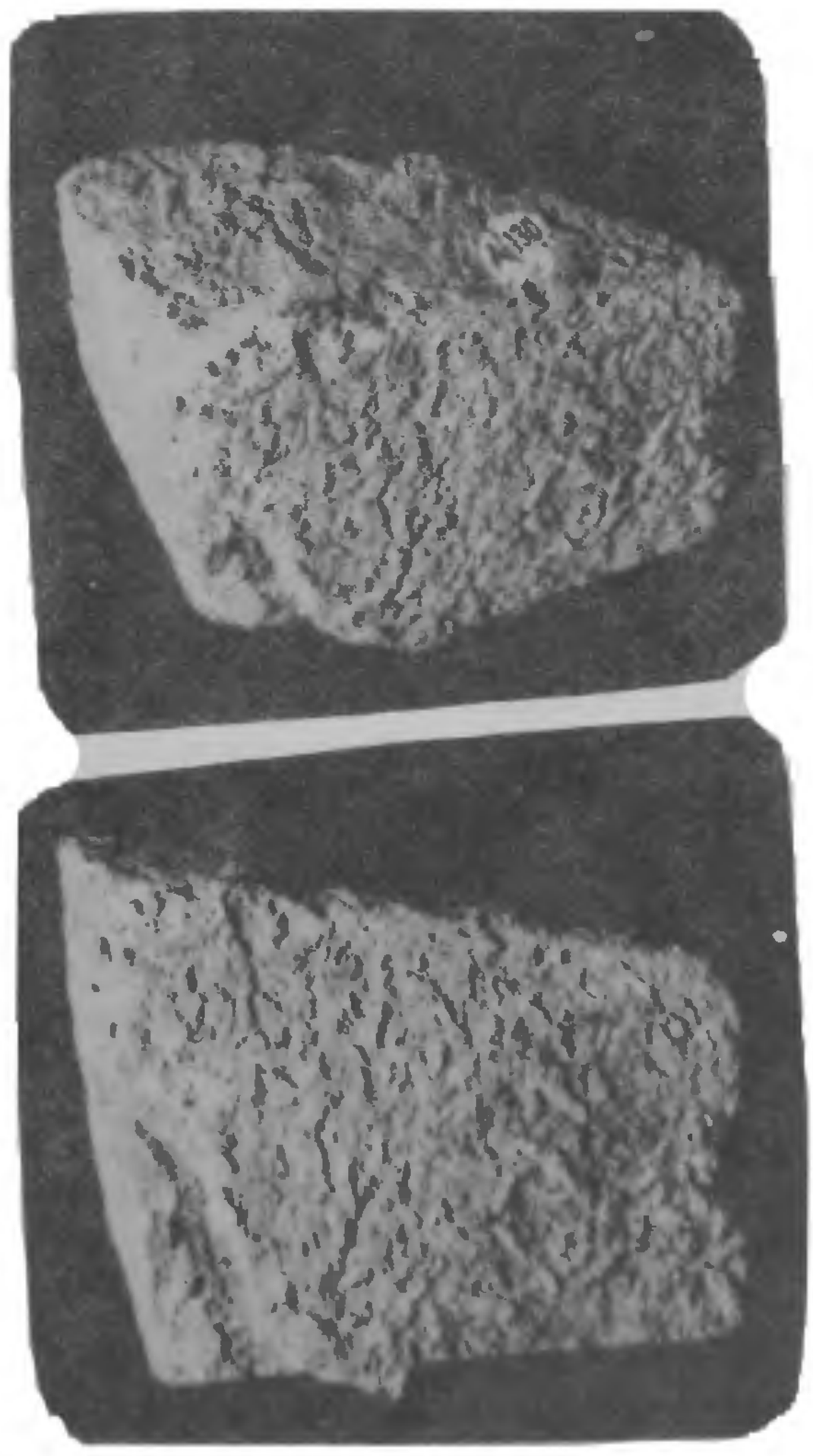

Figure 1. Trace fossils *Chondrite* sp.