

Hydrolysis of the glycoside with 6% HCl for 1 hr yielded myricetin (m.p., m.m.p. 358° and UV spectral studies<sup>8</sup>) and L-rhamnose (PC). The permethylated glycoside on hydrolysis afforded 5,7,3',4',5'-penta-methyl ether of myricetin, m.p. 224–25° which with AlCl<sub>3</sub> showed a bathochromic shift of 60 nm in band I absorption suggesting the glycosidation at C-3<sup>8</sup>. On acetylation with Ac<sub>2</sub>O and pyridine, it gave an acetate, m.p. 140°. The <sup>1</sup>H-NMR spectrum (CDCl<sub>3</sub>, 60 MHz) showed three alcoholic acetoxy groups between  $\delta$ 1.95–2.12 and five phenolic acetoxy groups between  $\delta$ 2.25–2.45, rhamnosyl methyl doublet at  $\delta$ 0.9 ( $J = 6$  Hz), aromatic proton signals at  $\delta$ 6.78 and 7.24 (d,  $J = 2.5$  Hz) for H-6 and H-8 respectively and two proton singlet at  $\delta$ 7.7 for H-2', 6', thus confirming that the compound was myricetin-monorhamnoside. The rhamnose C-1 proton at  $\delta$ 5.6 appeared as a doublet with  $J = 2$  Hz probably due to equatorial-equatorial coupling with H-2" thereby showing that the rhamnose formed  $\alpha$ -linkage to C-3. Thus the glycoside was identified as myricetin-3-O- $\alpha$ -L-rhamnoside<sup>9</sup>.

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## RESPONSE OF *TOLYPOTHRIX CEYLONICA* TO SODIUM STRESS

P. ROYCHOUDHURY, B. D. KAUSHIK and G. S. VENKATARAMAN

Division of Microbiology, Indian Agricultural Research Institute, New Delhi 110012, India.

MANY agricultural ecosystems are increasingly becoming salt-affected, thus rendering them inhospitable for good crop production. Such salt-affected soils cover at present an estimated 7 million hectares of potential crop land in India. Singh<sup>1</sup> was the first to suggest the feasibility of reclaiming such soils with cyanobacteria (blue-green algae). While most plants, with the exception of halophytes, fail to flourish on salt-affected soils, certain cyanobacteria have been found to grow successfully in such ecosystems. In general, cyanobacteria show considerable tolerance to salt and osmotic stresses and this property confers on them a reclamative potential in such problem soils<sup>2,3</sup>. However, the physiological basis of salt tolerance in cyanobacteria has not been adequately investigated<sup>4</sup>. The present communication deals with the response of *Tolypothrix ceylonica*, when challenged with increasing concentrations of Na<sup>+</sup> as sodium carbonate.

The algal strain was an isolate from an alkaline soil from Muketshwaram in Andhra Pradesh. The soil was highly alkaline with pH 10.5 and exchangeable sodium percentage of 96.3 (table 1). The alga was grown photoautotrophically in Fogg's nitrogen-free medium<sup>5</sup> (pH 9.5), fortified with different concentrations of Na<sup>+</sup> ranging from 0 to 200 mM at 29 ± 1°C under continuous illumination (2000 lux). Dry weight at the end of 10th day served as the index of growth. Protein was estimated by Lowry's method<sup>6</sup>. Na<sup>+</sup> was estimated by emission flame photometry<sup>7</sup>, using an Elico Flame Photometer Model CL 22A. Total extra-

Table 1 Physico-chemical properties of the soil from which *Tolypothrix ceylonica* was isolated

Soil status	Alkali
Sand %	41.0
Silt %	27.0
Clay %	23.5
pH	10.5
E C mmhos/cm	3.98
Exchangeable sodium percentage	96.3
Cation exchange capacity (meq/100 g)	49.37
Organic carbon (%)	1.13
Total nitrogen (%)	0.023
Available P <sub>2</sub> O <sub>5</sub> (kg/ha)	1.79

cellular polysaccharide was quantitatively estimated using anthrone reagent<sup>8</sup>. The sugars were chromatographically separated<sup>9</sup> and identified by spraying with ether benzidine reagent (250 mg benzidine + 100 ml acetic acid + 40 ml EtOH) or a mixture of equal volumes of 0.2% naphthoresorcinol in EtOH and 2% TCA in water (specially for sucrose and raffinose).

Under nitrogen-fixing conditions, the growth of the alga was stimulated by Na<sup>+</sup> upto a concentration of 50 mM in the medium (figure 1, G). Although there was a progressive decrease in growth at higher Na<sup>+</sup> concentrations, the total growth of the alga at 200 mM Na<sup>+</sup> was almost two and a half times greater than in the absence of Na<sup>+</sup>, indicating that the alga was fairly tolerant to high concentrations of Na<sup>+</sup>. Protein also showed a similar trend (figure 1, TP). Tolerance limits of *T. ceylonica* are comparatively lower than those known for halophiles<sup>10</sup> and certain halophilic cyanobacteria such as *Microcoleus chthonoplastes* (20–25% NaCl)<sup>11</sup>, *Spirulina subsalsa* (> 3 M)<sup>12</sup> and *Calothrix scopulorum* (5% NaCl)<sup>13</sup>, but higher than for *Anabaena torulosa* (J. Thomas, Personal communication).

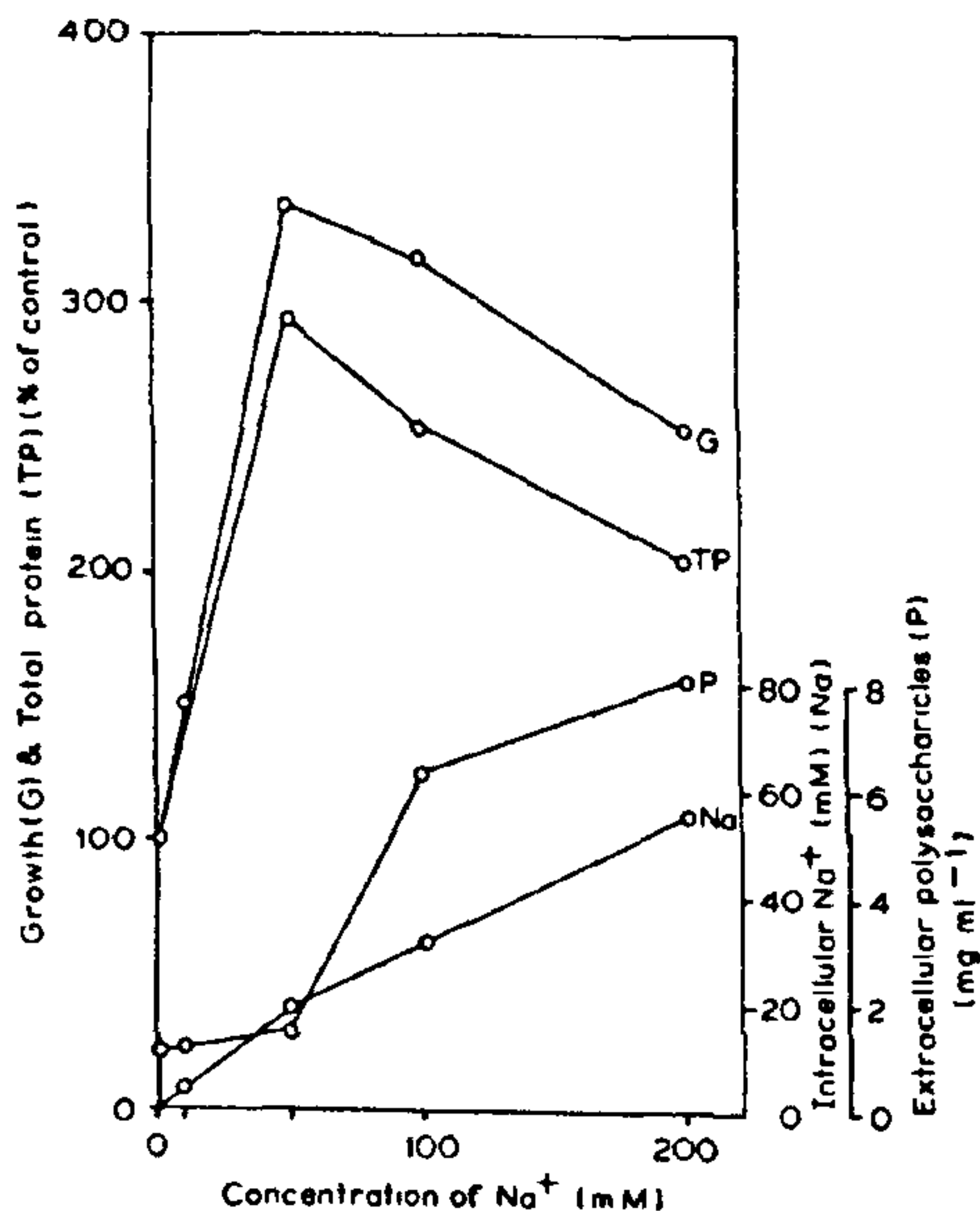


Figure 1. Effect of Na<sup>+</sup> on the growth (G), protein content (TP), Na<sup>+</sup> uptake (Na) and production of extracellular polysaccharides (P) by *T. ceylonica*.

The Na<sup>+</sup> uptake measured as intracellular Na<sup>+</sup> was almost linear with increasing concentrations of Na<sup>+</sup> in the medium upto 200 mM (figure 1, Na). While the growth at 200 mM Na<sup>+</sup> was about 20% lower than at 100 mM Na<sup>+</sup>, accumulation of intracellular Na<sup>+</sup> was about 70% more in the former. In *Anabaena* Na<sup>+</sup> uptake has been found to be carrier-mediated and regulated by the proton motive force, particularly the membrane potential of the cells<sup>14</sup>.

What is particularly interesting is the excess production of extracellular polysaccharides under Na<sup>+</sup> stress (figure 1, P). At 100 mM Na<sup>+</sup> level, the extracellular polysaccharides were quantitatively six times more than in the control, which further increased to eight times at 200 mM Na<sup>+</sup> level. However, no qualitative differences were observed with the sugar constituents under the presence or absence of sodium stress. The sugars detected in both the series were glucose, galactose, arabinose, xylose, rhamnose, raffinose and two unidentified sugars with *R<sub>f</sub>* values 0.04 and 0.08. Most of these sugars are known in several cyanobacterial species<sup>9, 15–17</sup> as well as a variety of osmoregulants such as glycerol derivatives<sup>18</sup>, and K<sup>+</sup> ions<sup>19</sup>.

Whether the triggering of excess production of extracellular polysaccharides under Na<sup>+</sup> stress is a contrivance to protect the cells against Na<sup>+</sup> injury by chelating the Na<sup>+</sup> in the immediate environment needs further investigation. The excess production of polysaccharides will also bind soil particles leading to increased soil aggregation as observed earlier<sup>2, 3</sup>.

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## EFFECT OF CYCLIC-AMP ON CATABOLITE REPRESSION OF CELLULASE COMPLEX OF *PENICILLIUM ISLANDICUM*

K. PRABAKARAN and H. C. DUBE\*

Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, India.

\* Present address: Department of Life Sciences, Bhavanagar University, Bhavanagar 364002, India.

CATABOLITE repression of inducible enzymes by glucose and other sugars is known to be caused by the lowering of cAMP (cyclic adenosine monophosphate) level. The addition of cAMP releases catabolite repression of pectate lyase synthesis by *Erwinia carotovora*<sup>1,2</sup>. Cyclic AMP has a regulatory role in bacteria, activating inducible operons at the level of transcription. However, it is not necessary for the utilization of glucose. Bacteria growing in the presence of glucose have a lower level of cAMP than those growing on a poor energy source such as lactose.

In the present study we have seen the ameliorating effect of addition of cAMP on catabolically-repressed cellulase synthesis by *Penicillium islandicum*,

Table 1 Showing the effect of some sugars (0.1M) and cAMP (20  $\mu$ M) on cellulase production by *P. islandicum*

Richards' CMC medium + sugars $\pm$ (cAMP)	$\beta$ -glucosidase		Exo-glucanase		Endo-glucanase	
	IU <sup>-ml</sup> (10 <sup>-2</sup> )	% inhibition	IU <sup>-ml</sup>	% inhibition	IU <sup>-ml</sup>	% inhibition
CMC medium control	1.86	—	0.98	—	1.32	—
+ cAMP	1.91	—	0.98	—	1.34	—
+ glucose	0.09	95	0.01	99	0.04	97
+ glucose + cAMP	1.79	—	0.97	—	1.33	—
+ cellobiose	0.06	97	0.02	98	0.06	96
+ cellobiose + cAMP	1.91	—	0.99	—	1.4	—
+ galactose	0.07	96	0.04	96	0.03	98
+ galactose + cAMP	1.78	—	0.97	—	1.3	—
+ arabinose	0.09	95	0.03	97	0.03	99
+ arabinose + cAMP	1.92	—	0.98	—	1.30	—
+ xylose	0.09	95	0.03	97	0.02	98
+ xylose + cAMP	1.82	—	0.98	—	1.34	—
+ fructose	0.07	96	0.07	93	0.02	99
+ fructose + cAMP	1.89	—	0.98	—	1.34	—
+ raffinose	0.04	98	0.02	98	0.08	97
+ raffinose + cAMP	1.85	—	0.99	—	1.37	—
+ mannose	0.07	96	0.04	96	0.048	96
+ mannose + cAMP	1.84	—	0.97	—	1.31	—
+ lactose	0.05	97	0.01	99	0.04	97
+ lactose + cAMP	1.96	—	0.99	—	1.37	—
+ maltose	0.04	97	0.02	98	0.03	98
+ maltose + cAMP	1.87	—	0.98	—	1.34	—
+ sorbose	0.6	96	0.03	97	0.05	96
+ sorbose + cAMP	1.83	—	0.996	—	1.33	—