

RESEARCH COMMUNICATIONS

Table 1. Germination (%) of stored embryos after transferring into the retrieval medium

Category	S1	S2	S3	S4	Mean
Mature	66.67	66.67	80.00	66.67	70.00
Immature	20.00	6.67	0.00	20.00	11.67
Mean	43.33	36.67	40.00	43.33	

No storage in case of S4, observations were made after 2 months of inoculation into the retrieval medium. The germination significantly differs between mature and immature embryos but not among media and also there is no interaction effect. The S.E. plot with regard to values after angular transformation is 17.54.

The germination of immature embryos after two months of storage was negligible (Table 1). In S4 too, their performance was not satisfactory when compared to the earlier results under controlled conditions (58 to 84%)¹.

The present results clearly show that coconut embryos can be stored for two months in sterile water alone. This finding has far-reaching applications for sterile water is

easily available and chances of contamination will be minimal compared to nutrient media. Further, the development of embryos will be arrested/negligible in sterile water compared to nutrient media and hence the effect of toxic substances at a later stage will also be minimal.

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Fatty acid profile of a marine *Nostoc calcicola* under saline and non-saline conditions

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A marine cyanobacterium *Nostoc calcicola*, when grown under non-saline condition synthesized quantitatively lower levels of fatty acids, except stearic acid. Salinity induced *de novo* synthesis of three unidentified fatty acids.

MANY cyanobacteria show adaptation to salinity and several physiological mechanisms underlying such adaptation have been identified¹⁻⁶. In this communication, we report on the fatty acid profiles of a marine cyanobacterium grown in the absence and presence of salinity.

A marine cyanobacterium *Nostoc calcicola* BDU 40302 was obtained from the National Facility for Marine Cyanobacteria, Bharathidasan University, Trichy. The alga was grown photoautotrophically at 28 ± 1°C with a 12 h light (3000 lux): dark regimen in nitrogen-free artificial seawater ASN III medium⁷ with 25 ppt NaCl. For the salinity-shift experiments, cultures

Figure 1. Fatty acid profile of a marine *Nostoc calcicola* grown in the absence (a) and presence (b) of NaCl (25 ppt). 1, undecanoic acid, 2, lauric acid, 3, tridecanoic acid, 4, myristic acid, 5, pentadecanoic acid, 6, palmitic acid, 7, palmitoleic acid, 8, heptadecanoic acid, 9, stearic acid, 10, oleic acid, 11, nonadecanoic acid, 12, arachidic acid, 13, heneicosanoic acid, 14, behenic acid (U, unidentified fatty acids).

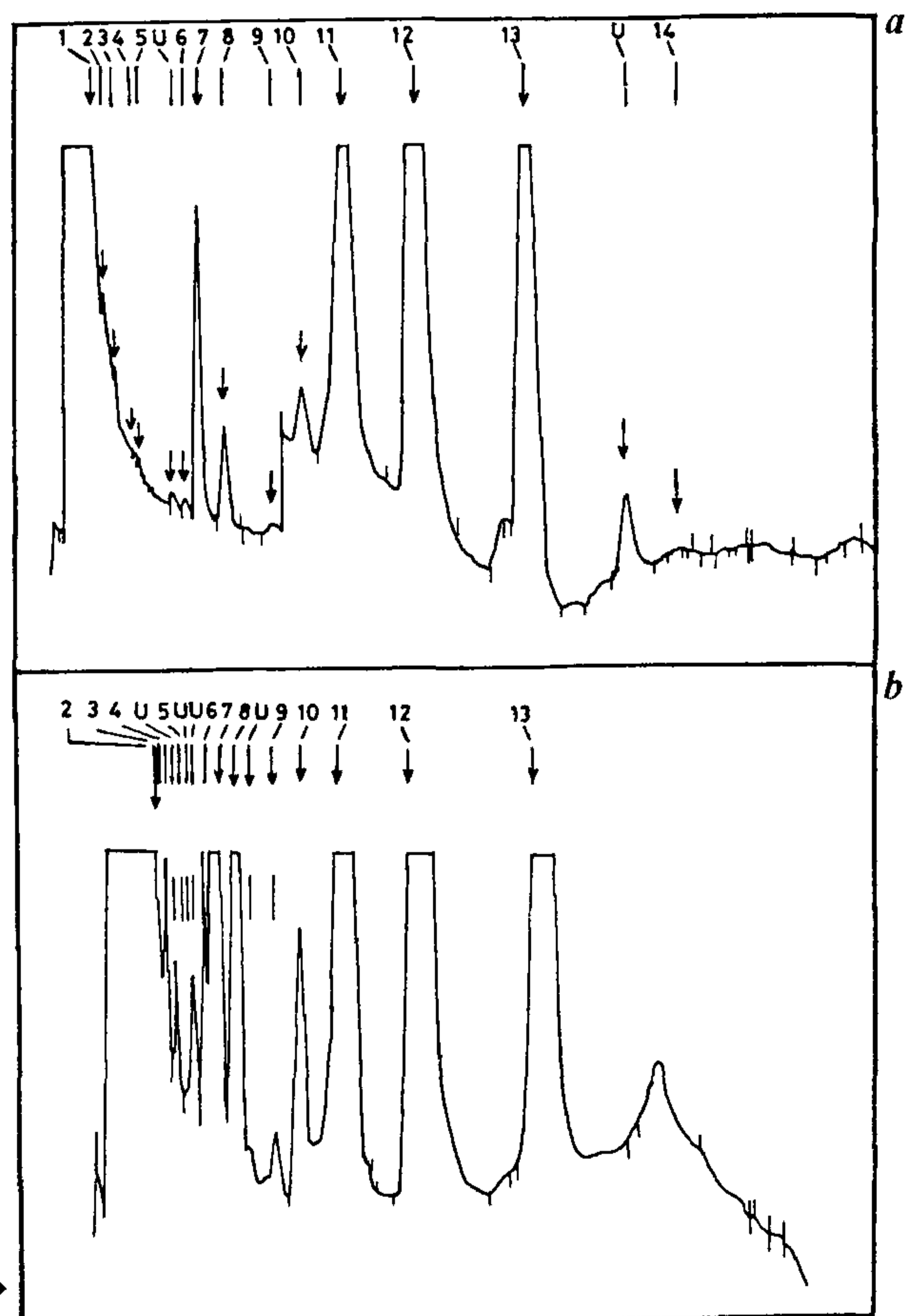


Table 1. Fatty acid (mg/100 g dry wt) profile of *Nostoc calcicola* BDU 40302 under saline and non-saline conditions [U I ('X1' to 'X6') = unidentified]

C No	Fatty acids	'O' NaCl	25 ppt NaCl	% of 'O' NaCl
11 0	Undecanoic acid	66 295	—	—
12 0	Lauric acid	10 346	23 043	222 72
13 0	Tridecanoic acid	15 226	44 780	294 10
14 0	Myristic acid	3 540	4 325	122 18
	U I	—	'X3'	—
15 0	Pentadecanoic acid	1 624	2 563	157 82
	U I	'X1'	'X4'	—
	U I	—	'X5'	—
16 0	Palmitic acid	13 612	30 008	220 45
16 1	Palmitoleic acid	335 069	763 803	227 95
17 0	Heptadecanoic acid	65 086	127 664	196 15
	U I	—	'X6'	—
18 0	Stearic acid	12 902	8 956	69 42
18 1	Oleic acid	26 616	62 383	234 38
19 0	Nonadecanoic acid	114 624	211 910	184 87
20 0	Arachidic acid	169 470	363 801	214 67
	U I	'X2'	—	—
21 0	Heneicosanoic acid	104 662	167 995	60 51
22 0	Behenic acid	1 370	—	—

at a late logarithmic phase were collected, washed well with phosphate buffer and transferred to fresh ASN III medium with no added NaCl. The cells were kept growing for 15 days before use.

Lipids were extracted with $\text{CHCl}_3/\text{CH}_3\text{OH}$ (2 : 1 v/v) according to Folch *et al.*⁸. Fatty acid methyl esters were prepared from lipids by transmethylation with 3% $\text{HCl}/\text{CH}_3\text{OH}$ and analysed by gas-liquid chromatography in a Hewlett Packard GLC Model 5890A equipped with a hydrogen flame-ionization detector. They were separated on a stainless steel column (3 m \times 3 m internal diameter) packed with 15% diethyleneglycol succinate on Chromosorb W run at 180°C. The fatty acids were identified and estimated by comparison of their retention time with those of standard fatty acid methyl esters.

When grown under saline (25 ppt NaCl) and non-saline conditions, *Nostoc calcicola* showed interesting differences in its fatty acid profiles. Under salinity, sixteen fatty acids were detected, including four unidentified ones ('X3' to 'X6'). The position of 'X3' was between myristic (14:0) and pentadecanoic (15:0) acids; 'X4' and 'X5' between pentadecanoic (15:0) and palmitic (16:0) acids and 'X6' between heptadecanoic (17:0) and stearic (18:0) acids (Table 1; Figure 1b). Under non-saline conditions too, sixteen fatty acids were detected with two unidentified ones ('X1, X2') (Table 1; Figure 1a). The unidentified fatty acid 'X1', like the 'X4' under saline condition, was also detected between pentadecanoic (15:0) and palmitic (16:0) acids, whereas the unidentified fatty acid 'X2' was found between heneicosanoic (21:0) and behenic (22:0) acids. It needs to be determined whether the 'X1' and 'X4' acids are the same or different. Thus, under salinity, the alga seems to synthesize three unidentified fatty acids

('X3', 'X5', 'X6') which are absent under non-saline condition (Table 1). These may be salt-specific fatty acids (SSFs). The identity and role of these three fatty acids in salinity adaptation need further study. Interestingly, the molecular species 11:0 (undecanoic acid) and 22:0 (behenic acid) detected under non-saline condition were absent under salinity. Strikingly, under salinity, all fatty acids except stearic acid, were quantitatively much higher, ranging from 22 to 194% over those under non-saline condition. Stearic acid (18 : 0) level was, however, only 69% of that under non-saline condition. Under non-saline condition, the C_{16} and C_{18} acids were 348.68 mg and 39.52 mg/100 g dry weight of the alga and under salinity, they were 2.23 and 1.8 times more, respectively. Enhanced fatty acid synthesis has been observed in other halotolerant algal systems such as *Botryococcus*, *Isochrysis* and *Dunaliella salina*^{9,10}. It appears that like other physiological mechanisms, lipid synthesis may also play an important role in the adaptation to salinity by cyanobacteria.

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