Carbohydrate Accumulation and Osmotic Stress in Cyanobacteria

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In a comprehensive survey of the carbohydrate accumulation profiles of more than 70 strains of cyanobacteria three organic osmotica (glucosylglycerol, sucrose and trehalose) have been identified in both freshwater and marine isolates under conditions of osmotic stress. While the trend was towards glucosylglycerol accumulation in marine strains and sucrose accumulation in freshwater forms, there were no absolute differences between cyanobacteria isolated from each habitat. There was also no clear link between genus and the type of carbohydrate accumulated.

INTRODUCTION

Cyanobacteria (blue-green algae) are world-wide in distribution, occurring in saline and non-saline habitats of diverse ionic composition (Fogg *et al.*, 1973; Golubic, 1980). The division of these organisms into marine and freshwater forms has been frequently regarded as a useful means of distinguishing between taxa (Rippka *et al.*, 1979; Stam & Holleman, 1979). However, more emphasis is now being placed on the importance of various metabolic features as taxonomic markers in cyanobacteria. Recently it has been suggested that soluble organic compounds, accumulated as internal osmotica in response to salinity stress, may provide a major biochemical character which distinguishes marine (Mackay *et al.*, 1983) and freshwater (Blumwald *et al.*, 1983) forms. Thus glucosylglycerol has been considered to be 'unique' to marine cyanobacteria (Mackay *et al.*, 1983) while sucrose has been reported to accumulate in response to osmotic stress in freshwater cyanobacteria (Blumwald *et al.*, 1983). Recently we demonstrated that the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (ATCC 27184), which was originally isolated from a freshwater habitat, was capable of growth both in freshwater and marine media and that, in response to osmotic stress, it produced glucosylglycerol as an internal osmoticum (Richardson *et al.*, 1983). Subsequently we have examined over 70 strains of cyanobacteria to see whether any distinctions between the types of low molecular weight carbohydrates which are employed as osmotica in cyanobacteria isolated from marine and freshwater habitats could be identified.

METHODS

Cyanobacterial strains were obtained from the sources shown in Table 1; freshwater isolates were maintained in BG11 medium (Rippka *et al.*, 1979) while marine strains were maintained in BG11 medium + 3.5% (w/v) sea salt (Gerrard Biological Centre, W. Sussex, UK). Carbohydrate accumulation profiles were obtained for marine cells exposed to salinities in the range 0.5-5.0% (w/v) sea salt; freshwater isolates were exposed to NaCl at concentrations up to 250 mM, to determine whether soluble carbohydrate levels varied with osmotic stress.

Low molecular weight, ethanol soluble carbohydrates were assayed following extraction in boiling 80% ethanol, followed by overnight extraction in 80% ethanol at 20 °C. Samples were then dried using a rotary evaporator and stored in a vacuum desiccator for 24-48 h prior to analysis. Trimethyl silyl derivatives were prepared in pyridine (Reed *et al.*, 1980); samples were analysed using a Varian 3700 gas chromatograph fitted with a 2 m column containing 2% SE52 on a diatomite support (Holligan & Drew, 1971). Chromatograms were recorded and quantified using a Hewlett Packard HP3390A integrator.

Table 1. Carbohydrate accumulation in response to osmotic stress in marine and freshwater cyanobacteria

Freshwater isolates*					Designation/Synonyms†	Origin/habitat	Carbohydrates‡
Source†	Strain no.						
CCAP	1403/7				<i>Anabaena ambigua</i> (IV)	Fresh water, India	Sucrose (—)
ANR	—				<i>Anabaena azollae</i> (IV)	From <i>Azolla</i> sp. (water fern)	Sucrose (F)
CCAP	1403/2b				<i>Anabaena cylindrica</i> (IV)	Pond water, UK	Sucrose (F)
BAW	D582				<i>Anabaena cylindrica</i> (IV)	Rice field, Iraq	Sucrose (F)
PCC	7122				<i>Anabaena</i> sp./ <i>Anabaena cylindrica</i> (IV)	Pond water, Cambridge, UK	Sucrose (—)
PCC	7120				<i>Anabaena</i> sp./ <i>Nostoc muscorum</i> (IV)	Habitat unknown	Sucrose (—)
JRG	1				<i>Anabaena</i> sp. (IV)	Limestone, Yorkshire, UK	Sucrose (F)
JRG	2				<i>Anabaena</i> sp. (IV)	Stagnant pond, Yorkshire, UK	Sucrose (—)
JRG	6				<i>Anabaena</i> sp. (IV)	Limestone cave, Yorkshire, UK	Sucrose (F)
JRG	9				<i>Anabaena</i> sp. (IV)	Pond water, Sudan	Sucrose (F)
PR/CF	A4				<i>Anabaena</i> sp. (IV)	Rice field, Senegal	Sucrose (F)
PR/CF	A7				<i>Anabaena</i> sp. (IV)	Soil (surface sample), Senegal	Sucrose (—)
PR/CF	A19				<i>Anabaena</i> sp. (IV)	Rice field, Senegal	Sucrose (F/M)
PR/CF	AS15				<i>Anabaena</i> sp. (IV)	Soil (surface sample), Senegal	Sucrose (F/M)
ATCC	29413				<i>Anabaena variabilis</i> (IV)	Habitat unknown	Sucrose (F)
BAW	D603				<i>Calothrix</i> sp. (IV)	Rice stem, Bangladesh	Sucrose (F/M)
BAW	D604				<i>Calothrix</i> sp. (IV)	Rice stem, Bangladesh	Sucrose (F/M)
BAW	D619				<i>Calothrix</i> sp. (IV)	Rice stem, Bangladesh	Sucrose (—)
BAW	D602				<i>Calothrix</i> sp./ <i>Gloeotrichia</i> sp. (IV)	Fresh water, Bangladesh	Sucrose (—)
PCC	6718				<i>Chlorogloeopsis</i> sp. (V)	Habitat unknown	Sucrose (F/M)
PCC	73103				<i>Fischerella muscicola</i> (V)	Rice field, India	Trehalose (F/M)
BAW	D612				<i>Fischerella muscicola</i> (V)	Soil/root sample, Bangladesh	Trehalose (—)
PCC	7414				<i>Fischerella</i> sp./ <i>Mastigocladus laminosus</i> (V)	Hot spring, New Zealand	Trehalose (—)
CCAP	1451/1				<i>Myxosarcina chroococcoides</i> (II)	Habitat unknown	Sucrose (F/M)
CCAP	1452/2				<i>Nodularia</i> sp. (IV)	Freshwater, Cambridge, UK	Glucosylglycerol (F/M)
ANR	—				<i>Nostoc aphithosa</i> (IV)	From <i>Peltigera aphithosa</i> (lichen)	Sucrose (F)
CCAP	1453/1				<i>Nostoc calcicola</i> (IV)	Soil sample, Holland	Sucrose (—)
BAW	D611				<i>Nostoc commune</i> (IV)	Soil/root sample, Bangladesh	Sucrose (F)
CCAP	1453/8				<i>Nostoc muscorum</i> (IV)	Habitat unknown	Sucrose (—)
PR/CF	Nmus				<i>Nostoc muscorum</i> (IV)	Soil (surface sample), Senegal	Sucrose (F)
PR/CF	N3				<i>Nostoc</i> sp. (IV)	Rice field, Senegal	Sucrose (F)
PR/CF	N4				<i>Nostoc</i> sp. (IV)	Rice field, Senegal	Trehalose (F)
BAW	D608				<i>Nostoc</i> sp. (IV)	Soil/root sample, Bangladesh	Trehalose (F/M)
PCC	7524				<i>Nostoc</i> sp. (IV)	Hot spring, Sri Lanka	Sucrose (—)
CCAP	1459/12b				<i>Oscillatoria redekei</i> (III)	Lough Neagh, Ireland	Sucrose (F/M)
CCAP	1462/9				<i>Phormidium inundatum</i> /LPP group (III)	Thermal freshwater, UK	Trehalose (—)
PCC	73110				<i>Plectonema boryanum</i> /LPP group (III)	Habitat unknown	Sucrose (F)
CCAP	1446/2				<i>Plectonema boryanum</i> /LPP group (III)	Habitat unknown	Sucrose (F)
CCAP	1464/2				<i>Pseudanabaena brunei</i> (III)	Habitat unknown	Trehalose (—)
PR/CF	82SO2				<i>Scytonema</i> sp. (IV)	Aquatic grass, Senegal	Trehalose (F)
PR/CF	82SO3				<i>Scytonema</i> sp. (IV)	Aquatic grass, Senegal	Trehalose (F)

PR/CF	82ST8	<i>Stigonema</i> sp. (V)		Rice field, Senegal	Sucrose (F/M)
PCC	6301	<i>Synechococcus leopoliensis/Anacystis nidulans</i> (I)		Fresh water, Texas, USA	Sucrose (—)
CCAP	1479/5	<i>Synechococcus</i> sp. (I)		Habitat unknown	Sucrose (F/M)
CCAP	1404/1	<i>Synechococcus</i> sp./ <i>Anacystis montana</i> (I)		Pond water, Cavendish, UK	Sucrose (F)
CCAP	1430/1	<i>Synechococcus</i> sp./ <i>Anacystis montana</i> (I)		Freshwater, California, USA	Sucrose (—)
CCAP	1408/1	<i>Synechococcus</i> sp./ <i>Aphanothece</i> sp. (I)		Blelham Tarn, UK	Sucrose (F)
PCC	6803	<i>Synechocystis</i> sp. (I)		Freshwater, California, USA	Glucosylglycerol (F/M)
PCC	6714	<i>Synechocystis</i> sp./ <i>Aphanocapsa</i> sp. (I)		Freshwater, California, USA	Glucosylglycerol (F/M)
Marine Isolates*					
Source†	Strain no.	Designation/Synonyms‡		Origin/habitat	Carbohydrate§
ATCC	33047	<i>Anabaena</i> sp. CA (IV)		Intertidal marine, Texas, USA	Sucrose (M)
CCAP	1410/9	<i>Calothrix pulvinata</i> (IV)		Salt marsh, Hayle, UK	Trehalose (—)
CCAP	1409/1	<i>Chroococcidiopsis</i> sp. (II)		Seawater off Lowestoft, UK	Glucosylglycerol (—)
PCC	7301	<i>Dermocarpa violacea</i> (II)		Marine aquarium, California, USA	Sucrose (M)
CCAP	1446/4	<i>Lyngbya majuscula/LPP</i> group (III)		Saline ditch, Gt. Yarmouth, UK	Trehalose (M)
CCAP	1446/5	<i>Lyngbya</i> sp./LPP group (III)		Seawater off Lowestoft, UK	Trehalose (M)
CCAP	1445/1	<i>Microchaete grisea</i> (IV)		Estuarine sample, Isle of Wight, UK	Trehalose (—)
SMBA	235	<i>Nodularia harveyana</i> (IV)		Salt marsh, Lincolnshire, UK	Sucrose (F/M)
CCAP	1452/1	<i>Nodularia harveyana</i> (IV)		Habitat unknown	Sucrose (F/M)
CCAP	1453/14	<i>Nostoc entophytum</i> (IV)		Marine supralittoral fringe, Scotland, UK	Sucrose (—)
CCAP	1459/9	<i>Oscillatoria nigro-viridis</i> (III)		Seawater off Lowestoft, UK	Sucrose (—)
PCC	6401	<i>Oscillatoria</i> sp. (III)		Marine mud, California, USA	Sucrose (—)
CCAP	1459/13	<i>Oscillatoria</i> sp. (III)		Seawater off Lowestoft, UK	Sucrose (M)
SMBA	245B	<i>Oscillatoria</i> sp. (III)		Seawater, Scotland, UK	Glucosylglycerol (M)
JRG	8	<i>Oscillatoria</i> sp. (III)		Intertidal marine, Landimore marsh, S. Wales, UK	Glucosylglycerol (M)
CCAP	1463/3	<i>Plectonema battersii/LPP</i> group (III)		Marine aquarium, California, USA	Trehalose (—)
CCAP	1464/3	<i>Pseudanabaena</i> sp. (III)		Habitat unknown	Glucosylglycerol (—)
CCAP	1475/2	<i>Spirulina subsalsa</i> (III)		Intertidal marine, Cullercoats, UK	Glucosylglycerol (F/M)
PCC	7002	<i>Synechococcus</i> sp./ <i>Agmenellum quadruplicatum</i> (I)		Marine mud, Puerto Rico	Glucosylglycerol (F/M)
PCC	73109	<i>Synechococcus</i> sp./ <i>Agmenellum quadruplicatum</i> (I)		Seawater, New York, USA	Glucosylglycerol (F/M)
SMBA	280	<i>Synechococcus</i> sp./ <i>Agmenellum quadruplicatum</i> (I)		Marine mud, Puerto Rico	Glucosylglycerol (F/M)
AGDE	—	<i>Synechococcus</i> sp. (I)		Seawater, Irish Sea	Glucosylglycerol (M)

* Freshwater isolates include cyanobacteria derived from soil samples, isolated and grown in freshwater-based media; marine strains (including strains from brackish habitats, isolated in saline media) were grown in seawater-based media.

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‡ The classification scheme of Rippka *et al.* (1979) has been used, wherever possible. Major sub-groups distinguished by Rippka *et al.* (1979) are shown in parentheses. CCAP designations (freshwater/marine) were taken from the List of Strains, 4th Revision (CCAP, Cambridge, UK, 1982).

§ Sustained growth in either freshwater (F) or marine (M) medium alone, or in both media (F/M) is indicated in parentheses, as appropriate. [Untested strains are designated (—)].

RESULTS AND DISCUSSION

All cultures grown in freshwater-based medium (BG11), including isolates which were originally derived from saline environments, had low intracellular soluble carbohydrate levels [usually 1–15 mmol (kg dry wt)⁻¹] while seawater-grown cells all contained in excess of 100 mmol (kg dry wt)⁻¹. Both marine and freshwater isolates produced either glucosylglycerol (a nine-carbon heteroside; Kollman *et al.*, 1979) which is structurally analogous to floridoside (galactosylglycerol) in the Rhodophyta (red algae), sucrose or trehalose (Table 1) in response to osmotic stress. In almost all cases (Table 1), a single carbohydrate represented more than 80% of the total soluble carbohydrate in any one strain.

No absolute correlation was found between soluble carbohydrate accumulation in response to salinity and genus, with different strains of each of the genera *Calothrix*, *Nodularia*, *Nostoc*, *Plectonema*, *Pseudanabaena*, *Oscillatoria* and *Synechococcus* showing different soluble carbohydrate accumulation profiles. Of the 22 marine isolates, 41% accumulated glucosylglycerol, 36% accumulated sucrose and 23% accumulated trehalose. Of the 49 freshwater isolates 76% accumulated sucrose in response to NaCl treatment, 18% accumulated trehalose and 6% accumulated glucosylglycerol. Thus, the trend is towards glucosylglycerol accumulation in marine isolates (as noted by Mackay *et al.*, 1983) and sucrose accumulation in freshwater isolates in response to osmotic stress but there is no absolute correlation of taxonomic or ecological value. For example, three freshwater isolates accumulated glucosylglycerol while various marine isolates with an absolute requirement for Na⁺ (Batterton & Van Baalen, 1971) accumulated sucrose or trehalose, among which was the marine sucrose-accumulating *Anabaena* sp. CA which has a high Na⁺ requirement for sustained growth (Stacey *et al.*, 1977). Overall, the data suggest that it would be unwise to redefine and reclassify cyanobacteria solely on the basis of their carbohydrate accumulation profiles since there is no absolute relationship between either habitat or genus and the type of internal osmoticum accumulated in response to salt stress. Thus carbohydrate accumulation profiles appear to offer less to the cyanobacterial taxonomist than to his physiological counterparts (e.g. Kremer, 1980).

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