

16 EFFECT OF SODIUM CHLORIDE SALINITY ON FREE
AMINO ACIDS OF SOIL STREPTOMYCETES

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Abstract

Actinomycetes were isolated from a number of saline and non saline salheya soils. From these isolates two isolates of streptomycetes were selected to assess their physiological response to salinity. Intracellular concentrations of the free amino acid pool increased in response to salt stress. Whereas the neutral free amino acids alanine and gamma aminobutyrate accumulated as salinity increased. Accumulation of free amino acids by streptomycetes under salt stress suggests a response typical of procaryotes-although the specific amino acids involved differ from those associated with other gram positive bacteria.

Introduction

The metabolic activities of micro-organisms vary considerably with changes in their growth environments. One of the parameters affecting microbial physiology is the medium osmolarity. Because salinity has become an ever increasing problem in irrigated agriculture,

it stimulated research on the mechanisms of response of microorganisms to salt stress. Sodium chloride concentration in medium has a gross effect on the intracellular levels of free amino acids in many bacterial species (Britten and McClure, 1962, Brown, 1976, Measure, 1975, Tempest and Meers 1970). Gram positive bacteria have been reported to accumulate the neutral amino acids proline (Brown, 1978, Measures, 1975 and Tempest and Meers, 1970) and gamma amino butyrate (Measures, 1975) in response to increasing salt stress, whereas gram negative bacteria accumulate glutamate (Britten and McClure, 1962, Hua et al, 1982, Makemson and Hastings, 1979). In contrast to the accumulation of free amino acids in procaryotes, glycerol, have been found to occur as osmotic agents in eucaryotic fungi and algae (Brown, 1978).

Streptomycetes are strictly gram positive bacteria of the order actinomycetales, because their morphology is sometimes considered to be transitional between the simpler eubacteria and fungi, Harris (1980) posed the question of wheather actinomycetes use free amino acids as heterotrophic procaryotes or as heterotrophic eucaryotes as inducible compatible internal solutes.

There is evidence to suggest that actinomycetes are of particular importance in saline soils (Gupta and Bajpai, 1974) and in environment of high alkalinity (Johnstone, 1947). Meiklejohn (1957) found that they become an increasingly dominant components of the soil microbiota during prolonged draught.

In this study an investigation was done on the occurrence of actinomycetes in a number of salheya soils of varies salinities. From these soils, two indigonus streptomycetes were selected to asses their physiological response to salinity. The effect of sodium chloride on the growth of these isolates and on composition and concentration of their internal solutes were reported. The data are discussed in relation to osmoregularity mechanisms and to the ecology of actinomycetes in saline soils.

Material and Methods

Soils: Saline surface soils (0-10 cm) were sampled from six agricultural sites in salheya district. A non saline soil was similarly sampled from an agricultural tract of Cairo - Ismailia. Soils were throughly mixed and sieved before analysis. Values for water soluble salts were determined by evaporating a

well known volume of soil extract solution and weighing the residue (Jackson, 1967). Soil pH was measured with a 1:1 distilled water soil slurry and a glass pH electrode of pH meter (Tacussel). Total soil organic carbon content was determined using Walkley's rapid method (Jackson, 1967).

Microbial counts and actinomycetes isolation: Microorganisms were counted by the dilution technique using starch-nitrate media of the composition: Soluble starch 10 g., Na NO₃ 1 g., K₂HPO₄ 0.3g, NaCl 0.5 g. Agar, 15 g. distilled water 1 litre (Waksman, 1967). To select for actinomycetes. For each soil dilution, five plates were incubated at 30°C for 4 days and then counted. Two streptomycetes isolates were preliminary identified by morphological examination and subcultured on to fresh media for use as batch culture inocula.

Preparation of cells:

An early stationary-phase cells, were harvested, centrifuged at 2400 r.p.m. for ten minutes and washed three times using isotonic solution, and then recentrifuged, the supernatant was discarded, and the resulting pellet were lyophilized using a Beta freez dryer (West Germany).

The amino acids from the lyophilized pellets were extracted by suspending in 5 ml 3.5% trichloroacetic acid (TCA) at 4°C for 24 hr with occasional blending in a vortex mixer. The suspensions obtained were used for amino acid analysis.

Analytical procedures :

Free amino acids were determined both qualitatively and quantitatively using a Beckman amino acid analyzer, model 118/119 CL with a single column (6 x 460mm) packed with W3 resin citrate buffers of pH 2.83, 3.70 and 3.75 were used. Amino acid concentrations were determined by reaction with ninhydrin reagent (flow rate 22ml/h.).

Results

Plate counts from the six soils (Table 1) indicated no clear relationship between soil salinity and number of actinomycetes.

Table 1: Soil characteristics

Soil No. ^a	pH	Chloride (mg)/g. soil	Organic carbon (%)	Water soluble salts (mg)/g.soil	No.of actinomyces per g (dry wt.) of soil $\times 10^8$
1	6.1	142	0.9	0.009	2.08
2	6.0	284	2.7	0.011	4.42
3	5.6	710	1.1	0.014	21.12
4	6.0	142.5	2.5	0.017	35.17
5	6.0	177.5	1.59	0.035	16.84
6	5.8	400	0.7	0.044	24.10

a Listed in order of increasing salinity. Soil No.1 nonsaline, soil Nos.2 through 6 saline.

Streptomycete No.1 isolated from soil No.5 was identified by morphology tests as a common actinomyces in all the saline soils. A second streptomycete isolate, streptomycete No.2. was found in soil No.3. These two streptomycetes were selected to assess their physiological response to salt stress.

Increasing salt concentration reduced the growth as manifested by g/dry weight (untabulated) of the two streptomycetes. This growth reduction was more pronounced in streptomycete No.2 than with streptomycete No.1. In fact, cells of streptomycete No.2 failed to grow at 1% NaCl medium (Table 3).

The free amino acid pool of cells grown in the basal medium was comprised largely of the acidic amino acids, glutamic and aspartic and neutral amino acids alanine and gamma amino butyric acid. With increasing salt stress there was marked change in both the concentration and composition of the free amino acids in both streptomycetes (Tables 2 & 3). The results indicated that most of the amino acids showed great reduction with salt stress. This reduction was more pronounced in streptomycete No.1 (Table 2) even at the low salt concentration, while in streptomycete No.2, this reduction was observed only at 1% NaCl salinity where cells did not grow. Acidic amino acids such as glutamic acid was greatly affected by salt stress, neutral amino acids such as alanine and gamma amino butyric acid were increased in response to NaCl salinity. Valine was not detected in the basal medium whereas at different levels of salinity considerable amounts were noticed especially in streptomycete No.2. Ammonia shows the same trend as amino acids, where it decreases with increase of NaCl salinity in streptomycete No.1 and increases with the increase of NaCl in streptomycete No.2. The intracellular concentrations of the assayed free amino acids cited in Tables 2 and 3 (glycine, leucine and isoleucine, Tyrosine, phenylalanine, Histidine, Lysine, arginine, serine and threonine, cystine, methionine and ornithine) showed the same trend whether in streptomycete No.1 or

streptomycete No.2. Total free amino acids, decrease or increase in response to NaCl salinity corresponding to streptomycete varieties.

Discussion

One of the goals of this study was to analyze the amino acid pool of two streptomycetes indigenous to saline soils, grown in media containing different amounts of NaCl and to assess their response to salinity. Growth of the two streptomycetes decreased as NaCl concentration increased. However streptomycetes No.1 could grow up to 1%, while the other one grow up to 0.5% only. These results were in full agreement with the results obtained by Kilham and Firestone (1984), who found that the specific growth yield of two streptomycetes indigenous to saline soils decreased with salt concentration increase. They also found that the yield reduction due to NaCl were consistently greater than those resulting from KCl stress. Moreover the intracellular concentrations of free amino acid did not consistently vary between the two salts. On the other hand, Hua et al. (1982) claimed that the growth rate of Rhizobium spp. strain WR1001 decreased with NaCl increased from 300 to 500 mM, however the bacterium could grow and survive in 500mM NaCl, although

most Rhizobium strains were very sensitive to salt and minimal media containing 45mM NaCl slows down the growth rate to a greater extent in some variants of R. japonicum (Upchurch and Elkan, 1977).

The observed accumulation of total free amino acids is similar to responses observed in other salt stress studies with gram positive bacteria (Brown, 1976 and Measures 1975). Intracellular accumulation of free alanine under salt stress, however, has previously been demonstrated in streptomyces griseus and streptomyces californicus by Kilham and Firestone, (1984), whereas the occurrence of gamma aminobutyrate as inducible compatible solute has been reported to accumulate in salt stressed streptococci (Measures, 1975). Betaine, which can also function as a compatible solute in Procaryotes (Galinski and Triiper, 1982) was not detected in cell extract.

Gram positive bacteria was found to accumulate the neutral amino acids proline (Brown, 1978 and Tempest and Meers, 1970) and gamma amino butyrate, (Measures, 1975), in response to increasing salt stress, whereas gram negative bacteria accumulate glutamate (Nakamura, 1979 and Hua et al., 1982) while, streptomyces indogenous

to saline soils were found to accumulate the neutral amino acids alanine which was not found before as a compatible solute in streptomycetes (Kelham and Firestone, 1984). Similarly experiments were done on some higher plants by Cusido et al. (1987) who found that some amino acids increased with NaCl increased in leaves and roots, of Nicotiana plants, those were aspartic, glutamic, ornithine, arginin and proline, the increase was significant especially in proline. In streptomycetes spp. glutamate was the dominant free amino acid in cells grown in the basal meduim, but it decreased with increasing salt stress. These streptomycetes therefore had several features of responses to salt stress that have not been found in other gram positive bacteria.

The reduced cell yields did not result only from the energy cost of amino acids synthesis. Apparently some of the energy cost of response to NaCl stress was specific to the Na ion.

There are numerous factors which might affect the accumulation of amino acids. The solubility of the amino acids may limit continued accumulation, although proline and alanine could have accumulated to far greater concentrations if solubility were the only factor

controlling intracellular solute accumulation. Another factor may affect accumulation of amino acids especially proline to concentration which are in some way inhibitory (Kilham and Firestone, 1984).

Generally in streptomycetes indigenous to saline soil, tolerance to high salinity is associated with intracellular accumulation of free neutral amino acids, alanine and gamma aminobutyrate. To relate this finding to the growth and survival of actinomycetes in saline environment, the energy costs of osmoregulatory solute control must be quantified under the conditions of energy availability characteristic of soil.

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Table (2): Influence of medium NaCl concentrations on the intracellular free amino acid composition of *Streptomyces no. 1*.

	Free amino acids	Amino acid concn(μ mol/g dry wt.) with NaCl concn(%)			
		0	0.25	0.50	1.0
1 -	Glycine	16.013	17.856	3.323	2.707
2 -	Alanine	21.723	7.072	3.448	1.964
3 -	Valine	25.880	6.780	5.827	7.0
4 -	Leucine and Isoleucine	30.654	10.234	9.703	7.571
5 -	Tyrosine	7.550	2.599	3.494	4.139
6 -	Phenylalanine	6.903	2.236	1.888	1.734
7 -	Histidine	4.906	1.082	0.608	1.0
8 -	Aspartic acid	22.409	5.283	-	-
9 -	Glutamic acid	34.679	10.764	15.343	21.234
10-	γ -Amino-n-butyric acid	4.017	1.404	1.162	2.881
11-	Lysine	11.107	4.243	3.541	3.515
12-	Arginine	10.101	3.484	1.396	1.202
13-	Serine and Threonine	28.860	7.561	11.841	12.500
14-	Cystine	1.864	0.333	Traces	0.193
15-	Methionine	4.953	1.768	0.554	0.587
16-	Ornithine	0.702	-	0.710	-
17-	Ammonia	5.569	4.337	-	0.954
Total free amino acids concn(μ mol/gm dry wt. of cells).		237.890	86.133	62.837	69.190

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Fig. (3): Influence of medium NaCl concentration on the intracellular free amino acid composition of Streptomyces no.2.

	Free amino acids	Amino acid concn ($\mu\text{mol/g}$ dry wt.) with NaCl concn (%)			
		0	0.25	0.50	1.0
1 -	Glycine	14.638	28.60	31.524	- ^a
2 -	Alanine	27.976	41.964	65.65	-
3 -	Valine	-	47.762	68.826	-
4 -	Leucine and Isoleucine	22.724	56.498	43.212	-
5 -	Tyrosine	4.407	12.376	12.324	-
6 -	Phenylalanine	4.121	13.416	9.079	-
7 -	Histidine	1.872	5.011	7.394	-
8 -	Aspartic acid	18.110	20.748	58.437	-
9 -	Glutamic acid	50.169	21.307	11.739	-
10 -	γ -Amino-n-butyric acid	6.591	11.063	21.746	-
11 -	Lysine	5.525	19.448	22.370	-
12 -	Arginine	0.936	9.542	2.465	-
13 -	Serine and Threonine	24.01	31.863	74.973	-
14 -	Cystine	1.261	4.03	3.151	-
15 -	Methionine	3.198	10.465	6.365	-
16 -	Ornithine	0.624	1.664	1.870	-
17 -	Ammonia	10.218	30.953	54.318	-
Total free amino acids concn ($\mu\text{mol/g}$ dry wt. of cells.		196.38	367.451	495.443	-

a- Cells did not grow.

Table(4): Percentage difference from control. Data were given from table 2.

Free amino acids					
		0	0.25	0.50	1.0
1 -	Glycine	16.013	+11.5	-79.3	-83.1
2 -	Alanine	21.723	-67.5	-84.1	-90.9
3 -	Valine	25.880	-73.8	-77.5	-72.9
4 -	Leucine and Isoleucine	30.654	-66.6	-68.3	-75.3
5 -	Tyrosine	7.550	-65.6	-53.7	-45.2
6 -	Phenylalanine	6.903	-67.6	-72.6	-74.9
7 -	Histidine	4.906	-77.9	-87.6	-79.6
8 -	Aspartic acid	22.409	-76.4	-	-
9 -	Glutamic acid	34.679	-68.9	-55.8	-38.8
10 -	α -Amino-n-butyric acid	4.017	-65.1	-71.1	-28.3
11 -	Lysine	11.107	-61.8	-68.1	-68.4
12 -	Arginine	10.101	-65.5	-86.2	-88.1
13 -	Serine and Threonine	28.860	-73.8	-58.9	-56.6
14 -	Cystine	1.864	-82.1	traces	-89.6
15 -	Methionine	4.953	-64.3	-88.8	-80.1
16 -	Ornithine	0.702	-	+ 1.2	-
17 -	Ammonia	5.565	-22.1	-	-82.8
		237.890	-63.8	-73.6	-70.9

Table (5): Percentage difference from control. Data were given from table 3.

	Free amino acids				
		0	0.25	0.50	1.0
1 -	Glycine	14.638	+95.4	+115.4	- ^a
2 -	Alanine	27.976	+50.0	+134.7	-
3 -	Valine	-	-	-	-
4 -	Leucine and Isoleucine	22.724	+148.6	+90.2	-
5 -	Tyrosine	4.409	+180.8	+179.6	-
6 -	Phenylalanine	4.121	+255.6	+120.3	-
7 -	Histidine	1.872	+210.4	+295.0	-
8 -	Aspartic acid	18.110	+14.5	+222.5	-
9 -	Glutamic acid	50.169	-57.5	-76.6	-
10 -	γ -Amino-n-butyric acid	6.591	+67.9	+230.0	-
11 -	Lysine	5.525	+252.0	+304.9	-
12 -	Arginine	0.936	+919.4	+163.4	-
13 -	Serine and Threonine	24.01	+32.7	+212.3	-
14 -	Cystine	1.261	+219.6	+149.9	-
15 -	Methionine	3.198	+227.2	+99.0	-
16 -	Ornithine	0.624	+166.7	+199.7	-
17 -	Ammonia	10.218	+202.9	+431.6	-
		157.950	+147.7	+223.0	-

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