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Rhizobia and bradyrhizobia under salt stress: possible role of trehalose in osmoregulation

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Seven *rhizobium fredii* strains and seven *Bradyrhizobium japonicum* strains were grown in defined medium with or without 20 mM trehalose in the presence or absence of NaCl. Trehalose had no effect on the growth rate of the strains in the absence of NaCl, but increased the growth rate of some strains in the presence of NaCl. *Bradyrhizobium japonicum* strain RCR 3827 was completely inhibited by 0·08 M NaCl in absence of trehalose, but multiplied when trehalose was added. The results indicate that trehalose may act as an osmoregulator in these strains of *Rhizobium* and *Bradyrhizobium*.

Under osmotic stress most bacteria accumulate K^+ and organic compounds such as amino acids, betaine and carbohydrates in their cytoplasm in order to prevent osmotic dehydration and to maintain turgor pressure (Imhoff 1986). Accumulation by uptake from the medium is preferred over biosynthesis (Imhoff 1986). Rhizobia (fast-growers) and bradyrhizobia (slow-growers) vary in their tolerance to salt, with rhizobia generally being more salt-tolerant than bradyrhizobia (Yelton *et al.* 1983; Singleton *et al.* 1982). This is possibly due to the ability of rhizobia to influence their internal osmotic potential (Chen & Alexander 1973) or accumulate particular solutes (Hua *et al.* 1982) more rapidly than bradyrhizobia.

Little work has been done on the biochemistry and physiology of rhizobia and bradyrhizobia under salt-stress. Hua *et al.* (1982) and Yelton *et al.* (1983) investigated the accumulation of amino acids (mainly glutamate), betaine and K^+. The aim of this work was to examine the ability of rhizobia and bradyrhizobia to utilize exogenously added trehalose in order to relieve salt-stress.

Materials and Methods

Slow-growing soybean strains (*Bradyrhizobium japonicum*) RCR 3407, RCR 3827, RCR 3824, 3Ib/143 were obtained from Rothamsted Experimental Station, Harpenden, UK; *Bradyrhizobium japonicum* strains USDA 110, USDA 122, USRA 136 and fast-growing soybean rhizobia (*Rhizobium fredii*) USDA 191, USDA 192, USDA 193, USDA 201, USDA 205, USDA 208 and USDA 217 were obtained from Dr H. Keyser, United States Department of Agriculture (USDA). The strains were maintained at 4°C on yeast extract mannitol (YEM) agar slopes incorporating 3·0 g/l $CaCO_3$ (Vincent 1970). For all experiments the strains were grown in glutamate-galactose-arabinose defined medium (Wood & Cooper 1988).

Trehalose was added as a filter-sterilized solution to defined media containing 0·08 M NaCl (for bradyrhizobia strains) or 0·34 M NaCl (for rhizobia strains) in addition to an unamended control, to give final concentrations of 0 or 20 mM trehalose. Aliquots (15 ml) were dispensed into 50 ml conical flasks and inoculated with

freshly prepared cultures of each strain in defined media to give an initial count of 10^4 cfu/ml. They were incubated on a shaker (90 rev/min) at 25°C. Duplicate cultures were checked twice daily for appearance of visible turbidity for 14 d.

Bradyrhizobium japonicum strain RCR 3427 was used for further investigations of the effect of trehalose. Trehalose was added as a filter-sterilized solution to defined media containing 0·08 M NaCl or 0·17 M NaCl in addition to an unamended control, to give final concentrations of 0, 1·0 or 50 mM trehalose. Next, 25 ml aliquots were dispensed into 100 ml conical flasks and inoculated with a freshly prepared culture of the strain to give an initial count of 10^4 cfu/ml. They were incubated on a shaker (90 rev/min) at 25°C. Viable counts of duplicate cultures were made every 2 d for 14 d using YEM agar.

Results and Discussion

Trehalose had no effect on all *Rhizobium fredii* and *Bradyrhizobium japonicum* strains in the absence of NaCl (Table 1). *Rhizobium fredii* strains USDA 192, USDA 205, USDA 208 and USDA 217 showed no response to 20 mM trehalose in the presence of 0·34 M NaCl; however, slight improvement in growth rate (in terms of the appearance of visible turbidity) of strains USDA 191, USDA 193 and USDA 201 was observed (Table 1).

Multiplication of *Bradyrhizobium japonicum* strains RCR 3827 and USDA 136 was not affected by 0·08 M NaCl, whereas the growth rate of strains USDA 110, RCR 3407 and 31b/143 was slightly increased (Table 1). The effect of trehalose in relieving salt-stress was most pronounced with *Bradyrhizobium japonicum* strains RCR 3827 and USDA 122.

Table 1. Estimated growth rate for soybean rhizobia (*Rhizobium fredii*) and bradyrhizobia (*Bradyrhizobium japonicum*) in defined liquid medium with or without additional NaCl or 20 mM trehalose (+ or − T), based on the time (days) taken for visible turbidity from an initial inoculum density of 10^4 cfu/ml at 25°C (means of two replications)

	No NaCl		0·34 M NaCl	
Strain	−T	+T		
Rhizobium fredii				
USDA 191	2	2	11·0	11·5
USDA 192	3	3	12·0	12·0
USDA 193	3	3	8·5	8·0
USDA 201	2	2	10·5	8·0
USDA 205	2	2	8·0	8·0
USDA 208	2	2	8·0	8·0
USDA 217	3	3	8·0	8·0
Strain	−T	+T		
Bradyrhizobium japonicum				
RCR 3407	3·5	3·5	4·0	3·5
RCR 3824	4·0	4·0	4·0	4·0
RCR 3827	4·0	4·0	NT	8·0
31b/143	4·5	4·5	6·0	5·5
USDA 110	4·0	4·0	5·5	4·5
USDA 122	5·5	5·5	7·5	5·0
USDA 136	4·0	4·0	4·5	4·5

NT = no turbidity observed within 14 days.

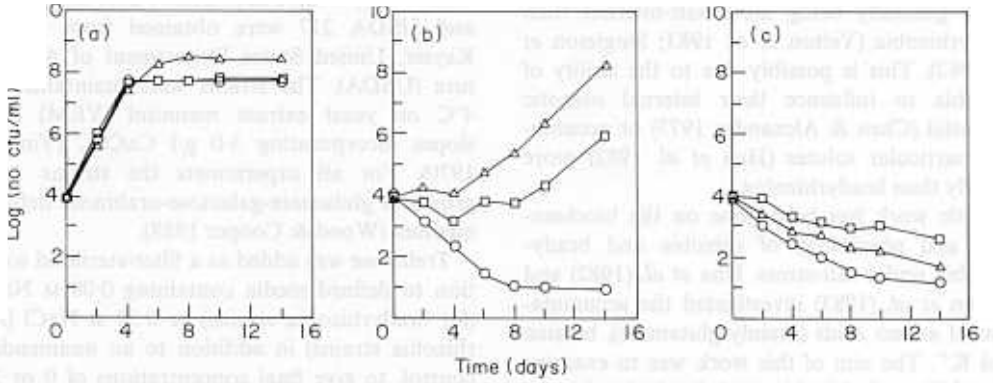

Fig. 1. The effect of trehalose on the growth of soybean *Bradyrhizobium japonicum* strain RCR 3827 under different NaCl concentrations. (a) No NaCl; (b) 0·8 M NaCl; (c) 0·17 M NaCl. ○, no trehalose; □, 1 mM trehalose; △, 50 mM trehalose. Two replicates per treatment.

Figure 1 shows the effects of trehalose on the growth of the salt-sensitive strain RCR 3827 with three different concentrations of NaCl. Both concentrations of trehalose (1 and 50 mM) protected the strain at 0·08 M NaCl, with the highest concentration having the greater effect. In the medium with 0·17 M NaCl both levels of trehalose failed to relieve the salt stress but survival was slightly improved.

The results suggest that trehalose may act as a means of osmoprotection in rhizobia and bradyrhizobia under saline conditions. Ahmed *et al.* (1980) suggested that trehalose, which has a low molecular weight, serves as an osmoregulator, formed in response to high osmotic pressures. Trehalose together with sucrose were the two most important disaccharides which were accumulated by salt-sensitive fresh water and brackish water cyanobacteria (Mackay *et al.* 1984).

Trehalose has been mentioned as a compound protecting the living cell by stabilizing membranes against damage induced by dehydration (Vanlaere & Slegers 1987). The results shown here (Fig. 1) indicate that trehalose protected the cells of strain RCR 3827 in salt-stressed medium. Trehalose may directly stabilize the conformation of protein (Booth *et al.* 1988), or may be rapidly broken down to lower molecular weight products, such as glycerol, which can protect enzymes (Vanlaere & Slegers 1987). The accumulation of trehalose by the cell can also lead directly to the release of K^+ and a lowering of the cytoplasmic ionic strength (Roller & Anagnostopoulos 1982). Trehalose also accumulates in cyanobacteria (Reed & Stewart 1985) and some fungal spores (Ahmed *et al.* 1980) in salt-stressed media, and in senescing nodules (Streeter 1985).

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