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20

MAINTENANCE OF DISCRETE BACTERIAL POPULATIONS IN ADJACENT MARINE HABITATS

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ABSTRACT - Some mechanisms of selection which operate on marine *Vibrio* and *Pseudomonas* strains during survival in dilute environments have been examined. Mannitol uptake and utilisation systems are more efficient in the *Vibrio* strain compared with the *Pseudomonas* strain during starvation survival. A *Vibrio* mutant strain lacking mannitol uptake showed no greater loss of viability during starvation survival than mannitol positive strains. The *Vibrio* strain appears to be constitutive with respect to mannitol uptake systems, whereas the *Pseudomonas* strain examined is inducible. Active uptake systems for glucose, glutamate, alanine and aspartic acid were efficiently maintained during starvation survival in the *Vibrio* strain. These results suggest that membrane uptake systems are maintained during starvation survival and that they could provide a basis for the segregation and maintenance of bacterial populations in the kelp ecosystem which was observed in previous work by our group.

Key words : starvation survival, mannitol utilization.

RÉSUMÉ - Quelques mécanismes de sélectivité influençant les souches marines de *Vibrio* et *Pseudomonas* pendant leur survie en milieu appauvri ont été étudiés. Le mannitol peut être assimilé et utilisé plus efficacement en période de jeûne par la souche de *Vibrio* que par celle de *Pseudomonas*. Une souche mutante de *Vibrio* incapable d'assimiler le mannitol peut aussi bien survivre à un jeûne qu'une souche ayant cette propriété. L'assimilation du mannitol est constitutive chez le *Vibrio* alors que ce mécanisme est inductible chez le *Pseudomonas* étudié. Pendant la période de jeûne, l'assimilation active du glucose, du glutamate, de l'alanine et de l'acide aspartique est maintenue chez le *Vibrio*. Ces résultats suggèrent que les systèmes d'absorption membranaire sont fonctionnels pendant le jeûne, et pourraient être à l'origine de la sélection et du maintien de certaines souches en milieu marin, comme ceci a déjà été observé par notre groupe.

Mots clés : survie à jeûn, utilisation de mannitol.

INTRODUCTION

Davis *et al.* (1983) have shown that different nearshore habitats contain bacterial populations with markedly differing component strains. These bacteria most probably originate in the water column and subsequently develop into various communities as a result of selection. The water column contains a largely dormant bacterial population with a low plate count. Several studies have examined the physiological adaptations of marine bacterial strains to starvation survival, and it is known that dormant bacteria maintain viability (Amy and Morita, 1983) and active membrane uptake systems (Faquin and Oliver, 1984) during long periods of dormancy. Our previous work (Davis *et al.*, 1983) indicated that bacterial communities on beached kelp consisted largely (94 %) of *Vibrio* strains which were able to ferment mannitol, the principle soluble carbohydrate in kelp (Newell *et al.*, 1980). This is in contrast with adjacent populations where a significant proportion of *Pseudomonas* strains occurred. The present study was undertaken in order

to compare the responses of the three main bacterial groups in our samples (*Vibrio, Pseudomonas* and *Flavobacterium)* to starvation survival. Physiological adaptations of mutated *Vibrio* strains are examined in relation to their mannitol utilisation phenotype, in order to establish the mechanisms of selection which might be operating on these populations.

MATERIALS AND METHODS

Isolation of bacterial strains

Water column bacteria were isolated as described in Seiderer *et al.*, 1984. Kelp associated bacteria were obtained by incubating 10 ml of sterile seawater (SW) with 2cm x 2cm pieces of kelp (*Ecklonia maxima*) that had been handled aseptically. Bacteria were plated out after 18hr incubation, and subsequently purified and maintained as described in Davis *et al.*, 1983.

Mannitol uptake and utilisation

Cells were grown overnight in one of 3 media made up with SW, a) 1 % casamino acid, b) SW broth (0.5 % peptone ; 0.1 % yeast extract) and c) 1/10 SW broth. Mannitol (1 % w/v) was added as an inducer where indicated. Bacteria were harvested, washed and resuspended in SM salts + 0.01 M Tris, pH 7.8 (Novitsky and Morita, 1976). The OD_{600} was adjusted to 0.2.

Mannitol uptake was measured after adding C^{14} mannitol (59 mCi/mmol, 12 μM) to the cells. Subsamples (50 μl) were taken at appropriate times, filtered onto 0.45 μm Gelman filters, (0.22 μm Millipore filters for starved cells) and washed with 1 ml SW.
Scintillation fluid was added to the samples and they were counted in a Packard 460 liquid scintillation counter.

Mutagenesis

A *Vibrio* strain was mutated using N-methyl—N'-nitro-N—nitrosoguanidine (NTG) and then grown in SWB. Counter-selection against wild type cells was carried out using growth in 1 % mannitol and 15 μg.ml^{-1} of ampicillin. Mutants were detected by plating onto mannitol tetrazolium plates (Lee *et al.*, 1983).

Starvation studies

Bacteria were grown overnight in 1/10 SWB, washed and resuspended in SM salts containing no nitrates or phosphates (Novitsky and Morita, 1976). The cells were kept in a 10°C water bath.

Viable cell counts were determined by plating onto SW agar (SWB + 1.5 % agar). Uptake and respiration of the following radiolabelled sugars and amino acids (Amersham) were measured during the starvation period : C^{14} mannitol (59 mCi/mmol) (11.3 μM), C^{14} glucose (28 mCi/mmol) (2.9 μM), C^{14} glutamate (280 mCi/mmol) (1.2 μM), C^{14} alanine (165 mCi/mmol) (1.9 μM) and C^{14} aspartic acid (224 mCi/mmol) (1.5 μM).

RESULTS AND DISCUSSION

Figure 1 shows the survival of *Vibrio, Pseudomonas* and *Flavobacterium* strains during a starvation survival experiment under conditions similar to those employed by Novitsky and Morita (1977). These strains represent the three major groups of kelp associated

bacteria (Davis *et al.*, 1983). The *Vibrio* strain shows greater survival than the *Pseudomonas* and *Flavobacterium* strains, expecially during the first four weeks. The initial increase in viability shown by the *Vibrio* strain is variable, and corresponds to the phase referred to as fragmentation by Novitsky and Morita (1977). The *Flavobacterium* strain underwent a relatively rapid loss of viability. There are a high number of *Vibrio* strains associated with kelp, and a lower abundance of *Vibrio* strains are recovered from offshore samples compared with *Pseudomonas* (Davis *et al.*, 1983). In general, the obligate aerobes (predominantly *Pseudomonas*) constitute the bulk of the other bacterial strains recovered from these samples. The experiment shown in Figure 2 was performed in order to establish whether possession of an effective mannitol uptake system would result in decreased long term survival. The survival rates of parallel cultures of a mannitol negative mutant strain and mannitol positive parent strain were identical. The mannitol uptake system is defective in the mutant strain, as shown in Table 1, where the uptake of C^{14} mannitol is measured in the mutant and wild type strains.

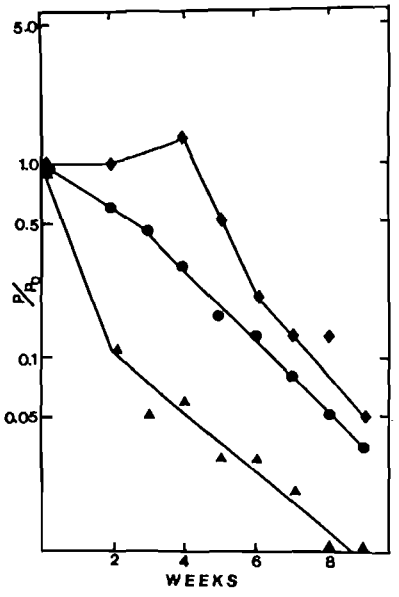

Figure 1 : The survival of three marine bacterial strains during starvation survival. Cultures were maintained at 10°C in SM medium as described in Materials and Methods. *Vibrio* (◆—◆), *Pseudomonas* (● —— ●), *Flavobacterium* (▲——▲).P/Po = fraction of surviving bacteria as measured by colony forming units.

Strain	Relative Mannitol uptake
Vibrio wild type	78
Vibrio mtl 5	2
Vibrio mtl 6	1

Table 1. : Relative mannitol uptake activities of the wild type *Vibrio* strain and two mutant strains defective in mannitol uptake. Cells were grown for 16 hr in 1/10 SWB at 10°C. Assay conditions are as described in Materials and Methods. Relative uptake refers to the percentage of mannitol available which is taken up by the cells.

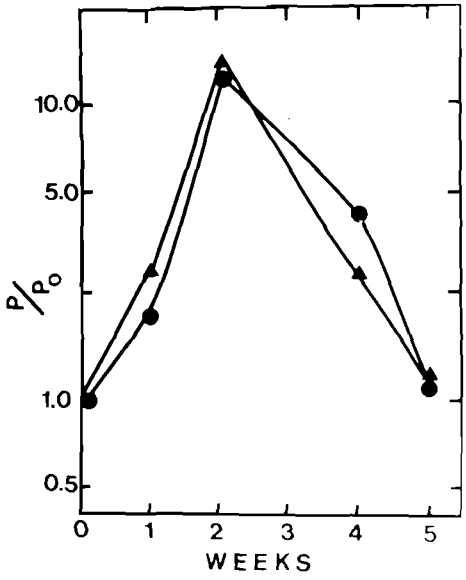

Figure 2 : The survival of mannitolytic *Vibrio* wild type (▲——▲) and *Vibrio* mtl mutant I (● —— ●) during starvation.

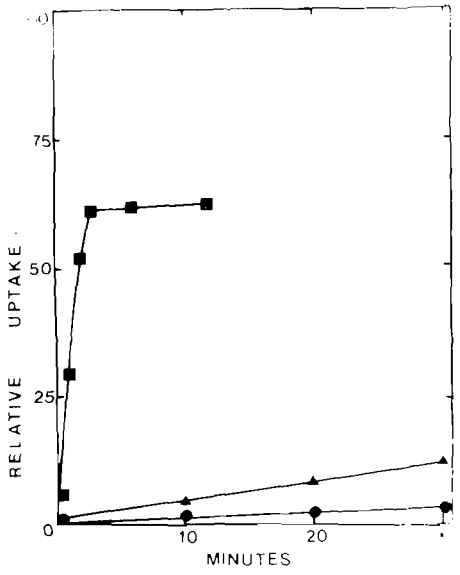

Figure 3 : Mannitol uptake by *Vibrio* wild type (■ —— ■) and *Pseudomonas* was grown in 1/10 SW broth either with (▲ ——▲) or without (● —— ●) the addition of 1 % D-mannitol. Relative uptake refers to the percentage of mannitol available which is taken up by the cells.

The *Pseudomonas* strain used here does not maintain detectable basal levels of mannitol uptake during starvation survival, (C. Davis, unpublished results) and is thus less able to respond rapidly to a pulse of exogenous mannitol.

There is a far higher maximal rate of mannitol uptake in the *Vibrio* strain isolated from a kelp frond, compared with the *Pseudomonas* from the water column, as shown in Figure 3. In addition, the mannitol uptake system is inducible by growth in the presence of 1% mannitol in the case of the *Pseudomonas* strain. The *Vibrio* strain used here is constitutive for mannitol uptake, in common with all the mannitolytic *Vibrio* strains we have tested (C. Davis, unpublished results).

The effects of long term starvation on several active uptake systems in the strains used here are shown in Table 2. This confirms the result that mannitol uptake is maintained in *Vibrio* strains during starvation, and also indicates that glucose, glutamate, alanine and aspartate uptake systems are present in starved cells. The recent work of Faquin and Oliver (1984) showed that arginine transport was maintained during starvation survival.

Substrate	Relative uptake	
	0 weeks	5 weeks
D-Mannitol	78	51
D-Glucose	84	50
L-Glutamate	73	81
L-Aspartate	55	46
L-Alanine	61	41

Table 2 : Relative uptake of several substrates by the *Vibrio* wild type strain at the beginning and end of a five week starvation period. Uptake was measured as described in Materials and Methods, over a period of ten minutes. The viable cell count was 6.3×10^8 at t = 0 and 7.0×10^8 at t = 5 weeks. Relative uptake refers to the pecentage of labelled compound available which is taken up by the cells in ten minutes.

CONCLUSION

We have shown that a marine *Vibrio* strain of a type which colonises degrading kelp, possesses physiological properties which would result in positive selection in competition with other marine bacterial types. In particular, the ability to take up and utilise manitol as a substrate is maintained in the *Vibrio* strain during prolonged starvation. The possession of this active uptake system does not affect starvation survival, but it may have a profound effect on the revival rate of dormant bacteria which come into contact with kelp. As a consequence, the kelp associated flora may be selected from the variety of bacterial forms in the water column.

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