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The production and release of an extracellular polysaccharide during starvation of a marine *Pseudomonas* sp. and the effect thereof on adhesion

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Abstract. A marine *Pseudomonas* sp. S9 produced and released an extracellular polysaccharide during complete energy and nutrient starvation in static conditions. The presence of the polysaccharide on the cell surface, demonstrable by immune transmission electron microscopy, correlated with changes in the degree of adhesion to hydrophobic surfaces. Polysaccharide coated cells showed a lower degree of adhesion than did cells devoid of the polymer. After 10 h of starvation, no ruthenium red stained antibody stabilized polysaccharides could be observed on the cell surface. The polysaccharide was not produced during growth since lysates of mid-log phase cells did not precipitate the antiserum. The relative proportions of sugars in the polysaccharide were 28% glucose, 35% N-acetylglucosamine and 37% N-acetylgalactosamine. The released polysaccharide did not significantly alter the physical parameters of surface tension and viscosity of the starvation regime. Cells starved in agitated conditions did not produce any extracellular polysaccharides and exhibited a different adhesion pattern to hydrophobic surfaces.

Key words: Starvation – Extracellular polysaccharides – Bacterial adhesion – Marine bacterium – *Pseudomonas*

Biopolymers such as polysaccharides have been shown to participate in pathogenic and symbiotic interactions between plants, animals and microorganisms (Costerton et al. 1985; Sutherland 1985). Polysaccharide biopolymers enhance attachment of marine bacteria to surfaces (Costerton et al. 1981; Fletcher and Floodgate 1976) but there are few reports which distinguish between polymers involved in the initial adhesion and those involved in the subsequent colonization process. It has been demonstrated that cell bound polysaccharide polymers can impede as well as enhance adhesion (e.g. Fattom and Shilo 1985; Hogt et al. 1983; Ofek et al. 1983; Pringle and Fletcher 1983; Rosenberg et al. 1983; Runnels and Moon 1984).

Interpretation of the significance in the natural marine ecosystems of polymers which are produced in the laboratory is extremely difficult (Marshall 1985). The marine environment is characterized by nutrient fluxes and predominantly low nutrient niches where bacteria must survive by adaption to long term starvation (Morita 1982). Bacteria are known to undergo an active process of starvation survival upon deprivation of exogenous energy and nutrient sources (Morita 1982). As a result, non-growing but metabolically active vegetative cells which are different from growing counterparts are formed. Large morphological, physiological and chemical changes occur (Dawson et al. 1981; Kjelleberg et al. 1982; Marden et al. 1985) but no data have been reported on the bacterial surface structure during starvation. Polymers produced under such conditions may be quite different from those produced in laboratory broth cultures and be more directly applicable to the situation in the natural ecosystem. This study demonstrates the production and release of an extracellular polysaccharide from a marine *Pseudomonas* during complete energy and nutrient starvation. The presence of this polysaccharide on the bacterial surface is correlated with the degree of adhesion to non-biological surfaces and aggregation of bacterial cells during starvation. Other marine isolates were also screened for polysaccharide production during growth and total energy and nutrient starvation.

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Non-standard abbreviations. FSS, Four salt solution; GLC, gas liquid chromatography; MS, Mass spectrometry; NSS, nine salt solution

Materials and methods

Organisms. The marine bacterium *Pseudomonas* sp. S9 (Humphrey et al. 1983) is a motile short rod, which reduces nitrate during facultatively anaerobic conditions. Cultures were preserved by freezing in glycerol from which precultures for each experiment were prepared.

A series of marine psychrophilic bacteria collected in the polar basin (Dahlbäck et al. 1982) was included in this investigation in order to screen for the frequency of polymer production during growth and starvation of marine bacteria.

Media and diluent. All cells were grown in VNSS broth which contained: peptone 1.0 g; yeast extract 0.5 g; glucose 0.5 g; starch (soluble) 0.5 g; $FeSO_4 \times 7\ H_2O$ 0.01 g; Na_2HPO_4 0.01 g; nine salt solution (NSS) 1000 ml. The diluent used in the starvation regimes, for washing bacterial cells and for viability estimations, was NSS which had the following composition: NaCl 17.6 g; Na_2SO_4 1.47 g; $NaHCO_3$ 0.08 g; KCl 0.25 g; KBr 0.04 g; $MgCl_2 \times 6\ H_2O$ 1.87 g; $CaCl_2 \times 2\ H_2O$ 0.41 g; $SrCl_2 \times 6\ H_2O$ 0.01 g; H_3BO_3 0.01 g; double distilled water 1000 ml. The pH was adjusted to 7.7.

The strains from the polar basin were grown on MSWYE-medium which has the following composition: yeast extract 1.0 g; proteose-peptone 1.0 g; four salt solution (FSS) 1000 ml. The starvation regime and diluents used for washing the cells was FSS which has the following composition: NaCl 24.0 g; KCl 5.3 g; $MgCl_2$ 5.3 g; $MgSO_4$ 7.0 g;

double distilled water 1000 ml. The pH was adjusted to 8.0. All media were autoclaved at 120° C for 20 min.

Growth and starvation of Pseudomonas sp. S9. The precultures in VNSS were incubated at 26° C overnight on a rotary shaker and a 1% inoculum was transferred to fresh VNSS growth medium. The culture was incubated as above, to mid-exponential phase which corresponded to an absorbance of 0.7 at 610 nm. The cells were then harvested by centrifugation (10 min at $13,300 \times g$), washed three times with cold NSS and resuspended in the starvation medium (NSS) to give a final concentration of approximately 5×10^8 cells/ml, as determined by a direct microscopic count. Starvation regimes were incubated both under static conditions and on the rotary shaker. No polymer production was observed during agitated starvation conditions and such cells served as non-polymer producing controls. Static regimes were sampled at various times for cell size and viability while shaken regimes were sampled for cell size analysis.

Measurements of cell size, numbers and viability. Samples for determination of cell size and numbers were fixed by adding 0.25 ml 2.5% glutaraldehyde per 2 ml aliquot. The size distribution was obtained by using a microcomputerized Elzone ADC-80 XY particle counter with a 12 µm orifice tube and NSS as both the internal fluid and diluent. The number of viable cells was determined in duplicate on VNSS agar using the spread plate method and expressed as the number of colony forming units (CFU) per ml. Plates were incubated at room temperature for 24 h and counted.

Adhesion of Pseudomonas sp. S9 to a non-biological surface. Siliconized glass cylinders, 13 mm in diameter with an inner diameter of 11 mm and 37.5 mm long, were used as the nonbiological surface. The test surfaces had been washed in detergent, rinsed with ethanol (95%) and double distilled water, dried at 450° C for 4–6 h and siliconized in 5% dichlorodimethylsilane in toluene with subsequent washings in methanol, ethanol and finally in double distilled water. The bacterial cells were grown in VNSS growth medium containing 3 µCi/ml (Methyl-3H) thymidine (80.2 Ci/mmol; New England Nuclear, NEN) and then starved in 800 ml NSS as previously described. After times 1, 4, 7, 10, 13 and 16 h of starvation, 100 ml aliquots were sampled, centrifuged ($13,300 \times g$, 10 min) and resuspended in the same volume of NSS in glass beakers. The siliconized cylinders were immersed in the bacterial suspensions for 15 min, rinsed in 100 ml NSS and then placed in scintillation fluid (Aquassure, NEN) and counted for 5 min in a Packard Model 3255 Tri-Carb. The disintegrations per minute were calculated using the counting efficiency determined by the external standard method. This reflected the total number of attached cells. The leakage of the radiolabel from the cells was tested by measuring the radioactivity in the supernatant of the bacterial suspensions.

Aggregation. After 1, 4, 7, 10, 13 and 16 h of starvation, 20 ml samples were taken from both agitated and static regimes and centrifuged ($2000 \times g$, 10 min). The supernatant was discarded and the pellet resuspended in an equal volume of NSS and resuspended by mixing for 15 s at the maximum speed using a tube mixer (Heidolph). The degree of aggregation was photographed using a Olympus OM-2 camera with a 50-mm lens and a 32 ASA Kodak Panatomic-X film.

Polysaccharide production and purification. Cells were starved in 20 l volumes at a cell concentration of approximately 4×10^8 per ml for 48 h, and harvested by centrifugation ($13,300 \times g$, 10 min) in 250 ml centrifuge bottles. The pellets containing bacteria and polymer, were collected and concentrated in one centrifuge bottle. This pellet was diluted to approximately 200 ml with NSS, thoroughly mixed and centrifuged ($113,000 \times g$, 1 h) in a Beckman L8-M ultracentrifuge equipped with a SW-28 rotor. This procedure removed the bacterial cells from the polymer.

The polymer containing supernatant was collected and dialyzed three times against double distilled water for 24 h. The concentrated and dialyzed polymer was freeze dried and stored for subsequent analyses.

Production of antibodies against Pseudomonas sp. S9 polymer. A rabbit, initially bled for pre-immune serum, was immunized by subcutaneous and intramuscular injection. The antigen consisted of an emulsion of 1.0 ml purified polymer (in 0.9% saline, 0.25% formaldehyde) and 0.5 ml of Freunds complete adjuvant. The procedure was repeated using Freunds incomplete adjuvant 2 times per week for a further 3 weeks. Two weeks after the last injection, a booster without adjuvant was injected intravenously using the marginal ear vein and the animal was bled 5 days later. The serum was assayed for the presence of antibodies against the polymer by a double diffusion precipitation test in narrow glass tubes.

Detection of intracellular polymer. Lysates of the bacterial strain S9 were prepared by harvesting mid-log phase cells, washing and then sonicating until no whole cells could be observed by light microscopy. Similarly, fully aerated shaken cells in starvation regimes were lysed. The tube precipitation test was used on the cell lysates to determine whether fractions or precursors of the polymer were present within the mid-log phase grown cells or produced during starvation.

Transmission electron microscopy. After 0, 1, 5, 10 and 50 h of starvation, 2 ml aliquots were sampled and incubated with 1 ml rabbit antipolymer whole serum at 37° C for 10 min. After centrifugation ($2000 \times g$, 10 min), the bacterial pellet was embedded in 2% (w/v) agar and fixed in 0.25% glutaraldehyde in phosphate buffer 0.01 M, pH 7.2 overnight. The samples were then washed three times, with a 30 min interval, in the same phosphate buffer and postfixed in 2% (v/v) osmium tetroxide in the buffer for 3 h at room temperature. Samples were then washed three times in phosphate buffer as above and dehydrated in a graded series of ethanol and embedded in a low viscosity epoxy resin according to Spurr (1969). Ruthenium red (0.15%) was included during fixation and washing in the ethanol series. Sections were cut with glass knives on an LKB Ultrotome III ultramicrotome, poststained with aqueous 4% (w/v) uranyl acetate and lead citrate (Reynolds 1963) and examined with a Philips EM 301 electron microscope.

Chemical analyses. The purified polymer was analysed both in order to check the degree of bacterial contamination and to determine the chemical composition.

Lipid analyses. Acyl lipids were extracted from the polymer with chloroform-methanol 2:1 (v/v). The extract was re-

duced to dryness on a rotary evaporator. The lipids were methanolyzed with 3% HCl in methanol at 70° C for 1 h. The fatty acid methyl esters were extracted with light petrol (b.p. 40–60° C). After evaporation to dryness the methyl esters were dissolved in n-heptane. Methyl esters of fatty acids were run on a Varian 1400 gas chromatograph with a flame ionization detector. The column (2 m × 3 mm i.d. stainless steel) was packed with 10% sp-2330 on a 100–120 mesh chromosorb W-AW (Chromatographic specialities Inc.); the column temperature was 180° C and nitrogen was used as the carrier gas. Fatty acid methyl ester peaks were identified by comparing their retention times with those of pure methyl esters and were quantitated using a Hewlett Packard 3390A integrator.

C/N-analyses. Replicate samples were dried at 150° C for 24 h, weighed and the C/N ratio was determined using a Carlo Erba CHN-analyser (Elemental analyzer Mod. 1106) with computer integration of the peaks.

Protein measurements. The amount of protein in the purified polymer was determined according to Bradford (1976) using bovine serum albumin as the standard.

Sugar analyses. The polysaccharide was hydrolysed with trifluoroacetic acid (2 M) for 2 h at 120° C after reduction with sodium borodeuteride and acetylated according to Sawardeker et al. (1965). The derivatives were analyzed on GLC-MS. For GLC a Hewlett Packard 5830A instrument, fitted with a flame ionization detector was used. Separations were performed on a fused silica capillary column (crosslinked) with 5% phenyl methyl silicone using a temperature program (150° C, 2 min: 150° C–220° C, 2° C per min). GLC-MS was performed on Hewlett Packard 5790–5970 GLC-MS system. All identifications of masspectra (Jansson et al. 1976) were unambiguous and will not be discussed here.

Surface tension measurements. After 1, 4, 7, 10, 13, 16, 30 and 50 h of starvation, 20 ml samples were transferred to clean sterile Petri dishes. The surface tension was measured with a platinum plate suspended from a torsion wire positioned in the center of the Petri dish. The torsion wire was attached to an electronic microbalance (Cahn 2000). The platinum plate was washed with concentrated dichromic sulphuric acid and rinsed several times with double distilled water and air dried before the measurements. All measurements were carried out at 20° C and with filtered NSS (0.2 µm pore size) as the reference.

Viscosity measurements. After 1, 4, 9, 20 and 120 h of starvation, 50 ml samples were taken for viscosity measurements using a Scott Gerate KP reverse flow capillary viscometer.

Screening for starving and non-starving polymer-producing cells. The frequency with which starving and non-starving bacteria produce and release polymers was examined. A series of marine bacteria isolated during the YMER 80 expedition in the polar basin (Dahlback et al. 1982) were screened for polymer production. Fifty randomly selected isolates were grown on MSWYE-medium at 4° C until turbidity was observed. The cells were harvested and washed as mentioned above and 50 ml starvation regimes were set up as for *Pseudomonas* S9. Polymer production was monitored by visual inspection for changes in apparent viscosity both for growing and starving cell suspensions.

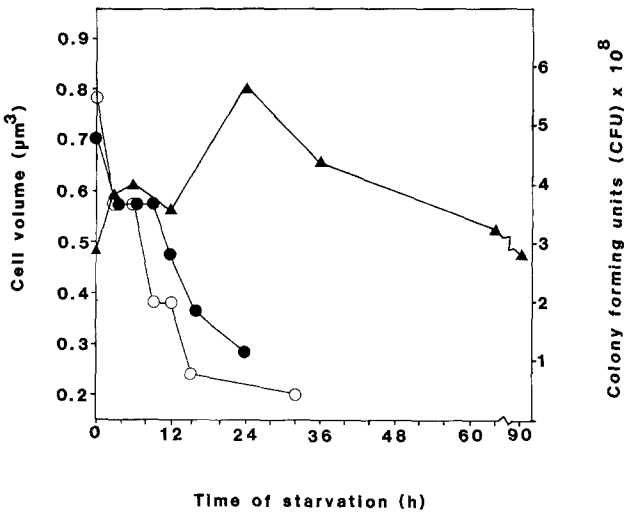

Fig. 1. Changes with time in the bacterial viable count (▲) and cell volume (○, ●) during complete energy and nutrient starvation in static (*open symbols*) and agitated (*filled symbols*) conditions using a *Pseudomonas* sp. S9. Data from a typical experiment presented

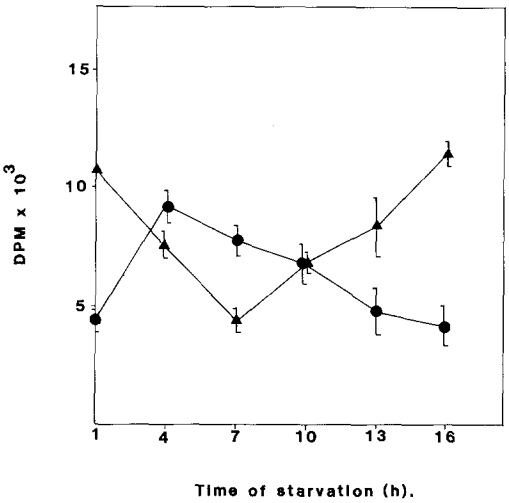

Fig. 2. Adhesion of radioactively labelled *Pseudomonas* sp. S9 to siliconized glass surfaces during complete energy and nutrient starvation in static (▲) and agitated (●) conditions. Adhesion was monitored by measuring the disintegrations per min (DPM) of the tritiated thymidine activity on the glass surfaces. Mean values ∓ standard deviations are the results of triplicate runs

Results

Cell volume and viability of Pseudomonas sp. S9 during starvation. The data presented in Fig. 1 show the size decrease and viability during starvation of the marine *Pseudomonas* S9. The previously noted (e.g. Humphrey et al. 1983) large drop in the cell volume during starvation was recorded and hence confirmed that an energy and nutrient deficient experimental system was used. The bacteria responded to starvation by fragmentation leading to an increase in numbers and a decrease in cell volume. The degree of the cell volume reduction was greater in the static than in the agitated starving

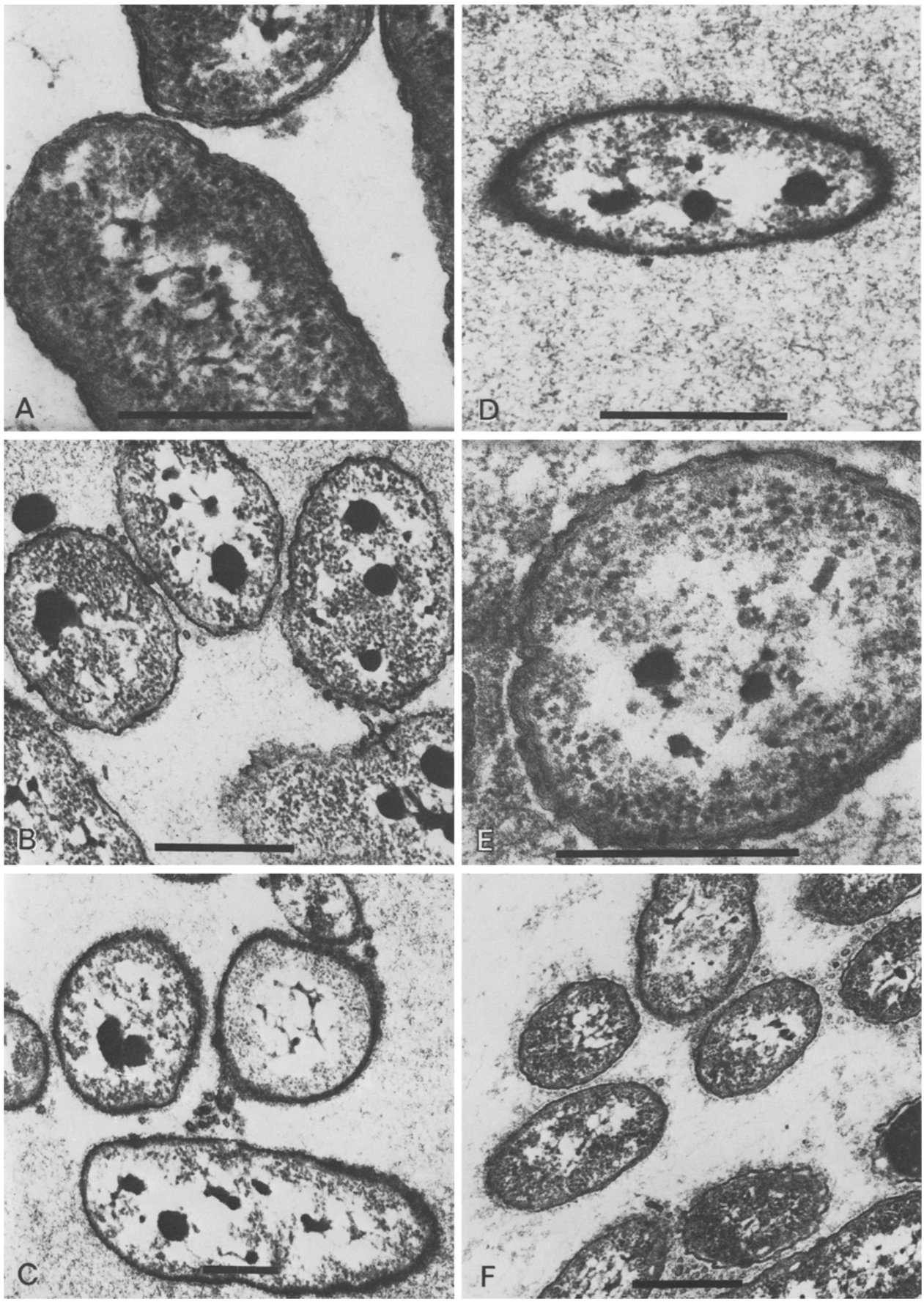

Fig. 3A–F. Transmission electron micrographs of ruthenium red stained, antibody stabilized preparations of *Pseudomonas* sp. S9. **A** shows mid-log phase cells, while **B, C, D** and **E** show cells during complete energy and nutrient starvation for 1 (**B**), 5 (**C** and **D**) and 10 (**E**) h in static conditions. **F** shows cells after 5 h of starvation in agitated conditions. Note the layer of ruthenium red stained material around the cell envelope in **C** and **D** and the absence of the same in **F**. The bar indicators equal 0.5 μm

regime. The viability of cells starved in static conditions decreased after 24 h but still exceeded the number of viable cells at the start of the starvation experiment. This was also the case after 86 h of starvation.

Polymer production by Pseudomonas sp. S9 during starvation in different conditions. Polymer production by the *Pseudomonas* S9 was visualized as changes in the apparent viscosity of the fluid and was only recorded for the static starvation regime. Agitation prevented polymer production and polymer production was not observed during growth of the *Pseudomonas* S9. Lysates from sonicated cells before starvation did not precipitate with antipolymer antibodies. This indicated that fractions or detectable precursors of the polymer did not exist internally at the onset of starvation but was rather produced during static starvation.

Adhesion and aggregation experiments. The adhesion to hydrophobic, siliconized, glass surfaces of cells starved under agitated and static conditions were significantly different (Fig. 2). The adhesion of the agitated cells increased between 1 and 4 h of starvation followed by a slow decrease in attachment with time. The adhesion of cells starved under static conditions decreased between 1 and 7 h prior to an increase with extended starvation. The leakage of radioactive material from the cells did not exceed 10% in any of the adhesion experiments. Figure 4 illustrates the aggregation of bacterial pellets from static starvation regimes after resuspension in NSS. The aggregation study showed that cells were freely suspended in NSS at times 1 and 4 h, but after 7 h of starvation aggregates were clearly visible. Subsequently, there was a gradual increase in the degree of aggregation until by 13 h, no free cells could be observed.

Transmission electron microscopy. Transmission electron micrographs of *Pseudomonas* sp. S9 cells sampled from a mid-log phase culture (Fig. 3A) and a static starvation regime at times 1, 5, 10 h are presented in Fig. 3B, C, D, E and a representative photograph of 5 h starved cells from an agitated suspension is seen in Fig. 3F. An antibody stabilized, ruthenium red stained material developed around the bacterial cells between 1 and 5 h of starvation. With extended starvation, the cells appeared to gradually lose the extracellular layer of polysaccharides. While virtually no layer of antibody stabilized ruthenium red stained material could be observed after 10 h, 50 h starved cells were completely devoid of such material. No antibody stabilized ruthenium red stained material could be detected around cells that had been starved in agitated cell suspensions (Fig. 3F), irrespective of the length of the starvation period. This material was undetected when cells were not stabilized by antibodies before embedding and staining. No data using non-immunized serum is presented as the lack of the extracellular layers with extended starvation and in shaken cultures serve as negative controls.

Surface tension and viscosity of the starvation fluid. The data presented in Tables 1 and 2 show that the polymer formation and release into the starvation regime had small effects on the physical parameters of surface tension and viscosity. The only differences observed in these parameters were detected during the first 3 h of starvation. The correlation between these changes in physical parameters and the adhesion pattern was negligible.

Table 1. Relative changes in surface tension of cell suspensions of *Pseudomonas* sp. S9 during energy and nutrient starvation. The surface tension after 1 h was assigned 100%

Time of starvation (h)	Relative changes in surface tension (%)
1	100
4	99.2
7	98.9
10	98.9
13	98.9
16	98.9

Table 2. Viscosity of the cell suspension of *Pseudomonas* S9 during complete energy and nutrient starvation

Time of starvation (h)	Viscosity (mm^2/s)
0	0.7836
3	0.7912
7.5	0.7988
20	0.7988
120	0.8292

Table 3. Composition and relative amount of fatty acids in the purified polymer from *Pseudomonas* S9. The polymer contained 0.01% dry weight of fatty acids

Fatty acid composition	Relative amount (%)
14:0	7
16:0	53
16:1	17
18:0	16
18:1	7

Chemical analyses. The composition of fatty acids in the purified polymer from *Pseudomonas* S9 is shown in Table 3. The fatty acid profile was typical of marine bacteria (Oliver and Colwell 1973). The relative amount of fatty acids in the polymer was low however. The polymer contained 0.02% dry weight of fatty acids. The corresponding figure for the amount of protein was 2–4%.

The carbon:nitrogen ratio was found to be 9.28 ($\pm$0.84, $n = 2$). The polymer contained 323.6 µg C and 40.8 µg N per mg dry weight.

Gas chromatographic/masspectrometric analysis revealed the following composition of the polymer: Glucose 28%; N-acetylglucosamine 35%; and N-acetylgalactosamine 37%.

Screening of starving and non starving polymer producing marine bacteria. The investigation of polymer production during both growth and starvation of 50 marine psychrophilic isolates showed that two of these strains rendered the medium apparently more viscous during growth, and two different strains produced material that resulted in an increased apparent viscosity of the starvation regimes.

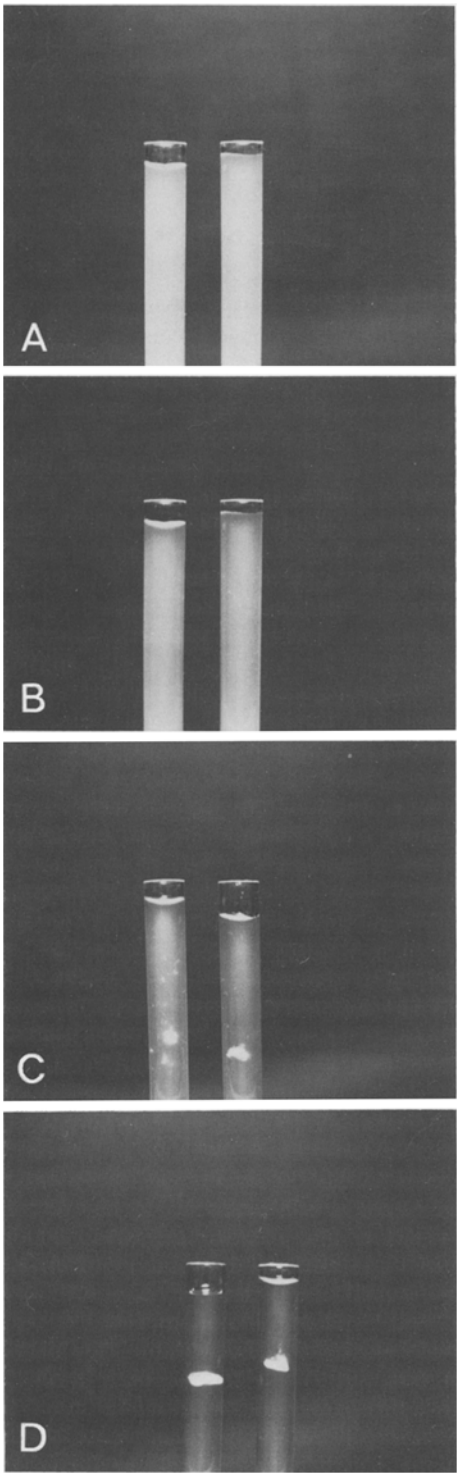

Fig. 4 A – D. Cell aggregation when bacterial pellets of *Pseudomonas* sp. S9 were resuspended in NSS, at starvation times 1 (**A**), 4 (**B**), 7 (**C**) and 10 (**D**) h

Discussion

The production and release of polymeric material is considered to be a relatively common phenomenon during bacterial growth (Costerton et al. 1985). While many species have been shown to produce and/or release polymers, the ecological significance of the polymers is still not completely elucidated. Several reports have indicated that the polymers enhance adhesion of the bacterial cell to surfaces, for example by bridging across the repulsion barrier and thereby anchoring the cell to the surface (e.g. Geesey 1982; Rutter 1980). Transmission electron microscopy studies also suggest that polymers promote adhesion (Costerton et al. 1981). The role of polymers has also been ascribed as a protection barrier against phagocytosis, bacteriophages, antibiotics, biocides, surfactants and antibodies (e.g. Costerton et al. 1985).

More recently, Fattom and Shilo (1985) suggested that the production of a polymer by a *Phormidium* J-1 (*Cyanobacteria*) serves as a dispersal mechanism in the normal life cycle, which enables the bacteria to colonize new surfaces. A similar desorption effect of *Acinetobacter calcoaceticus* RAG-1 caused by the release of a polysaccharide containing polymer called Emulsan, has also been reported by Rosenberg et al. (1983). Several examples of such effects are cited in Rosenberg and Kjelleberg (1986). There are several explanations for the observed differences and the proposed role of polysaccharide containing polymers in the adhesion events. The adhesion of bacterial cells to surfaces may be enhanced by polymer encapsulated envelopes (Rutter 1980), provided that the polymer can interact specifically with the surface bound material. This may be of relevance for adhesion to inanimate surfaces which are covered with a conditioning film of macromolecular substances (Marshall 1985), or animate, mucin-covered surfaces.

Conversely, if both the polymer on the bacterial cell and the surface are hydrated, the bacterium could be sterically hindered from approach to the surface for the van der Waals forces of attraction to operate (Rutter 1980). Maroudas (1975) found that surface bound polymers on adsorbed serum proteins inhibited cell attachment because of their steric exclusion volume. Other workers (Paul and Jeffrey 1985) considered it unlikely that slimes and extracellular polymeric substances could be involved in the initial adhesion process of bacteria to substrata in the marine environment because of their common hydrophilic properties and loose connection to the cells.

Even though energy and/or nutrient deficient situations occur frequently in natural habitats (e.g. Morita 1982), there have been no reports of bacteria producing polymer during total nutrient and energy deprivation. The effect of aeration and limitation of inorganic nutrients and the composition, amount and physical properties of extracellular polysaccharides has recently received attention (reviewed by Sutherland 1985). From these data, it seems that changes in environmental conditions influence the types and relative concentrations of sugars in bacterial extracellular polysaccharides. This effect may be of importance for marine bacteria continually exposed to rapid changes in nutrient and energy levels.

This study demonstrates that energy and nutrient starvation of a marine *Pseudomonas* sp. S9 induced the production and release of an extracellular polymer, with resulting pronounced effects on the degree of adhesion and aggregation of the bacterial cells. From Figs. 2 and 3, it is clear that the presence of the polymer on the cell surface corresponds with a decrease in the degree of adhesion to hydrophobic surfaces. After 10 h of starvation, the polymer was undetectable on the cell surface and this was concomitant with an increase in adhesion. Since lysates of mid-log phase cells did not precipitate with the antiserum, the polymer must be synthesized during the starvation conditions. Facultatively anaer-

obic metabolism may be a prerequisite for the polymer production as the fully aerated and agitated cells did not produce and release the polymer. It should also be noted that facultatively anaerobic conditions induced a faster and more pronounced cell volume decrease during starvation, as compared to aerobically starved cells. Similar findings were presented by Davis (1985). It is known that the initial phase of starvation survival of *Pseudomonas* S9 is an active energy consuming process (Kjelleberg et al. 1983). It is noteworthy that this energy expenditure appears to coincide with the production of polymers as described in this paper.

There is also a possibility that the pattern of aggregation correlated with the production and release of the polymer although this behaviour did not correlate with either the adhesion pattern or the occurrence of polymers around the cell surface. The reason for this effect is at present not understood.

Chemical analyses of the purified polymer revealed a low degree of contamination of bacterial cell material. The lipid content corresponded to 0.01% of polymer dry weight. The sugar monomers of the polymer produced by starved *Pseudomonas* S9 cells are also known to be constituents of other bacterial exopolysaccharides. Sutherland (1977) states that the presence of glucose, N-acetylglucosamine and N-acetylgalactosamine in bacterial polymers are widespread.

The results of the control experiments using agitated starved cells eliminate the possibility of differences due to variations in various biological parameters during the time course of the experiment. All cells were viable throughout the adhesion and aggregation study and during the time at which the polymer was produced and released. The effects on changes in viscosity and surface tension of the bacterial as well as the polymer suspensions appeared to be insignificant. It is known that changes in surface tension of the fluid influence the thermodynamic force of bacterial adhesion (Absolom et al. 1983). The small reduction in surface tension observed in this study would not result in such effects on the adhesion process. The number of collisions between bacterial cells and the test surface was probably not reduced. The small increase in viscosity did not decrease the rate of motility of the bacterial cells as seen in the microscope.

The frequency at which marine bacteria produce polymers during growth or starvation is not known. The screening of 50 isolates from our collection of bacteria from the polar basin revealed that a low number of bacterial strains were able to do so. The observation of a low percent of polymer producing bacteria during growth was supported by similar figures given by a recent study of 160 marine isolates from the air/water and bulk water samples at various stations along the Swedish west coast (Hermansson, M pers. comm.). It is well known, however, that the fraction of culturable bacteria rarely exceeds 1% of the total number of bacterial cells per unit volume of seawater. The importance and generality of polymer production, particulary as a result of energy and nutrient down shift conditions, remains to be elucidated. It is also important to learn more about the energy costs in synthesizing, assembling and transporting of the polymer, as well as the effects of the formation and release of the polysaccharide capsule on processes such as uptake of substrates and membrane and enzyme stabilization. The possible effect of polymers on a series of bacterial cell processes were recently discussed by Fletcher (1985). Changes in uptake rates of soluble substances have been reported (Morita 1982; Davis 1985; Mården, Nyström and Kjelleberg, to be submitted for publication) and may reflect tactics of prime importance for the successful survival of starved bacteria.

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