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EFFECTS OF ENVIRONMENTAL CONDITIONS ON THE SESSILE EXISTENCE OF AN ESTUARINE SEDIMENT BACTERIUM

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ABSTRACT - A submerged glass coverslip technique was developed to determine dissolved organic nutrient uptake by adherent cells of a sediment isolate of *Enterobacter cloacae*. Cells which colonized the coverslips in swirling culture flasks during anaerobic growth remained firmly attached during manipulations employed to determine uptake of radiolabeled glucose by the adherent population. The attached cells were capable of a more rapid rate of glucose uptake than free cells. Lineweaver-Burk plots demonstrated different glucose uptake kinetics for the 2 cell populations. The data suggest that physiological changes occur in cells soon after they become attached to surfaces. These changes appear to enhance the metabolic activity of the adherent population.

Key words : bacterial attachment, glucose uptake, estuarine sediment.

RÉSUMÉ - Pour déterminer le taux d'absorption de composés organiques dissous, par les cellules fixées d'une souche d'*Enterobacter cloacae*, isolée d'un sédiment, une technique d'immersion de lamelles de verre a été mise au point. Les cellules qui colonisent les lamelles en anaérobiose, le flacon étant sous agitation constante, restent fermement fixées durant les manipulations de détermination de l'absorption de glucose marqué par la population adhérente. Les cellules fixées présentent un taux d'absorption du glucose supérieur à celui des cellules libres. Les tracés de Lineweaver-Burk montrent des cinétiques d'absorption du glucose différentes pour les deux types de cellules. Ces observations suggèrent que des changements physiologiques apparaissent chez les cellules, dès qu'elles se fixent aux surfaces. Ces changements semblent accroître l'activité métabolique de la population fixée.

Mots clés : fixation bactérienne, absorption du glucose, sédiment estuarien.

INTRODUCTION

Sessile or attached microorganisms are found in great abundance in aquatic environments. They are commonly concentrated on the surfaces of submerged macrophytes, detritus, and sediment particles. Their densities are often many orders of magnitude greater than the free-floating, planktonic bacterial population (Geesey *et al.*, 1978).

One of the characteristic features of sessile bacteria in various aquatic systems is their tendency to elaborate extracellular polymers. These polymers appear to serve as holdfasts, anchoring the cell to a surface and ensuring its sessile existence. Recently, Moriarty and Hayward (1982) demonstrated that most sediment bacteria are enveloped in an extracellular network of fibers. Coral reef sediments, in particular, are heavily populated by slime-embedded bacteria. These exopolymers, which are synthesized by the bacteria, restrict the dissemination of replicating cells and lead to microcolony development on the surface.

It is known that some bacteria fluctuate between a sessile and free or "swarmer" existence (Costerton *et al.*, 1981). We know very little, however, about the environmental conditions which control a cell's tendency to shift from a free-floating to a sessile nature. Zobell (1943) suggested that the sessile state provided bacteria better access to nutrients concen-

trated on surfaces, but clearly there may be other controlling factors involved. A better understanding of the conditions which promote attachment and the effect attachment has on subsequent physiological activities should provide a more complete knowledge of the importance of sessile bacteria in aquatic environments.
The present study describes the effect of surface attachment on nutrient uptake by a brackish water sediment isolated of *Enterobacter cloacae*.

METHODS AND MATERIALS

Bacteria were isolated from sediments of Cerritos Channel in Long Beach, California in an area where salinity varies seasonally from 0-12 %. Isolates were selected on the basis of their ability to : a) attach to surfaces, b) produce capsular exopolymers, and c) grow in the presence and absence of O_2. A bacterium exhibiting these characteristics that was frequently isolated from the sediments was subsequently identified as *Enterobacter cloacae*.

The isolate was cultured in a broth medium that encouraged capsule production (Salas and Geesey, 1983). Cells were cultured anaerobically by placing inoculated culture flasks in Gas Pak 100 jars (BBL) containing Gas Pak anaerobic generators. The jars were incubated with agitation at 14°C. Subcultures were made, using as an inoculum, cells that grew as a collar on the inner surface of the swirling culture flask. All transfers and manipulations were conducted under a stream of sterile N_2 gas. In this way, an adherent cell population which produced capsular exopolymers was cultured and maintained under anaerobic conditions.

Surface colonization of glass coverslips by cells of *E. cloacae* was performed according to procedures outlined by Salas and Geesey (1983). Attached cell densities were determined by epifluorescence microscopy.

Glucose uptake studies were conducted with cells attached to coverslips shortly after maximum adherent cell density was obtained. Individual coverslips containing the attached cells were dipped in fresh sterile growth medium lacking organic constituents to remove loosely attached cells and then introduced into a 20 ml volume of the same medium supplemented with ^3H- glucose at a final concentration which ranged from 125-2,500 pg/ml. After various periods of incubation with agitation , the coverslips were removed and rinsed in fresh medium without labeled glucose and transferred to scintillation vials for counting. Uncolonized coverslips treated in a similar manner were used to determine non-specific adsorption of radiolabeled glucose to the surface, and the resulting counts subtracted from those obtained from colonized coverslips to estimate cell-associate uptake.

Free cells were recovered from the culture flask at the time maximum adherent cell density was achieved and washed free of culture medium by centrifugation. The cells were resuspended in growth medium lacking organic constituents at a density which provided a total cell number in a 5 ml volume approximately equal to that attached to a single coverslip. Radioactive glucose was added at the concentrations described for the attached cells and, after various incubation times, the reaction was stopped by filtering the cells onto a 0.45 μm membrane. The membranes were dried and the associated radioactivity determined by liquid scintillation. Corrections were made for differences in counting efficiency caused by the different scintillation geometries of glass coverslip and membrane samples. All uptake studies were conducted at 14°C under anaerobic conditions using an N_2 atmosphere.

RESULTS AND DISCUSSION

Cells colonized glass coverslips submerged in broth culture medium within 35 h of inoculation at 14°C (Fig. 1). Maximum adherent cell density was established 45 h after inoculation and was maintained for at least an additional 4 h. During the 4 h period, coverslips were transferred to growth medium without organic constituents, rinsed, agitated in fresh medium for up to 30 mn, rinsed again, and examined by epifluorescence microscopy following staining with acridine orange. Table 1 reveals that very little change in the attached cell density occurred as a result of the manipulations. These results suggested that coverslip-associated cells could be used to measure uptake of radiolabeled nutrients by the adherent population without losing significant numbers of attached cells during the uptake reaction. Previous studies have shown that coverslips are colonized uniformly over their surfaces and that the number of cells that colonize multiple coverslips introduced into the culture do not differ significantly from one coverslip to another (Salas and Geesey, 1983). These features thus enabled us to use colonized coverslips as replicate samples of an attached cell population in a manner similar to the way uniform volumes of a homogeneous cell suspension are used to obtain replicate samples of a free cell population.

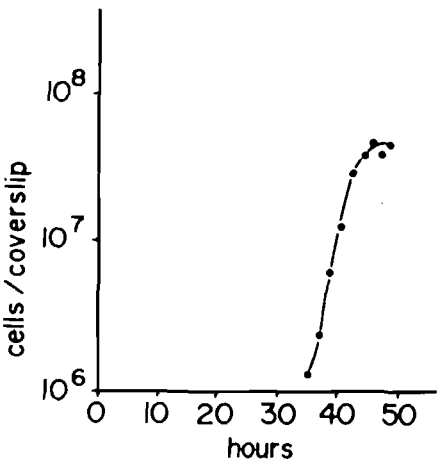

Figure 1 : Colonization of glass coverslips submerged in anaerobic cultures of *Enterobacter cloacae*.

Agitation time (mn)	Cells/field (±1 SD)
0.33	98.6 ±12.6
2.0	96.8 ±13.3
10.0	100 ±12.6
30.0	94.3 ±12.9

Table 1 : Effect of manipulation of coverslips on the retention of attached bacteria : Coverslips were removed from the culture 45 h after inoculation and subjected to a series of manipulation used for the determination of radiolabeled substrate uptake by the attached bacteria.

Preliminary experiments indicated that while some radiolabeled substrates displayed unacceptably high non-specific adsorption to the glass coverslips, others such as glucose exhibited low levels of binding. The latter substrate was therefore chosen to evaluate nutrient uptake kinetics by free and attached cell populations. As shown in Table 2, glucose uptake by the attached cells was greater than that of free cells at a glucose

Reaction time	uptake (10^{-6} fg glucose/ cell)	
	Free cells	Attached cells
10 s	24	173
30 s	121	281
2 mn	190	785
10 mn	697	2456
30 mn	1187	4771

Table 2 : Uptake of glucose by free and attached cells of *E. cloacae*.

^{3}H-glucose was added at a final concentration of 1.25 ng/ml. Total adherent cells per coverslip = 6.2×10^7. Total free cell population assayed was 6.0×10^7.

concentration of 1.25 ng/ml over a period ranging from 10 s to 30 mn. The 2 populations of cells also displayed different uptake kinetics over glucose concentrations ranging from 125-2,500 pg/ml (Fig. 2). These data indicate that attachment to surfaces results in a rapid alteration in the ability of cells to take up glucose and support the work of Bright and Fletcher (1983) which showed that assimilation of leucine by attached cells was greater than that of free-living cells.

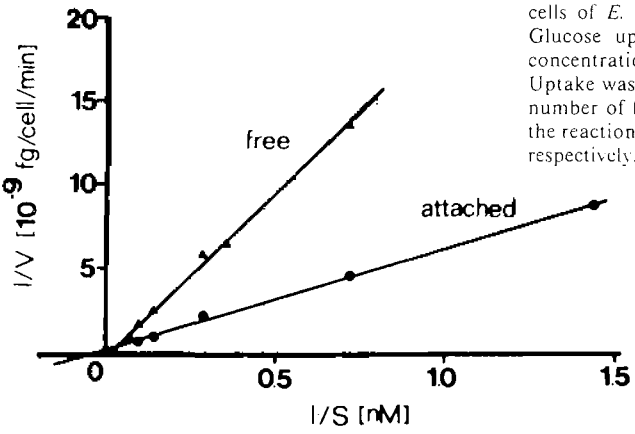

Figure 2 : Glucose uptake by free and attached cells of *E. cloacae* under anaerobic conditions. Glucose uptake was determined over glucose concentrations ranging from 125-2500 pg/ml. Uptake was determined over a 2 mn period. The number of free and attached bacteria present in the reaction mixture was 4.0×10^7 and 4.3×10^{-7}, respectively.

Addition of citrate and succinate to the glucose uptake reaction mixture resulted in a reduction in the maximum velosity of glucose uptake by the attached cell population but did not significantly alter the transport constant (Table 3). These results suggest that citrate and succinate do not share common transport components with glucose. They do appear to non-competatively inhibit glucose uptake, however.

Parameter	Glucose only	Citrate and succinate present (1)
K_t (nM)	25	25
V max (10^{-6} fg cell/mn)	5000	1000

Table 3. Effect of citrate and succinate on glucose uptake by attached bacteria

1. Citrate and succinate present at 0.5 and 0.2 g/l, respectively. Glucose uptake determined over concentration range of 125-2500 pg/ml.

Multiple coverslips submerged in swirling liquid cultures were uniformly colonized by encapsulated cells of a sediment isolate of *E. cloacae*. The colonized coverslips provided a means of determining glucose uptake characteristics of the adherent cells shortly after their attachment. The technique should also be useful in evaluating other physiological responses elicited by bacteria when they switch from a free-living to a sessile existence.

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