GROWTH OF BACTERIA AT SURFACES: INFLUENCE OF NUTRIENT LIMITATION

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1. Introduction

Bacterial growth in natural environments is often associated with attachment to surfaces. This may involve adherence to surfaces of material degraded by the bacteria (e.g., detritus particles) to provide nutrient, or to inert surfaces (e.g., stones, rock and glass). The attachment may often be strong and while some bacteria have developed specialized structures for this purpose (e.g., holdfasts in Caulobacter spp.), for most organisms it has been suggested that the production of a 'sticky' extracellular polysaccharide is responsible [1–5]. The reasons for this preferential growth at surfaces of inert material are not clear, but it is apparent that bacterial growth is slow and is likely to be nutrient-limited in natural aquatic environments. Since the limiting nutrient could be concentrated at a surface/water interface, bacteria attached to the surface could have a distinct ecological advantage.

To date most studies have involved attachment to clay particles, glass, plastics and to the walls of fermentation vessels (see review of Hattori and Hattori [3]) with relatively little attention being paid to the 'physiological state' of the organisms involved. We have therefore carried out experiments on the attachment of bacteria from a natural aquatic environment (river water) when they have been grown in the defined conditions of a chemostat. In addition, we have used alternatively carbon- and nitrogen-limited systems for comparative purposes. These were essentially enrichment experiments, the outcome of which depended on the behaviour of mixed cultures when grown in a chemostat. In this type of culture, assuming no interaction occurs between organisms, then a steady state is reached in which one organism predominates, the remainder being washed out [6–8]. Previously we suggested that while this argument held for organisms in free suspension, surfaces in the chemostat could provide a site in which mixed populations of bacteria could survive the selective pressures of chemostat growth [9].

The present experiments, therefore, had two objectives; firstly to establish whether this mixed culture hypothesis held in practice, and secondly to determine whether surface adhesion was a function of the nutritional state of the organisms present.

2. Materials and methods

A glucose/mineral salts medium buffered with phosphate at pH 7.0 and able to support a bacterial population of approximately $10^9$ organisms/ml was used throughout. This medium had the following composition — per litre water — $0.775 \text{ g NaH}_2\text{PO}_4$, $5.5 \text{ g Na}_2\text{HPO}_4$, $1.75 \text{ g K}_2\text{SO}_4$, $0.1 \text{ g MgSO}_4 \cdot 7 \text{ H}_2\text{O}$, $0.75 \text{ g Na}_2\text{ EDTA}$, with trace concentrations of Ca, Mn, Co, Fe, and Zn. For carbon-limited medium a glucose concentration of $0.5 \text{ g/l}$ was employed together with $3.82 \text{ g/l NH}_4\text{Cl}$, while nitrogen-limited medium contained $2 \text{ g glucose/l}$ with $1.91 \text{ g/l NH}_4\text{Cl}$.

The source of bacteria was water from the River Bourne at Gomeldon, Wiltshire. Chemostats of 500 ml capacity were filled to capacity with river water and medium added continuously at a dilution rate of $0.05 \text{ h}^{-1}$. The temperature was maintained at $20^\circ\text{C}$.
which approximated to the river temperature at the
time of the experiments.

3. Results and discussion

In accord with the theory of mixed cultures in a
chomstat, one organism (a Vibrio sp.) predominated
in the vessels and accounted for 70–90% of the popu-
lation after 3 days. At this stage aluminium foil strips
included in the vessels for surface attachment of bac-
teria were removed, washed in saline and dried in air.
Circles of approximately 2 mm diameter were
removed and viewed in a scanning electron micro-
scope after shadowing with Au/Pd. The aluminium
surface taken from the chemostat supplied with nitro-
gen-limited medium was coated with some unknown
material, presumably of bacterial origin since the
culture contained an abundance of extracellular
polymer. Preliminary experiments suggest that the
material on the aluminium foil was glucose-containing
polysaccharide. There were very few bacteria present,
and these were of similar shape and size (fig. 1). In
contrast the surface from the chemostat supplied with
glucose-limited medium had attached to it a wide
variety of bacterial types (fig. 2) including rods,
cocci and spirals but no surface polymer was appa-
rent. Some of these bacteria have been isolated and
found to be Aeromonas sp., Flavobacterium sp. and
Pseudomonas sp., although many have resisted isola-
tion using conventional methods. These results corre-
lated well with parallel experiments using glass micro-
scope slides suspended in the culture vessels. The
experiment was repeated twice further samples of
river water with essentially similar results.

These results are somewhat surprising in that the
accepted view is that extracellular polysaccharides
act as adhesives, and that these materials are pro-
duced maximally under conditions of carbon excess
such as in the nitrogen-limited chemostat system. It
was therefore expected that the greatest attachment
of bacteria would occur in the chemostat supplied
with the nitrogen-limited medium. This clearly was
not the case, even though extracellular polysaccharide

![Fig. 1. Scanning electron micrograph of aluminium foil after 3 days in the nitrogen-limited culture, showing a few bacteria em-
bedded in extracellular material.](https://academic.oup.com/femsle/article-abstract/1/3/163/494406)
was apparently produced by the culture. Indeed when compared with the carbon-limited system it may be that this polymer actually prevented the attachment of many bacterial types. In the carbon-limited system, without polymer formation, a film of bacteria built up with time. This supports our earlier suggestion that attachment of bacteria to surfaces during an enrichment would allow a more heterogeneous population of organisms to develop there than in the stirred culture in the vessel, although this is apparently dependent upon the nature of the overall substrate limiting growth.

These results are similar to those reported by Marshall et al. [5] who showed that addition of glucose to a culture of bacteria growing in artificial sea water inhibited attachment to glass. His interpretation of this result was to suggest that glucose receptors on the bacterial envelope were saturated by the excess of glucose and were not available to glucose molecules held by the glass surface. While there is no direct evidence to support this view, it is significant that attachment to surface is often associated with growth at very low nutrient levels (see, for example Zobell and Anderson [2]). The same reasoning provides a possible explanation for the results described above. In the nitrogen-limited experiment (containing an excess of glucose) any receptor or adsorption sites for glucose would be saturated and not available for initial attachment, even though extracellular polysaccharide was produced. Conversely the bacteria growing in the glucose-limited medium would possess a maximum number of glucose receptor sites which would not be saturated. Glucose could bind to a aluminium as a molecular film and act as a bridge for the attachment of the bacteria by the binding of the receptor sites to the glucose molecules. This could effect the initial absorption of bacteria which could then grow and give rise to a bacterial film composed of several types of organism, even though in the culture suspension they could not compete with the predominating organisms. If growth in natural aquatic systems is carbon-limited for the growth of heterotrophic bacteria, these results suggest that in such conditions growth would occur preferentially at the surface/water interface. Whether or not other carbon substrates produce similar results to those obtained.
with glucose is not known although similar results have been obtained in an enrichment using glycerol.

These results may be useful in interpreting the behaviour of bacterial populations in natural environments.

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References