ADHESION OF DIFFERENT MARINE BACTERIA, OUTERMEMBRANE PROTEINS AND LIPOPOLYSACCHARIDE PRODUCTION

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Abstract

Marine bacteria were identified by fatty acid analysis and their ability to attach to surfaces was tested. We found that bacteria belonging to *Cytophaga-Flavobacteria* (*CF*) displayed stronger adhesion properties than the *γ-Proteobacteria* isolates, thus suggesting that *CF* might be favoured in aggregate colonisation. These differences are also reflected in different outer membrane proteins and lipopolysaccharide composition.

Key words: marine bacteria, adhesion, mucilaginous aggregates, northern Adriatic

Introduction

Microbial exopolysaccharides (EPS) are carbohydrate-enriched polymers produced by microalge and bacteria that bind aggregates and form dense biofilms near the sediment-water interface. EPS synthesized by attached bacteria strengthen their binding to surfaces, but outer membrane proteins (OMP) are required for the early stages of adhesion (1).

Our aim was to determine the differences in EPS at the cell-surface of diverse strains commonly found in sediment and mucilaginous aggregates, and in attachment of these strains to solid surfaces.

Materials and Methods

Six Gram-negative bacterial strains isolated from northern Adriatic seawater were grown in Marine Broth, at 25°C with vigorous shaking. Each isolate was saponified, methylated and their fatty-acid (FA) patterns were analysed by GC/MSD. OMP and LPS of the strains were extracted and analysed by one-dimensional sodium dodecyl sulfate-polyacrylamide (SDS-PAGE) and polyacrylamide (PAGE) gel electrophoresis, respectively (2). For adhesion assays, cultures were washed and re-suspended in phosphate-buffered saline (PBS). The suspensions were loaded onto columns filled with pure sea sand (Fluka), fractions were collected and cell adhesion to sand was measured as the ratio of the optical density (OD_{280}) in each column fraction to the OD_{280} of the initial bacterial suspension (2).

Results and discussion

Bacterial isolates had FA patterns corresponding to *Cytophaga-Flavobacteria* (7, 25, 30, 31) and *Proteobacteria* (6, 38). PAGE analysis of EPS demonstrated a marked difference between marine bacteria 7/25, 38 and other three strains (Fig. 1). LPS analysis of strain 7 and 25 gave the same band, but slightly more expressed in strain 7. Strains 30 and 38 had a strong LPS and OMP analysis highlighted the differences in the extracellular composition of different bacteria: strains 7 and 25 produced acidic EPS that ran as a smear in PAGE (Figure 1A). In contrast, strains 30 and 38 produced high MW LPS, detectable as a dense band at the top of the gel. In addition, strain 38 produced lower MW EPS. Analysis of OMP highlighted the production of different dominant bands, expressed in strains 7, 25 (around 30 kDa) and 38 (around 40 kDa). In addition, higher MW bands were present in strains 7 and 25. Differences in the extracellular structures were reflected in adhesion properties: adhesion tests showed efficient attachment by strains 7, 25, 30 and 31 belonging to the *CF* group while 6 and 38 (*Proteobacteria*) displayed poor adherence (Fig. 2).

Favourable adhesive capabilities of *Cytophaga-Flavobacteria* indicate that these bacteria are well adapted for colonising solid surfaces. Since they mainly utilise decomposing refractory macromolecules, they can meet their nutritional requirements in the mucous matrix of aggregates or on epipelic biofilms (3), without having to compete with *Proteobacteria*, which mainly utilise lower MW substances. In mucilaginous aggregates found during periods of unusually massive aggregation in northern Adriatic, increasing proportions of branched fatty acids on aged mucilaginous aggregates (4) indicate more efficient aggregate colonization by *Cytophaga-Flavobacteria* over other bacterial populations. Differences between *CF* strains in OMP and LPS expression could allow us to define different mechanisms of adhesion control and should be further investigated.

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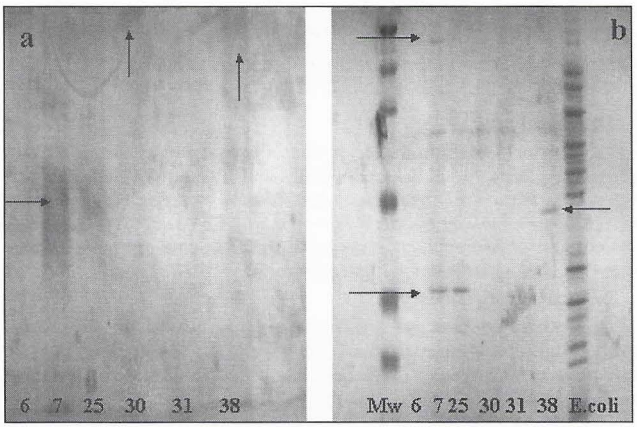

Fig. 1. a) PAGE analysis of bacterial LPS, and b) SDS-PAGE analysis of bacterial OMP.

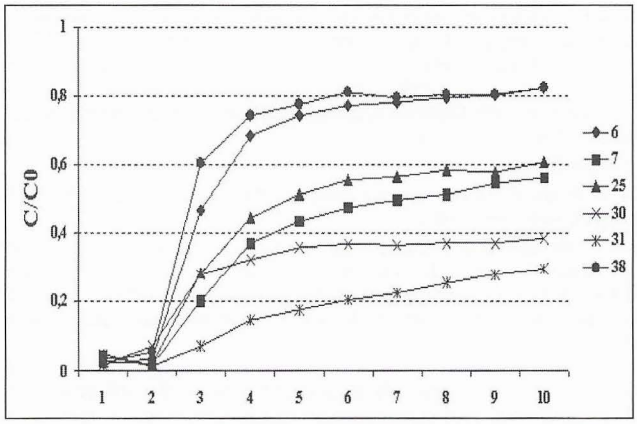

Fig. 2. Adhesion to a sand column. The C/C_0 value was calculated as the ratio of bacteria recovered from the flow-through column to bacteria loaded onto the column for each fraction.

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