PROTEOMIC APPROACHES APPLIED TO ADHESION FACTORS IN MARINE BIOFILM-FORMING BACTERIA

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Introduction

Biofouling is ubiquitous in marine environments, and bacteria are among the first organisms to colonize solid surfaces. They form biofilms which serve as a foundation for attachment and growth of other organisms, such as invertebrates, sessile plants, and animals (Huis in’t Veld, 1998). Marine biofilm communities are complex, highly dynamic ecosystems (Fig. 1) and once established are extremely difficult to eradicate (Holmstrom et al., 2002). For this reason the understanding of the mechanisms leading to marine bacterial attachment and subsequent biofilm development are of great biological importance with obvious potential industrial usages. This development is conditioned by complex processes involving bacterial attachment to surfaces, growth, cell-to-cell communication, mobility and production of exopolymers constituting the biofilm matrix. Concerning attachment, the molecular strategies used by bacteria are diverse. They can employ pili, fimbriae and a plethora of proteins engaged under the term “adhesins” that recognize different elements of the target (living or inert) surfaces (Figueras-Gordo and Cassart, 2004; Frances et al., 2000). Although there is consistent data on human pathogenic bacteria attachment mechanisms, scarce information is available for marine bacteria. For this reason this project focused in the marine biofilm-forming bacterium Pseudoalteromonas sp. D41 (P. D41). This organism displays strong and competitive adhesiveness onto a wide variety of substrates, promoting subsequent biofilm development (Fig. 2). Previous phenotypical studies in this strain revealed the high outer-membrane protein content in its adhesion properties (Traud et al., 2006; Leray et al., 2008). For this reason, we attempted to unravel the molecular mechanisms responsible for these adhesive and competitive properties through a proteomic strategy, with particular attention to the outer membrane (OM) fraction.

Results

In order to screen for proteins regulated during biofilm formation in P. D41, we carried out 2D-PAGE proteomic profiling of the total soluble and the OM protein fractions (Fig. 3). The differential expression of 10 biofilm-related proteins was detected and they were subsequently identified by MS-MS of nude sequencing. These proteins were identified as involved in primary cellular metabolic functions, membrane transport, stress resistance and cell adhesion. Particular interest, we detected four strongly induced OM proteins preventing high biofilm to a TonB-dependent receptor, TAC125 (PAO1, which is a widely used bacterial model for biofilm studies. The inactivated mutants with the corresponding P. D41 homologues genes. P. aeruginosa PAO1, which is a widely used bacterial model for biofilm studies. The unaltered PorR and OprC were not found in any of the biofilm studies (Fig. 4).

Conclusion and perspectives

Bacterial adhesion and biofilm development implies profound physiological changes and particularly with relation to cell surface structure. Our study report for global changes in physiology during biofilm formation of a marine bacterium, Pseudoalteromonas sp. D41. Interestingly, P. D41 upregulates a type IV bacterial (PAO1) under biofilm conditions. In E. coli, OmpA is a key adhesion for colonization of non-mucin-producing cells, through the direct interaction between OmpA and a glycoprotein (Prasadarao et al., 1996). Besides, OmpA exerts its effects on surface populations. A B C D

Fig 2. Characterization of Pseudoalteromonas sp. D41 adhesion and biofilm formation features. (A) Electron microscopy images of P. D41 entrapped in its own extracellular matrix after 3 hours of attachment on stainless steel surfaces. (B) Biofilm formation by P. D41 monitored by RT-qPCR. Expression profiles of ompA, pilF, TonB-receptor and ompF genes were followed on nickel (Nickel) and glass (Glass) biofilm cultures for 1, 3 and 5 days. The pilF, TonB and ompF genes were used as internal controls. The relative variations in gene expression were calculated as fold changes in the biofilm when compared control conditions and presented as 2ⁿ-fold variation. Results are means ± SE from three independent biological samples.

References

RR RR uu uu ll llaa aa nn nndd dd,,,, KK KK uu uu ll llaa aa nn nndd dd,,,, WW WW uu uu ll llaa aa nn nndd dd,,,, (1995) Marine Chemistry 55 55 00 00, 117-138

Fig 6. Biofilm formation by Pseudoalteromonas sp. D41 and the outer membrane mutants p pilF and p pilF. Biofilm were grown at 24°C for 5 days in LB broth. Biofilms were stained with 5 µM of SYTO 61 red and observed by confocal laser scanning microscopy. (A) Z-stack observations are shown for P. D41 and p pilF. Mean values (dotted lines) and mean± standard deviations were calculated from three biological samples.