PSEUDOMONAS FLUORESCENS - PRODUCTION OF BIOSURFACTANTS AND IMPACT ON THEIR BIOADHESIVE BEHAVIOUR

MEYLHEUC T.[(1)], **NGOYA S.**[(2)], **DUCLAIROIR C.**[(2)], **FEUILLOLEY M.G.J.**[(2)], **BELLON-FONTAINE M.-N.**[(1)], **ORANGE N.**[(2)]

[(1)] INRA - Unité de recherche en Bioadhésion et Hygiène des Matériaux, 25, avenue de la République F-91744 Massy Cedex, France

[(2)] Laboratoire de Microbiologie Du Froid, UPRES 2123, Université de Rouen, 55 rue saint-Germain 27000 Évreux, France

AIM OF THE STUDY

Pseudomonas fluorescens is a spoilage microorganism frequently isolated in the food industry that could be considered as a potential pathogen on account of its ability to adhere to nervous cells. The ability of microorganisms to adhere to different substrates is determinant in the process of contamination of patients or food products. Microbial adhesion, the first stage in the formation of biofilms, depends principally on the physicochemical surface characteristics of microorganisms and substrata. In addition, several strains of *P. fluorescens* have been shown to produce surface active compounds called biosurfactants, capable of lowering the surface tension and the interfacial tension of aqueous or hydrocarbon solutions. By modifying the surface properties of the substrate, the adsorption of these biosurfactants to such inert materials may have consequences that are of particular importance to the expression of bioadhesive phenomena. Thus, recent studies applied in a medical setting showed that adhesion of uropathogenic microorganisms could be inhibited by a biosurfactant produced by a *Lactobacillus* (Velraeds *et al.*, 1996; Busscher *et al.*, 1997).

On this basis, the aim of this study was to evaluate the impact of *P. fluorescens* biosurfactants on the bioadhesive behaviour of two strains of *P. fluorescens* on stainless steel, glass, and polystyrene surfaces conditioned or not by these surface-active compounds.

MATERIALS AND METHODS

•Bacterial strains and growth conditions

Pseudomonas fluorescens 3608 and *Pseudomonas fluorescens* MF0 isolated from nosocomial infection and crude milk, respectively were used in this study. They were cultured on Citrate Mineral Medium (CMM) at 8°C, 12°C and 17°C with orbital agitation (180 rpm). for 30 to 54 h at the relevant temperature.

- Preparation of suspensions tests

Cells were harvested in early stationary growth phase and cleaned twice by successive centrifugation (Sigma, France) for 10 min. at 7000*g* in physiological saline. Then inoculum was diluted in physiological saline to obtain an optical density of 0.8 at 400 nm, corresponding to a cell concentration of 1 to 3 x 10^8 CFU . mL^{-1}.

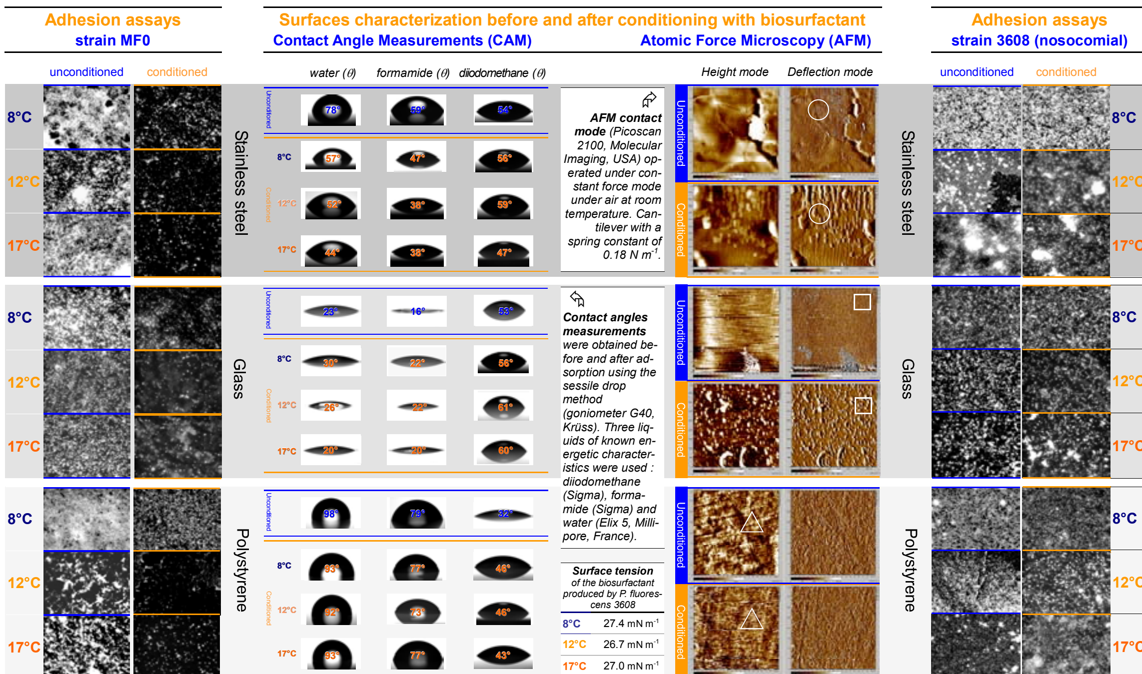
•Biosurfactant: production and ionic characterization

Strains were cultured 4 days on CMM agar for each temperature. After incubation, cells were suspended in mineral water (Volvic, France) and shaken strongly. Supernatant containing the biosurfactant was separated from the cells by two centrifugations and filtered through a 0.22-µm pore-size filter (Millipore Inc., Bedford, MA) before storage at 4°C. The activity of biosurfactants was determined by surface tension measurements (Wilhelmy's blade method) using an automatic tensiometer (K12, Krüss, Germany). The ionic characteristic of the biosurfactant was determined by the gel double diffusion technique (van Oss and Heck, 1963) by using different surface-active agents of known charges (SDS, $BaCl_2$).

• Adhesion assay

Inert surfaces (stainless steel 304, polystyrene, glass) were cleaned before each test of biosurfactant adsorption or cell adherence by soaking in a solution of detergent RBS 35 (2% v/v) followed by rinsing with warm water then with deionized water. Adsorption of biosurfactant on solid surfaces was achieved by immersion of coupons in the solution of biosurfactant during 24h followed by rinsing in sterile water. Each surface was characterize before and after conditioning using CAM and AFM analysis. Surfaces characterization before and after adsorption was deduced from contact angles measurements using an automatic goniometer (G40, Krüss, Germany). Adhesion assays on supports (conditioned or not by the relevant biosurfactant solution) were performed by sedimentation of a suspension of *Pseudomonas fluorescens* (10^8 CFU ml^{-1}) during 24 hours at 20°C. Supports were then rinsed by pouring 400 ml of distilled water to remove non sticking bacteria. Washed surfaces were stained with acridine orange 0.01% for 10 min. The stained surfaces were examined under an epifluorescence microscope which was connected to a CDD camera and a PC computer for image acquisition and storage.

RESULTS



CONCLUSION

The nosocomial strain, *P. fluorescens* 3608, isolated from a patient, was found to be a good biosurfactant-producing strain (surface tensions values from 26.4 to 27.0 mN m^{-1}) in a large range of temperature using citrate as C-source. Whatever the surface tested, we observed that pre-treatment of the substratum with the biosurfactant (adsorption) produced by *P. fluorescens* 3608 caused a considerable reduction in the number of adherent cells of *P. fluorescens* MF0. This effect may be related to a change in the physicochemical characteristics of the substrata as showed by contact angles and AFM analysis. Considering adhesion of the two strains on unconditioned surfaces, we demonstrated a substratum-dependent behaviour with higher contamination levels on stainless steel (fairly hydrophilic) compared with those obtained on glass (hydrophilic) or polystyrene (hydrophobic) surfaces. Concerning the adhesion of by *P. fluorescens* 3608, it was interesting to note that surface conditionig by its biosurfactant had none significant effect whatever the growth temperature and substratum tested. These results suggested that the production of biosurfactant by the nosocomial strain *P. fluorescens* 3608 did not modified its own attachment on inert surfaces, whereas it reduced the implantation of another strain of *P. fluorescens*. These first results suggest that the synthesis of biosurfactant by *P. fluorescens* may be implicated in nosocomial infections.

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