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Biofilm Morphology and Effects of Bacterial Cell-to-Cell Communication on Biofilm Formation by *Escherichia coli* K-12

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The study describes the morphology of biofilms formed by two *E. coli* K-12 strains as shown by reflected light microscopy and the dynamics of the bacteria-substrate contact during cultivation in liquid medium as registered by plasmon microscopy. The effects of quorum sensing on *E. coli* K-12 biofilm formation is also examined. Quorum sensing influences biofilm growth but has no apparent effects on initial attachment of bacteria.

Key words: microbial biofilm, autoinducers, quorum sensing, plasmon microscopy.

Introduction

In most natural environments bacteria do not exist as solitary cells but are colonial organisms. They associate with an inert or living surface and form structures known as biofilms. Microbial biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix [3, 4]. There are four stages of biofilm formation: reversible adhesion, irreversible adhesion, biofilm maturation and detachment of biofilm cells [12]. Biofilm bacteria communicate with each other using chemical signal molecules, or autoinducers, excreted from the cells. This process, termed quorum sensing, allows bacteria to count the members in the community and to synchronously alter gene expression of the population [12]. Previous literature data show that many *E. coli* K-12 biofilms are poorly developed if they lack a conjugative plasmid [7, 10]. The aim of the present study is to compare the structure of two strains *E. coli* K-12 – *E. coli* W1655F+ and *E. coli* W3110F-, examine the dynamics of the bacteria-substrate contact, and characterize the effects of quorum sensing on biofilm formation.

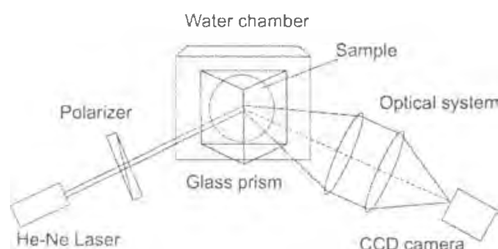

Fig. 1. Scheme of the plasmon microscope with the water chamber

Materials and Methods

For examination of morphology, biofilms were cultivated for 24h on glass slides covered with methyl metacrilate. The observations of native hydrated biofilms were done on a light microscope with reflected light. To examine cell contact with the substratum, we used an optical plasmon sensor [2], supplied with a specially constructed cell (Fig. 1.) for real-time observation in a liquid medium. The sample slide was covered with bacterial suspension, incubated for 20 min. and washed in sterile saline prior to closing of the cell filled with M63 medium [9]. The laser beam was fixed in definite position, and serial photographs were taken at 5 min intervals.

The strains of *E. coli* K-12 used in this study were *E. coli* W1655 F+ and *E. coli* W3110 F-. The biofilm formation assay was done in 96-well microtitre plates, following two protocols. In the first experimental series 10 ml of the overnight bacterial cultures in TSB were suspended in 100 ml of M63 medium with addition of sterile supernatants of *E. coli* and *Yersinia enterocolitica,* used as sources of quorum sensing. M63 medium was supplemented with the supernatants in proportions 5:1, 5.5:0.5 and 5.8:0.2. The plates were incubated for 24h at 20°C. The planktonic cells were washed. The adherent bacteria were coloured with 0.1 % water solution of crystal violet, solubilized in 75 % ethanol and the absorbance at 620 nm was determinated by ELISA reader.

In another experiment 100 ml of the overnight bacterial cultures were placed in the wells. Quorum sensing was added to reach the concentrations described above. Cells were left to adhere for 4h at 20°C. The non-adherent cells were removed, wells were gently washed, and 120 ml of pure M63 medium were placed in each well. The plates were incubated for 24 h at 20°C.

Results and Discussion

Morphology of E. coli K-12 in relation with the F plasmid. We first compared biofilm morphology of two *E. coli* K-12 strains: one F+ and another – F-. Within the 24h interval tested, the F+ strain formed thick, well-structured biofilm with well-expressed cellular masses interposed by channel-like structures (Fig. 2A). The F-strain was loosely adherent, forming thin cellular filaments organised in fractal-like pattern (Fig. 2B). This supports previous observations on the role of the F plasmid in biofilm formation [7, 10]. Reflected light microscopy to this moment had only limited application in biofilm examination [8]. One very important advantage of this is simplicity of use, and the possibility to examine morphology of the biofilm in its native hydrated state.

Plasmon microscopy. The setup we used for surface plasmon microscopy is shown in Fig.1. The p-polarized beam from a He - Ne laser falls onto the sample through a glass prism (index of refraction n=1.78). The sample consisted of 45-nm Au layer evaporated

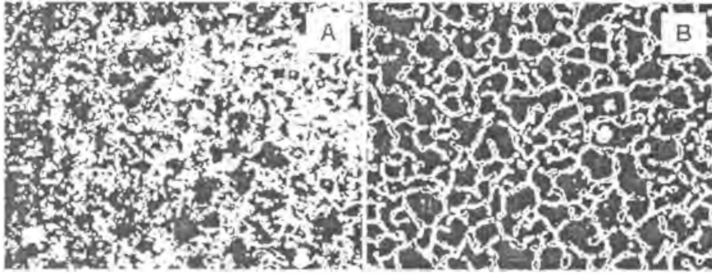

Fig. 2. Reflected light microscopy images of 4h biofilms of (A) *E. coli* K-12 W1655F+ and (B) *E. coli* W3110F-. Scale bar = 20 μm

on a cover glass (n=1.78) and covered with 780-nm PMMA. A 2-nm Cr layer was deposited before the Au to increase the adhesion of the gold layer. The sample was in contact with a water chamber. The optical contact between the glass prism and the sample was realized using a special immersion liquid. The sample surface was imaged onto the CCD camera by an imaging system. The plasmon sensor used is constructed so that it can visualize objects as small as 1μm [2] which exceeds previous applications of plasmon microscopy that allowed observation of large cells, e.g. neurons [6].

Strain *E. coli* W1655F+ which forms pronounced biofilms was used. Only after a short contact with the polymer substratum, numerous bacteria were attached to the slide. The illustrations (Fig. 3 A-C) show changes in cell density and distribution on a fixed point of the sample, and describe a dynamic cell-substrate interaction.

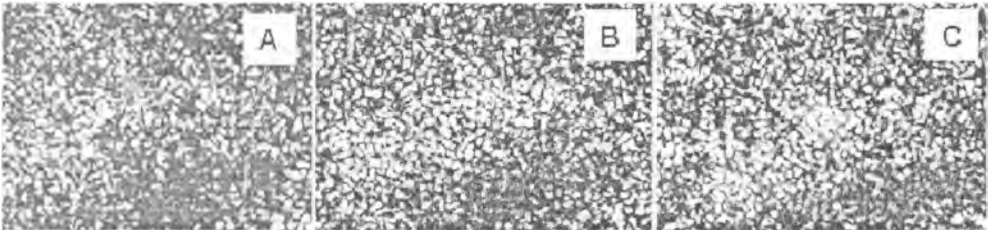

Fig. 3. Changes in the number and the position E. coli K-12 W1655F+ cells that are in a close contact with the substrate. Pictures taken with optical plasmon sensor in a fixed position of the laser beam at 30 min intervals. Scale bar = 1 μm

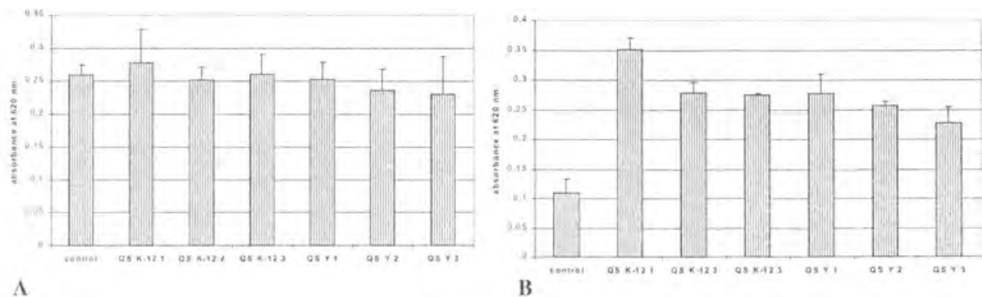

Fig. 4. Effect of QS K-12 and QS Y on the adhesion and the growth of 24h *E. coli* K-12 W1655F+ biofilm formation. Addition of 48h sterile supernatants

Effect of quorum sensing on E. coli K-12 biofilm formation. Further examinations of *E. coli* W1655F+ were directed to the effect of quorum sensing. As a source of signals, 48h sterile supernatants of *E. coli* K-12 (QS K-12) containing autoinducer 2 and *Yersinia enterocolitica* (QS Y) containing also autoinducer 1 were used. [1, 11]. First we examined the effect of QS K-12 and QS Y during adhesion stage of biofilm development (Fig. 4A). The obtained values of absorbance showed no statistically significancant differences between control and test groups and between test groups themselves. In another series of experiments, quorum sensing was present throughout 24 h biofilm growth (Fig. 4B). It was found that both QS K-12 and QS Y stimulate biofilm formation in a dose-dependent manner. QS K-12 has stronger effect than QS Y ($p < 0.001$).

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