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Enhancement of Luminescence in *Vibrio harveyi* by Culture Extracts of Biofilm-Forming Sulfate Reducing Bacteria

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Microbiologically-influenced corrosion (MIC) of metals is common in the natural environment, and sulfate reducing bacteria (SRB) are known to be representative microorganism responsible for MIC [1]. In the course of MIC, biofilm formation of SRB on the surface of metals is an essential step. We observed biofilms of *Desulfovibrio desulfuricans* and *D. vulgaris* on the copper coupons using the atomic force microscope (AFM) and scanning electron microscope (SEM) (Fig. 1). The dense biofilm induced pitting corrosion of the metal, which was seen with the microscopy and an energy dispersive X-ray analyzer (EDX).

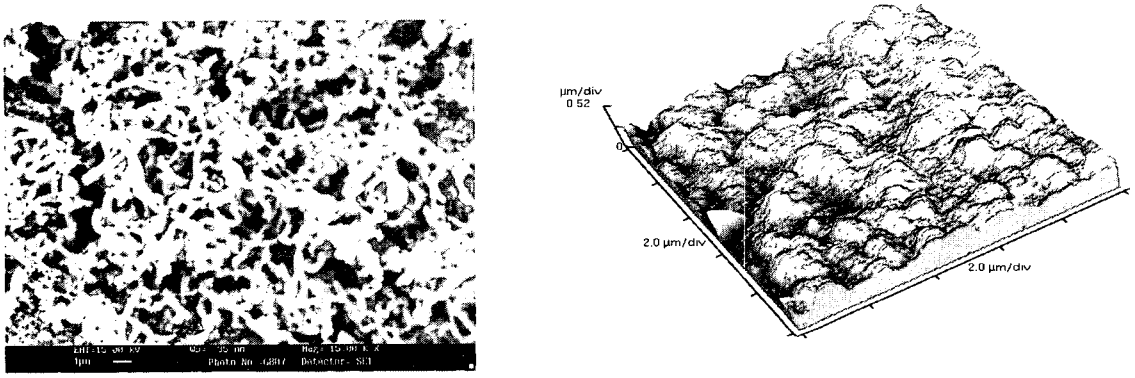

Fig. 1. Biofilm formation on the surface of a polished copper coupon. SEM (left) and AFM (right) images show tangled biomass of *D. vulgaris* grown for 6 days.

We also found that biofilm formation by the SRB on the metal surface might be controlled by a cell-density dependent manner, quorum sensing. As cell-free culture fluids (spent media) of *D. desulfuricans* and *D. vulgaris* were analyzed using quorum sensing test strains, it was found that spent media enhanced luminescence of *Vibrio harveyi* BB886 (sensor 1^+, sensor 2^-) and BB170 (sensor 1^-, sensor 2^+) [2]. TLC-bioassay confirmed that quorum sensing signal molecules, AI-1 and AI-2, were contained in the spent media of two SRB (Fig. 2).

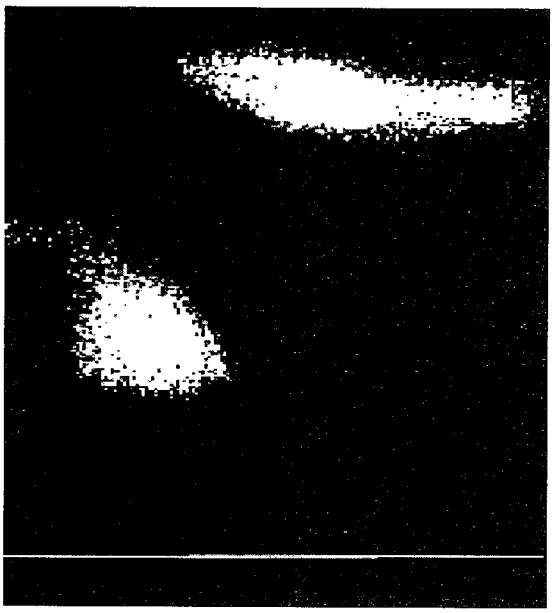

Fig. 2. TLC-bioassay for chloroform extracts of spent media of *D. vulgaris* and *D. desulfuricans*. Culture extracts were developed on a silica plate and overlaid with soft agar containing *V. harveyi* D1 (a dark mutant) [3], followed by 10-12 hrs of incubation at 30°C. Luminescence was monitored with an image analyzer. A luminescence inducer contained in the chloroform extract was regarded as AI-1 like substance of *V. harveyi*. Water soluble AI-2 like substance was also seen on the reverse-phase TLC-bioassay (data not shown).

Production of autoinducers from *D. desulfuricans* and *D. vulgaris* appeared to be growth-associated, i.e., it was low in the lag phase, highly increased in the exponential phase, and reached a maximal plateau in the stationary phase. Interestingly, however, induction of luminescence in *V. harveyi* BB886 and BB170 by a unit cell mass of the SRB showed a maximal peak in the late lag phase. From this result, it was suspected that quorum sensing-related properties of these two SRB might play unknown roles in shifting cells from dormant to growth stages.

References

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