Micro-patterned surface for *Phaeobacter inhibens* biofilm growth in a flow-cell system for biosynthetic production of the antibacterial compound TDA

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Aquaculture, as the fastest-growing food production over the last five decades, is providing a sustainable source of intaking protein for humans.[1] However, the fish industry is facing a challenge of infectious disease control. Growing probiotic bacteria in the aquaculture, to biosynthesize antibacterial compounds, has become a promising way to control the infections. *Phaeobacter inhibens*, the most commonly used probiotic bacteria, produce the non-chemically-synthesizable compound tropodithietic acid (TDA).[2]

To enhance the production of TDA, a microfluidic chamber[3] is used for bacterial culture, and the effect of the microstructure on culturing is studied. In our research, surface morphology and surface energy of the surfaces are investigated. We proposed a polymeric microfluidic chamber, incorporated with three different surfaces of planar, pits, and pillars arrays. Polymeric surfaces were replicated from a Ni master mold which in turn was originated from a Si wafer by steps of lithography, dry etching, and electroplating.[4]

The polymer surfaces with pillar arrays showed lower amounts of bacterial biofilm biomass, suggesting that there is likely easier detachment of microbial biofilms. However, the higher biomass on the pit surfaces did not result in higher production of TDA, whereas more TDA was measured from the pillar surfaces, indicating its effect on facilitating the antagonistic effect.

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[1] FAO. 2021 COFI declaration for sustainable fisheries and aquaculture. (2021)
[2] Grotkjær. T et al., *Syst. Appl. Microbiol.* 39(3), 180-188. (2016)
[3] Sternberg. C et al., *Curr Protoc Microbiol.* 1, 1B-2. (2006)
[4] Droumpali. A et al., *Micromachines.* 12(8), 926. (2021)

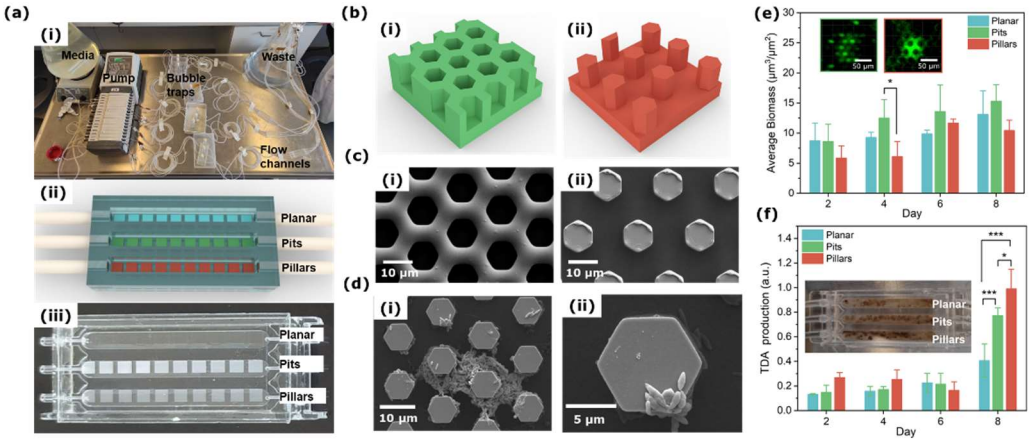

Figure 1: The micro-patterned flow-cell *Phaeobacter inhibens* culturing system. (a) The flow-cell culture system. (i) The overview of the flow chamber system. (ii) The schematic illustration of the culture chamber. (iii) The image of the fabricated flow chamber. (b) The micropatterns on the polymer surfaces of (i) pit and (ii) pillar arrays surface for probiotic bacteria growing. (c) Scanning electron microscope (SEM) images of fabricated polymeric (i) pit and (ii) pillar micro-patterned surfaces. (d) cryoSEM images indicating the location and colony like behavior of the bacteria. (e) The biomass of bacteria growing on different surfaces was calculated, to investigate the adhesion of bacteria. The fluorescent images show the dyed bacteria growing on pit and pillar arrays. (f) Production of the antibacterial compound TDA was compared, to determine the optimal micro-patterns for improving the antibacterial effect. The brown pigment is related to the TDA production.