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Spatial Organization of Cells over the Process of Pellicle Formation by Pseudomonas alkylphenolica KL28

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Abstract : Numerous aerobic bacteria have the ability to form multicellular communities on the surface layer of the air-liquid (A-L) interface as a biofilm called a pellicle. Pellicles occupied at the A-L interface will benefit from the utilization of oxygen from air and nutrient from liquid. Buoyancy of cells can be obtained by high surface tension at the A-L interface. Thus, formation of pellicles is an adaptive advantage in utilization of excess nutrients in the standing culture where oxygen depletion is easily set up due to rapid cell growth. In natural environments, pellicles are commonly observed on the surface of lake or pond contaminated with pollutants. Previously, we have shown that when cultured in standing LB media an alkylphenol-degrading bacteria Pseudomonas alkylphenolia KL28 forms pellicles in a diameter of 0.3-0.5 mm with a thickness of ca 40 μm. The pellicles have unique features for possessing flatness and unusual rigidity. In this study, the biogenesis of the circular pellicles has been investigated by observing the cell organization at early stages of pellicle formation and cell arrangements in pellicle, providing a clue for highly organized cellular arrangement to be adapted to the air-liquid niche. Here, we first monitored developmental patterns of pellicle from monolayer to multicellular organization. Pellicles were shaped by controlled growth of constituent cells which accumulate extracellular polymeric substance. The initial two-dimensional growth was transited to multilayers by a constraint force of accumulated self-produced extracellular polymeric substance. Experiments showed that pellicles are formed by clonal growth and even with knock-out of genes for flagella and pilus formation. In contrast, the mutants in the epm gene cluster for alginate-like polymer biosynthesis were incompetent in cell alignment for initial two-dimensional growth of pellicles. Electron microscopic and confocal laser scanning microscopic studies showed that the fully matured structures are highly packed by matrix-encased cells which have special arrangements. The cells on the surface of the pellicle lie relatively flat and inside longitudinally cross packed. HPLC analysis of the extrapolysaccharide (EPS) hydrolysate from the colonies from LB agar showed a composition with L-fucose, L-rhamnose, D-galactosamine, D-glucosamine, D-galactose, D-glucose, D-mannose. However, that from pellicles showed similar neutral and amino sugar profile but missing galactose. Furthermore, uronic acid analysis of EPS hydrolysates by HPLC showed that mannuronic acid was detected from pellicles not from colonies, indicating the epm-derived polymer is critical for pellicle formation as proved by the epm mutants. This study verified that for the circular pellicle architecture P. alkylphenolica KL28 cells utilized EPS building blocks different from that used for colony construction. These results indicate that P. alkylphenolica KL28 is a clever architect that dictates unique cell arrangements with selected EPS matrix material to construct sophisticated building, circular biofilm pellicles.

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