Availability of Congo Red Agar as a Simple Isolation Method for Oral Biofilm-forming Bacteria

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Abstract: It is widely known that most bacteria produce exopolysaccharide and exist in the form of communities, commonly referred to as biofilm. Since the ability of bacteria to form biofilm is considered an important factor in persistent infections, it is important to isolate these bacteria from lesions and study their mechanism of biofilm formation. The screening of biofilm-forming bacteria is difficult because it requires the measurement of viscosity of culture supernatants and scanning electron microscopic (SEM) observation of cell surface structures. We used Congo red agar (CRA) plates to screen biofilm-forming bacteria from the oral cavity. Whole saliva from five healthy volunteers was diluted to $10^{-4}$ to $10^{-6}$ and used to inoculate CRA plates. The bacterial cultures were incubated aerobically at 37°C for 24 hours. Using a stereoscopic microscope, the colonies were divided into four groups: black colonies with rough periphery (RB), lucent or red colonies with rough periphery (RL), black colonies with smooth periphery (SB) and lucent or red colonies with smooth periphery (SL). The cell surface structures of the bacterial strains obtained from arbitrarily selected colonies in each group (RB: 24; SB: 24; RL: 8; SL: 13) were further studied by SEM, and each strain was identified by 16S rRNA gene sequencing.

Bacterial strains that formed rough periphery colonies (RB and RL types) on CRA under aerobic conditions expressed dense fibrillar structures, which is a typical phenotype for biofilm-forming bacteria. On the other hand, more than 90% of bacterial strains that formed colonies with smooth periphery (SB and SL types) showed biofilm-negative cell surfaces. In addition to Congo red reaction, the reaction of bacteria obtained from each colony type to calcifluor white (CFW), which binds to β1-3 and β1-4 carbohydrate linkages and fluoresces under long-wavelength UV light, was examined. CFW reaction did not correspond to biofilm phenotype.

These results suggest that oral bacteria that form colonies with rough periphery cultured under aerobic conditions on CRA have the capacity to form biofilm. We also found that CRA can be applied as a simple method for screening oral biofilm-forming bacteria.

Key words: Congo red, Oral bacteria, Biofilm