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## Effect of cultivation conditions on biofilm formation of *Bacillus subtilis*

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### Abstract

Biofilms are the moving, constantly changing, heterogeneous bacterial communities. Microorganisms growing in a biofilm are highly resistant to both antimicrobial agents and immune mechanisms. Objective of this paper was to study the effect of extracellular and membrane-bound proteases *B. subtilis* on the formation of biofilms in liquid media. The strains *B. subtilis* 168 (wild type) and *B. subtilis* BRB14 (protease-deficient strain) were examined on the ability to form biofilms in liquid medium. The LB and 1% glucose LB media were used as rich culture medium, and the synthetic E-medium was chosen as poor culture medium. A decreased formation of biofilms by 20% was observed during growth in the glucose-containing LB medium as compared with the glucose-free LB medium. The level of biofilm formation in the synthetic E-medium was 7 times higher than the total level in both LB media. Under identical culture conditions, the ability of mutant strain *B. subtilis* to form biofilms was 10%-15% higher than the level of biofilm formation by a wild strain. By the 48th hour of growth, the level of biofilm content in the culture of both strains reached maximum and declined significantly by the 72nd hour of growth. pH-Optimum of biofilm formation by both strains *B. subtilis* was 7.4. The temperature optimum for the formation of biofilms for both strains *B. subtilis* also ranged from 22°C to 45°C. The results of the studies allow us to conclude that the extracellular and membrane-bound proteinases *B. subtilis*, deleted in the mutant strain, do not play a key role in the formation of biofilm, however, their effect is more significant during growth of bacteria in a nutrient-rich environment rather than in synthetic one.

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### Keywords

*Bacillus subtilis*, Biofilms, Culture medium, PH optimum, Proteinase-deficient strain, Temperature optimum