Production of antifungal active substance using Biofilm by *Bacillus subtilis*

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Abstract

we examined the influence of medium concentration on biofilm formation and the production of antibiotic substance. The relationship between biofilm formation and antifungal substance production was clearly observed, and it was shown that antifungal substance was produced after biofilm formation. We have previously observed the biofilm formation in the medium with the agriculture residues. Using these properties, the experiment to increase the production amount of antifungal substance is under consideration.

Introduction

In order to achieve higher food production, the burden on the environment has to be smaller and more sustainable. In view of this fact, microbial pesticides have attracted attention in recent years. Microbial pesticides are agents that use cells and/or the substances produced by microorganisms and inhibit the growth of phytopathogenic fungi. Compared with chemical pesticides, microbial pesticides are less likely to remain in the environment, and it is difficult to develop drug-resistant bacteria. Therefore, we focused on *Bacillus subtilis* in this study.

B. subtilis are broadly distributed microorganisms in the soil. It is reported that *B. subtilis* produce antibacterial substances and form biofilms. Biofilm is a mass of microorganisms and is formed on the surface of a solid and/or liquid in which the microorganisms are encapsulated with extracellular polysaccharides. Biofilm-formed microorganisms show extremely high resistance to environmental stress and antibiotics. We have *B. subtilis* RB14 is known to produce iturin A, an antifungal substance and surfactin (Fig. 1), so we examined the influence of medium concentration on biofilm formation and the production of antibiotic substance.

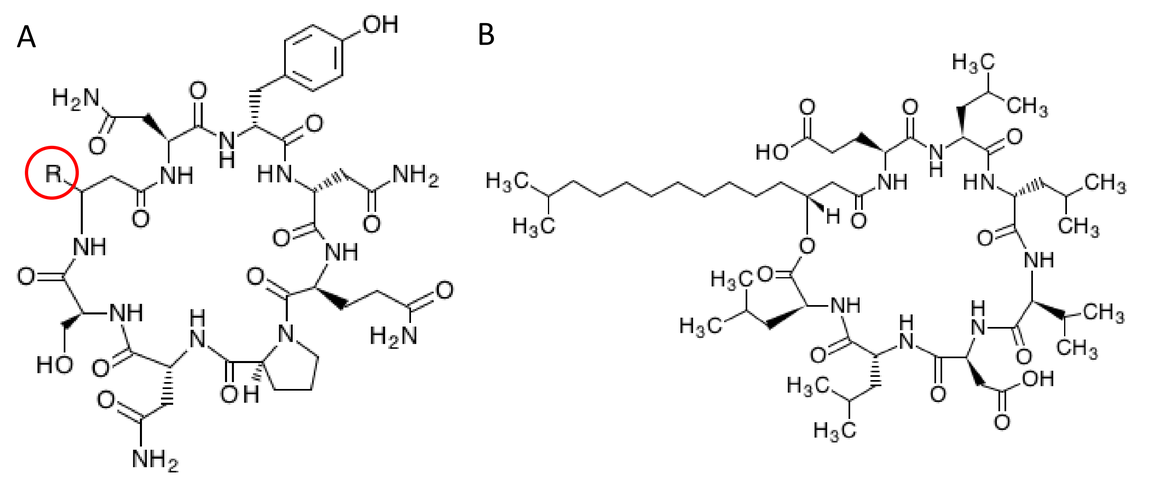


Fig. 1. Antibiotics iturin A (A) and surfactin (B) produced by *Bacillus subtilis* RB14. iturinA is classified into eight depending on the side chain indicated by R in red circle.
source : Kyouto Encyclopedia of Genes and Genomes

Experiment 1. Biofilm formation and antifungal activity by *B. subtilis* RB14 under different medium concentration

B. subtilis RB14 precultured in LB medium was inoculated into polypepton medium with the concentration of 0.5%, 3.0%, 10%, and was cultured to the stationary phase at room temperature. As a result, biofilm formation was observed at all concentrations, and the biofilm became thicker depending on the number of days of culture. In addition, robust biofilm formation was observed as the concentration of polypepepton increased. Each culture was collected and sterilized by filtration,and a bioassay for the plant pathogen *Rhizoctonia solani* K1 was performed.

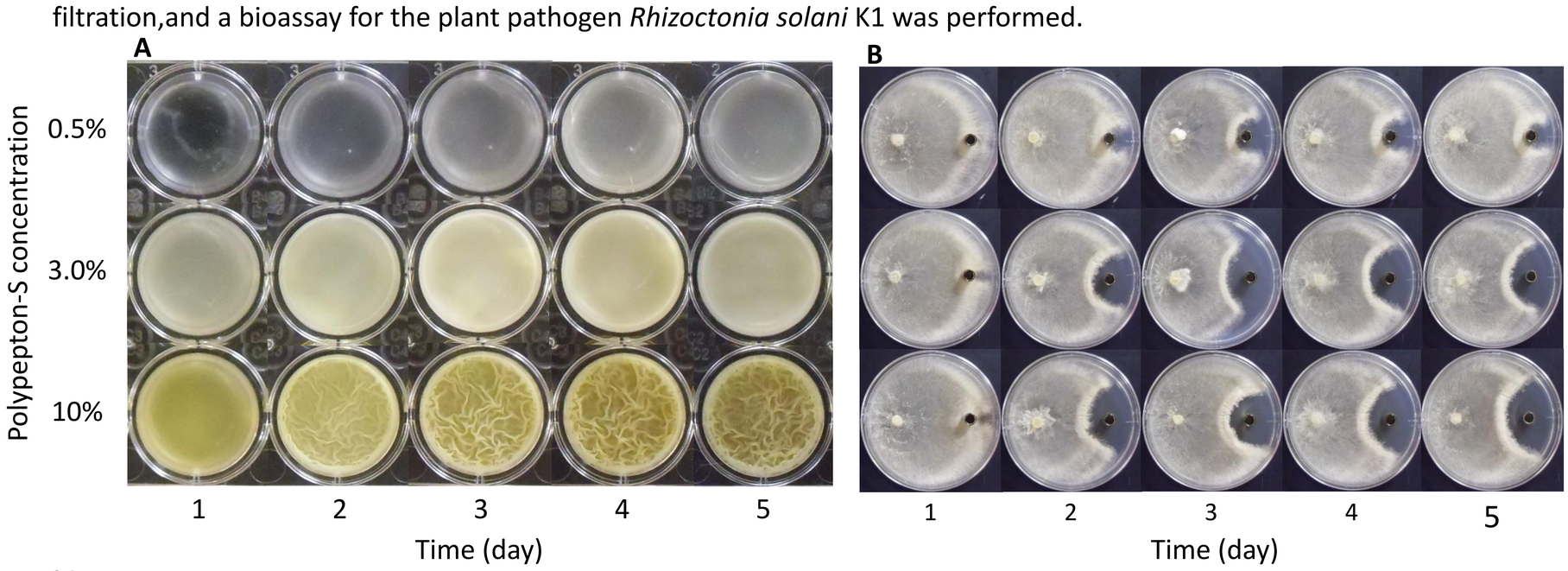
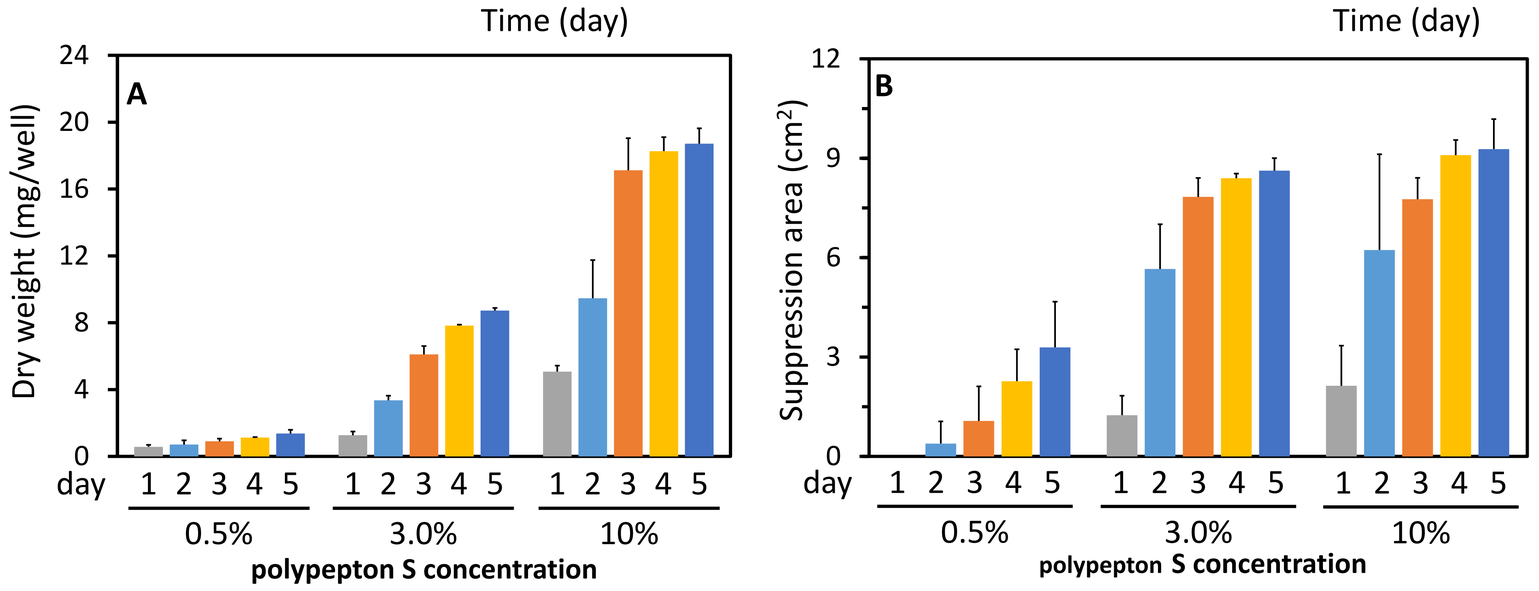


Fig. 2. Biofilm formation by *B. subtilis* RB14 under different medium concentration in 12 wells (A). Antifungal activity of 200 µL sterilized culture of *B. subtilis* RB14 of each condition (B).



All contents of the wells were centrifuged at 13,000 rpm for 10 minutes, and the supernatant portion was discarded. The precipitated part was washed and treated with a freeze dryer. The dry weight of the recovered biofilm was measured.

Fig. 3. Dry weight of biofilm by *B. subtilis* RB14 under different medium concentration in 12 wells (A). Suppression area (B).

Experiment 2. Biofilm formation and antifungal activity by *B. subtilis* RB14 under high medium concentration

We thought that *B. subtilis* could grow on higher concentrations of peptone water. *B. subtilis* RB14 strain was inoculated into polypepton medium with the concentration of 10 - 30%. As a result, biofilm was formed at all concentrations and biofilm was maintained in high concentration medium. 1 mL of the culture solution was sterilized by filtration, and was used for the bioassay for the plant pathogen *R. solani* K1 strain. The bioassay showed the highest antifungal activity in 10% peptone water.

From the above, it was suggested that *B. subtilis* can proliferate and produce antibacterial substances even in high concentration medium. In the future, we will try to culture
using agricultural residues such as rice bran, and the product useful substances from waste.

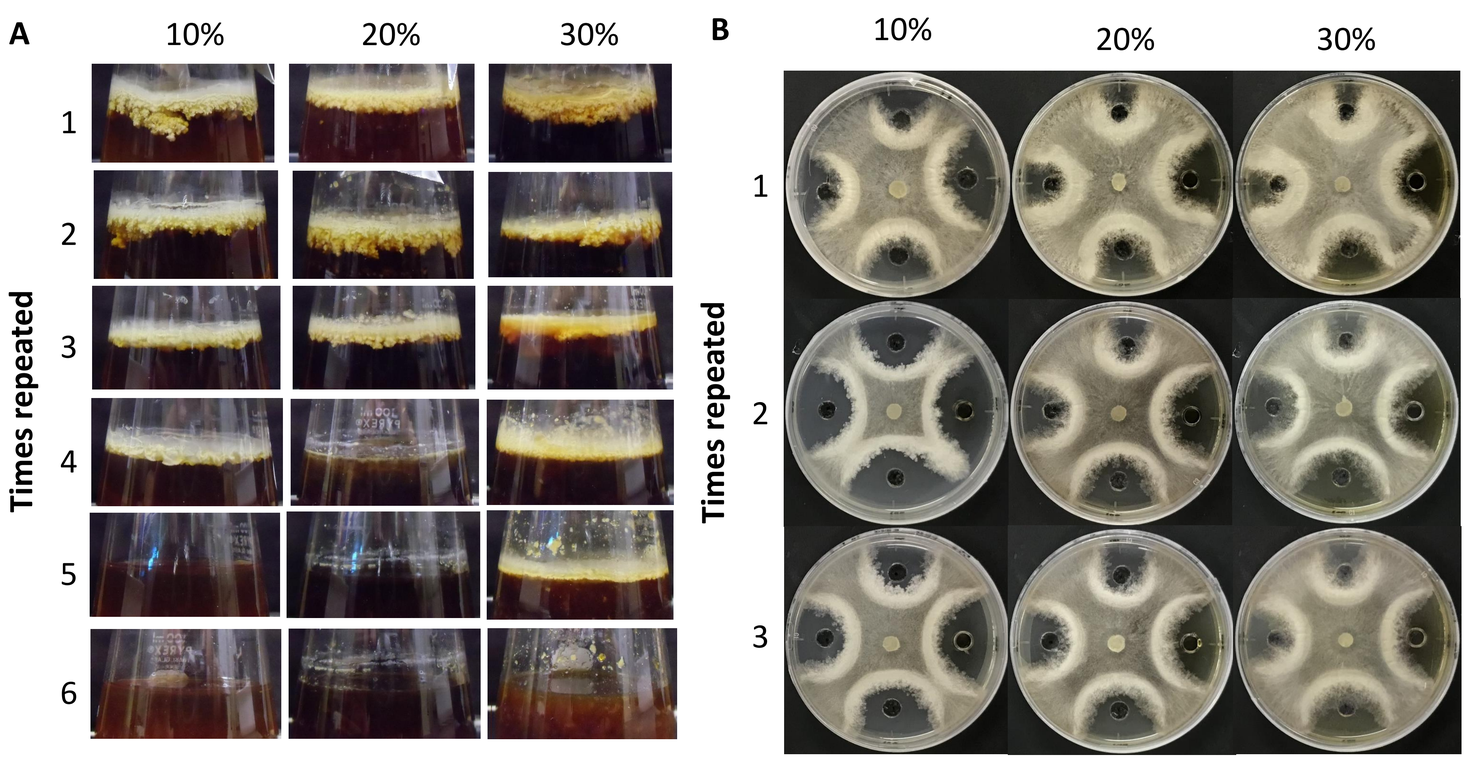


Fig. 4. Biofilm formation and reproduction by *B. subtilis* RB14 under different medium concentrations. Antifungal activity of the culture solution of *B. subtilis* RB14 under each condition (A). Each petri dish contains 30, 50, 100 and 200 µL of sterilized culture of *B. subtilis* RB14 in the counterclockwise direction from the top position (B).

Conclusion

- The culture of *B. subtilis* RB14 showed antifungal activity.
- *B. subtilis* RB14 formed robust biofilm under high concentration pepton medium.

We believe that antibiotics can be produced from waste water rich in protein from food factories by using *B. subtilis* RB14.