Quorum sensing system influences the synthesis of isoquercitrin catalyzed by recombinant *E. coli* BL21-pET21a-*rhaB1* biofilm

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Introduction

As flavonoids, isoquercitrin is an intermediate for the synthesis of new food additive Enzymatically Modified Isoquercitrin (EMIQ). But it is very rare in nature (1-5‰). However, isoquercitrin can be synthesized by hydrolyzing rhamnosyl of rutin, which can be found abundantly in nature. Biofilm is a structured community formed by the adhesion and aggregation of a large number of bacteria, and its advantages such as good self-regeneration, sustainability and scalability have great application potential in the field of biocatalysis. Previous studies have shown that quorum sensing system plays a key role in regulating biofilm formation. Therefore, it is of great significance to explore the relationship between quorum sensing and biofilm catalysis in engineering bacteria.

Methods

E. coli BL21-pET21a-*rhaB1* expressing RhaB1 were used to grow biofilm. Crystal violet was used to assess the total biomass assay of recombinant *E. coli* biofilm. *V. harveyi* BB170 was used as indicator bacteria to detect the activity of quorum sensing signal molecule AI-2. Finally, the biofilm was used to catalyze the synthesis of isoquercitrin from rutin.

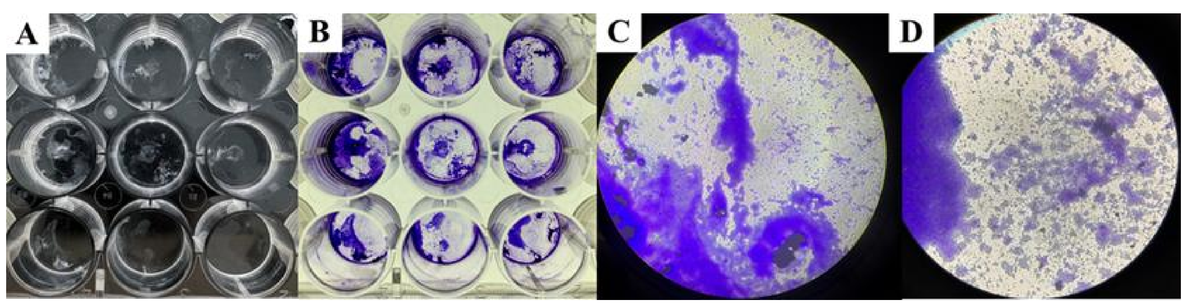

Fig. 1 Crystal violet staining and microscopic observation of biofilm. (A) Recombinant *E.coli* BL21-pET21a-*rhaB1* biofilm, (B) Crystal violet staining of the biofilm, (C) Microscopic observation of biofilm (100×), (D) Microscopic observation of biofilm (400×).

Results & Discussion

Fig. 2A shows that when pH increased from 3.5 to 8.5, the AI-2 activity of recombinant *E. coli* increased first and then decreased, and the activity was the highest at ph5.5. Fig. 2b monitored the dynamic changes of AI-2 activity in recombinant *E. coli* within 24 h. The AI-2 activity increased rapidly during the exponential growth period and decreased slowly after the stable period. Fig. 2C and Fig. 2D showed that the optimum growth condition of recombinant *E. coli* biofilm was pH 6.5 and temperature 35℃.

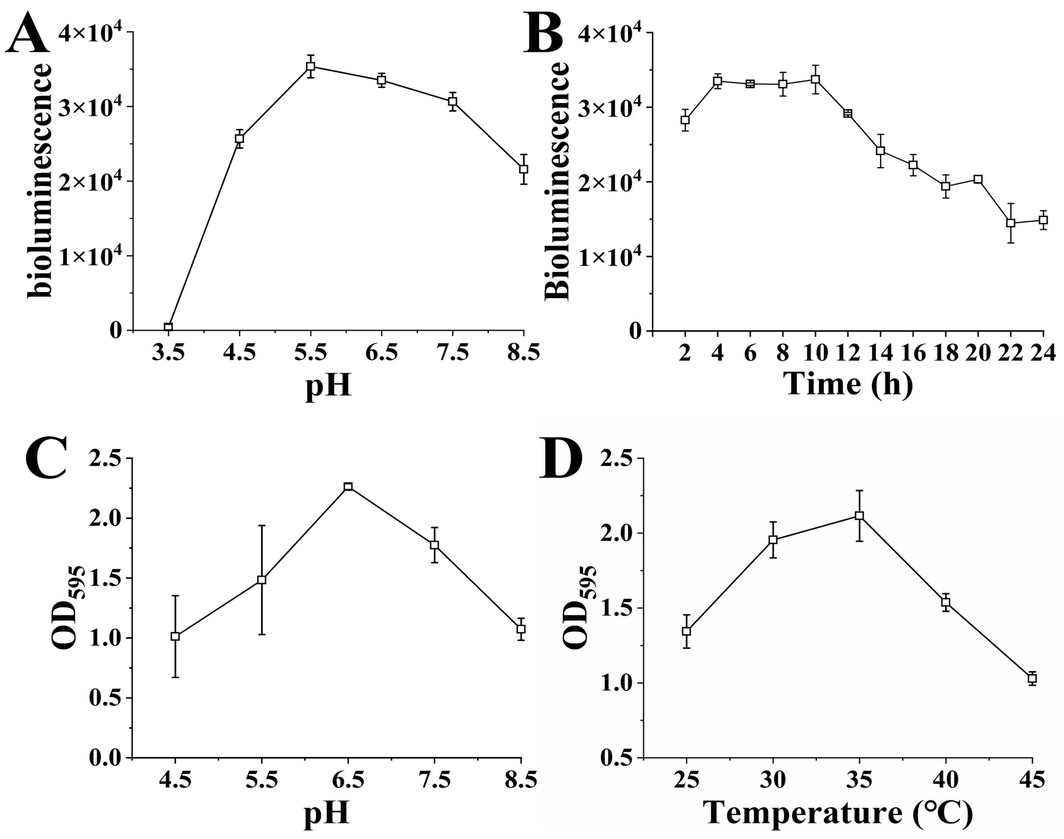

Fig. 2 Effects of environmental factors on QS system and biofilm biomass of recombinant *E. coli*. Effect of pH (A) and time (B) on AI-2 activity of QS system, Effect of pH (C) and temperature (D) on biofilm biomass.

Fig. 3 provides the observation of biofilm formation by scanning electron microscope (SEM) and fluorescence inversion microscope (FIM). recombinant *E. coli* biofilm was formed on a glass slide. Bacteria adhere to glass surfaces and secrete various highly organized, coordinated, and functional extracellular substances to form biofilms.

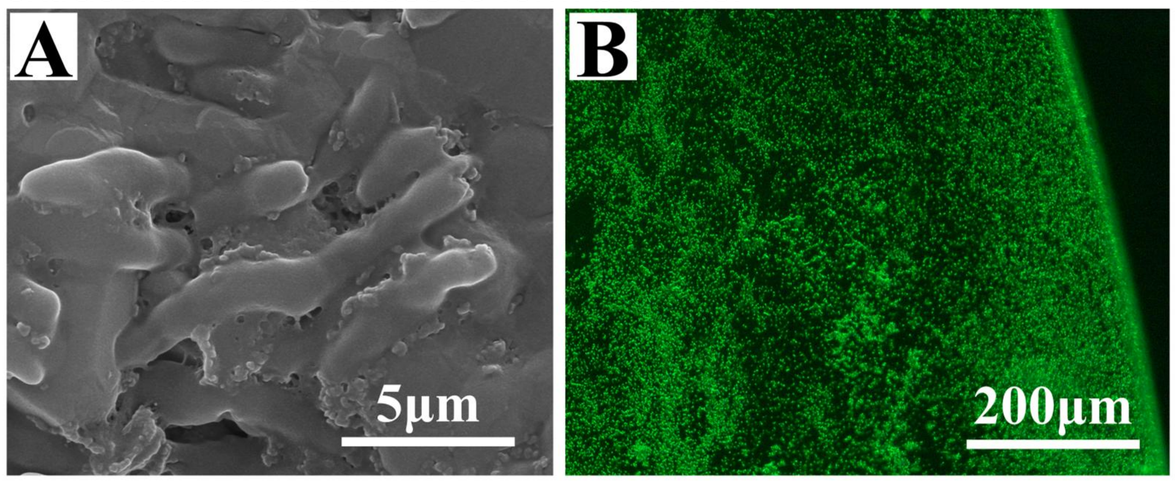

Fig. 3 Observation of recombinant *E. coli* biofilm by SEM (A) and FIM (B).

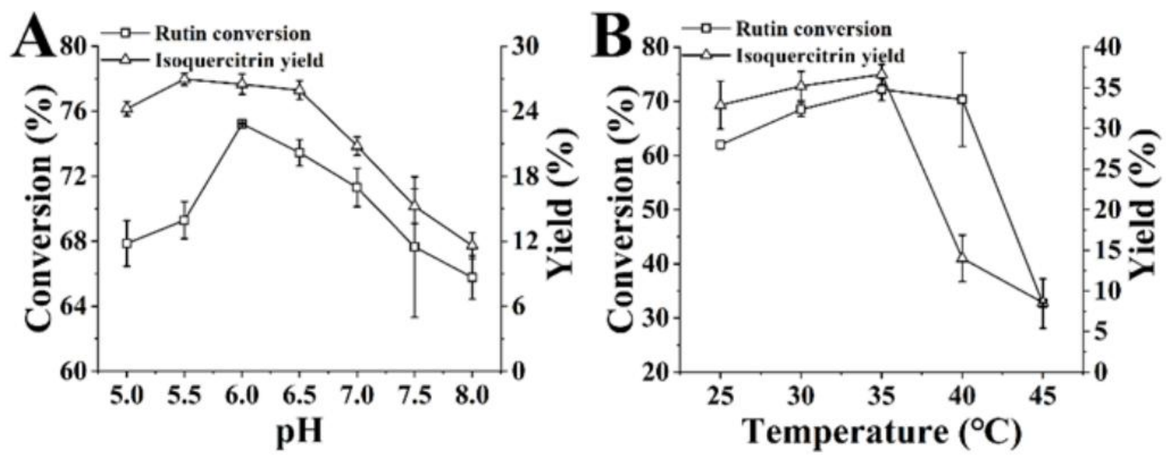

Fig. 4 Effect of pH (A) and temperature (B) on rutin conversion and isoquercitrin yield.

Fig. 4A shows that when pH is between 5.0 and 8.0, the rutin conversion rate increases first and then decreases, the maximum value is 75.22±0.05%, and the maximum isoquercitrin yield was 26.96±0.55%. Fig. 4B shows that the rutin conversion rate maintained a high level between 25℃ and 40℃ and then decreased rapidly, with the highest conversion rate reaching 72.20±8.67%, and the maximum isoquercitrin yield was 36.65±1.21%.

Conclusions

- AI-2 activity was highest at pH 5.5 and increased rapidly during the exponential growth period.
- The optimum growth condition of recombinant *E. coli* biofilm is pH 6.5 and temperature 35℃.
- The acidic and low-temperature environment were more suitable for the reaction in the catalytic process of recombinant E. coli biofilm.

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