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

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As flavonoids, isoquercitrin has many pharmacological effects such as antioxidant, anti-tumor, anti-depression, antihypertensive, and lipid-lowering(Pennesi et al., 2017). It is an advanced food additive, auxiliary drug, or effective component of drugs that are developed internationally, and an intermediate for the synthesis of new food additive Enzymatically Modified Isoquercitrin (EMIQ). But isoquercitrin is very rare in nature (1 - 5 ‰), and its chemical synthesis is complex. It has been found that rutin which has one more rhamnosyl than isoquercitrin in the molecular structure can be found abundantly in nature (Šimčíková et al., 2014). Therefore, it is of great significance to catalyze the hydrolysis of rutin widely existing in nature to synthesize isoquercitrin.

Biofilm acts as a living catalyst has great advantages in the field of biological resources transformation (Maksimova and Yu., 2014). Biofilms are the main way for microorganisms to survive in the natural environment. Biofilms are mainly formed by bacteria and extracellular polymeric substances (EPS) secreted by bacteria. Bacteria only account for 5 % – 35 % of the biofilm. The microorganisms are embedded with the EPS secreted by itself, which is conducive to the adaptation of the growth environment. EPS directly provides the extracellular environment and survival conditions of bacterial biofilms, separates cells from the external environment, and provides mechanical stability for biofilms to protect bacteria in biofilms. The formation of biofilm multi-cell structure is a dynamic process, including the stages of bacterial initial adhesion, biofilm development, maturation, diffusion, and re-adhesion (Latif, Majid, May, Oba, and E., 2018). However, in industrial production, bacterial biofilms grow disorderly and loosely and cannot be reused, resulting in limited mass transfer and reduced reactor efficiency (Muffler et al., 2014). Quorum sensing (QS) is considered to be associated with biofilm formation. QS is a kind of colony behavior in which bacteria regulate their gene expression according to the change of cell density in the population. The biofilm formation and resistance to various environmental stresses of bacteria are regulated by LuxS/AI-2 system (Gu, Li, Tian, Wu, and He, 2018). Therefore, it is of great significance to explore the relationship between quorum sensing and biofilm catalysis in engineering bacteria.

E. coli is considered that sense and respond quickly to external signals, which are an important part of inducing host surface adhesion to enable biofilm formation (OS, G, KB, MR, and CD, 2021). Meanwhile, *E. coli* is a widely used engineering strain, and the growth of biofilm is beneficial to improve catalytic efficiency. Rhamnosidase is a hydrolytic enzyme that hydrolyzes naringin, rutin, quercitrin, hesperidin, etc. to release rhamnose, which has many potential industrial applications. On this basis, the recombinant *E. coli* BL21-pET21a-*rhaB1* secreting rhamnosidase was the research object. As shown in Fig. 1, biofilms were cultured in 24-well plates for catalytic studies.

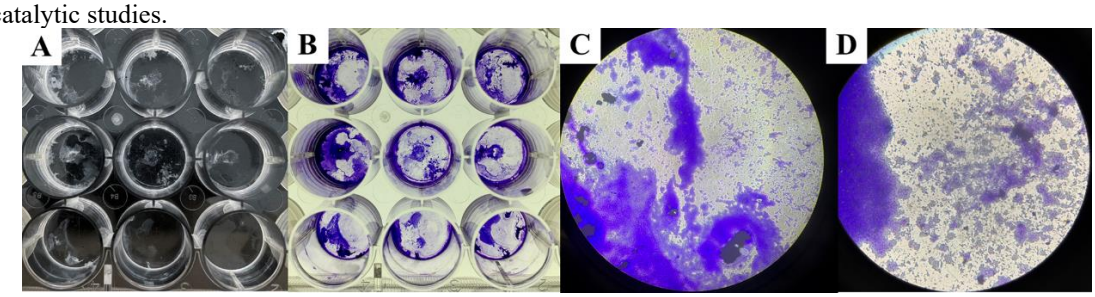

Fig. 1 Crystal violet staining and microscopic observation of biofilm. (A) *Recombinant E.coli* BL21-pET21a-*rhaB1* biofilm, (B) Crystal violet staining of the biofilm, (C) Microscopic observation of biofilm (100×), (D) Microscopic observation of biofilm (400×).

The effect of environmental factors on the QS system AI-2 activity and biofilm biomass of recombinant *E. coli* was studied. Fig. 2a shows that when pH increased from 3.5 to 8.5, the AI-2 activity of *E. coli* increased first and then decreased, and the activity was the highest at ph5.5, which may be related to the optimal pH for the growth of the strain. Fig. 2b monitored the dynamic changes of AI-2 activity in *E. coli* within 24 h. The AI-2 activity increased rapidly during the exponential growth period and decreased slowly after the stable period.

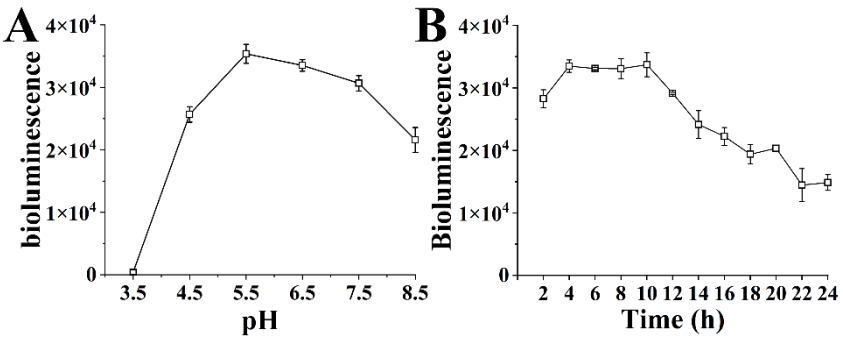

Fig. 2 Effects of environmental factors on QS system and biofilm biomass of recombinant *E. coli*. Effect of pH (A) and time (B) on AI-2 activity of QS system, Effect of pH (C) and temperature (D) on biofilm biomass.

Fig. 3 studied the effects of different pH and temperature on the conversion rate and yield of isoquercitrin from rutin catalyzed by recombinant *E. coli* biofilm. Fig. 3a 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 %. The isoquercitrin yield rate was maintained at a high level between pH5.0-6.5, the highest reached26.96 ± 0.55 %. When pH > 6.5, the yield rate decreased rapidly. Fig. 3b 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 %. The isoquercitrin yield rate maintained a high level between 25 ℃ and 35 ℃ and then decreased rapidly, with the highest formation rate reaching 36.65 ± 1.21 %. The results showed that the acidic and low-temperature environment were more suitable for the reaction in the catalytic process of recombinant E. coli biofilm.

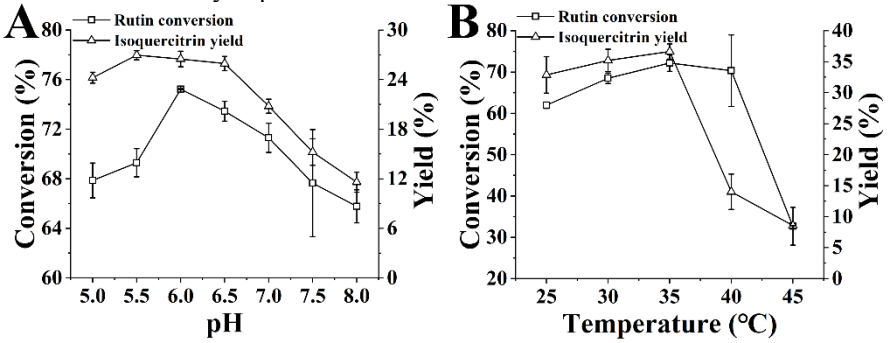

Fig.2 Effect of pH (A) and temperature (B) on rutin conversion and isoquercitrin yield.

In conclusion, AI-2 activity was highest at pH 5.5 and increased rapidly during the exponential growth period. The optimal pH and temperature for recombinant *E. coli* catalysis were 5.5 and 35℃, respectively.

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