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Efficient Production of Recombinant Proteins in *B. Subtilis* by Disruption of Cell Wall Protease

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Staphylokinase (SAK), a polypeptide secreted by *Staphylococcus aureus*, is a plasminogen activator with a therapeutic potential in thrombosis diseases (1). SAK expression plasmid pSAK704 carrying a promoter and a signal sequence of subtilisin was constructed and introduced into *Bacillus subtilis* strain which is multiply deficient in exo-proteases. However, the amount of SAK secretion was marginal and there was a severe degradation in the purification process. PMSF prevented the proteolytic degradation of secreted SAK in the extracellular medium. Thus, it was suspected that residual protease activity is responsible for the degradation of SAK.

B. subtilis LB700 was constructed from six-exoproteases-deficient *B. subtilis* WB600 (2) by disruption of *wprA* gene which encodes a PMSF-sensitive cell wall protease (3), using a temperature-sensitive suicide vector, pDWPRA. The cultivation of *B. subtilis* LB700 carrying pSAK704 at 37°C for 20 h in LB medium resulted in a 4-fold increase (181mg/liter) in production of staphylokinase. In addition, the extracellular stability of mature SAK was ten-fold enhanced. These data indicate a significant role of the *wprA* gene product in degrading SAK, both during secretion and in the extracellular milieu (4).

Another plasminogen activator, streptokinase (SK) was expressed in *B. subtilis* LB700. Recombinant *B. subtilis* LB700 produced 4×10^6 IU/liter of SK in the cultivation at 37°C for 16h in the LB medium, which is a 2-fold increase compared with that of WB600. Western blot analysis showed SK fragments specifically digested by a WprA protease.

In order to evaluate the significance of WprA in foreign protein production, disruption of *wprA* gene in different versions of mulfiple-protease-deficient *B. subtilis* strains. Inactivation of *wprA* in *B. subtilis* 168, DB104 (5) and DB428 (6) resulted in LB100, LB300 and LB500, respectively. SAK secretion in LB500 increased as LB700. However, SAK secretion in LB100 and LB300 was declined compared with 168 and DB104. Oversecretion of native extracellular proteases was observed in *wprA*-disrupted *B. subtilis*. The secretion of native bacillopeptidase F was promoted by 20% in *wprA*-disrupted strain. These data indicate that *wprA* gene disruption not only strongly promotes the production of foreign proteins, but also slightly improves the secretion of self-extracellular protease that is involved in proteolytic degradation of secreted recombinant proteins. In conclusion, *wprA* gene disruption is indispensable for the efficient production of foreign proteins in highly engineered *B. subtilis*.

References

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