Development of low-temperature protein production systems by using cold-adapted bacteria, *Shewanella livingstonensis* Ac10 and *Pseudoalteromonas nigrifaciens* Sq02

Soichiro Kawai

Recombinant proteins produced by microbial hosts are widely used in the fields of life science and biotechnology. However, there are still many proteins that cannot be produced efficiently by the previously constructed protein production systems. In this study, to provide a new option for production of these proteins, I constructed low-temperature protein production systems by using *Shewanella livingstonensis* Ac10 and *Pseudoalteromonas nigrifaciens* Sq02 as the hosts. Low-temperature protein production alleviates heat-denaturation of proteins and suppresses enzyme activities. Thus, the system is useful for the production of thermolabile proteins and for the production of enzymes that are toxic to the host due to their catalytic activity. In Chapter I, I constructed a regulatable low-temperature protein production system by using *S. livingstonensis* Ac10 as the host and a promoter of the *trp* operon of this bacterium. In Chapter II, I isolated and characterized a cold-adapted bacterium, *P. nigrifaciens* Sq02, as a prospective host for heterologous protein secretion at low temperatures. The mechanism of selective protein secretion of this strain was also

analyzed. In Chapter III, I constructed a low-temperature protein expression system by using *P. nigrifaciens* Sq02 as the host for secretory production of foreign proteins. I demonstrated that foreign proteins can be produced as a single major protein in the culture supernatant by using the system constructed in this study.

CHAPTER I

Development of a regulatable low-temperature protein expression system using the psychrotrophic bacterium, *Shewanella livingstonensis* Ac10, as the host

An Antarctic bacterium, *Shewanella livingstonensis* Ac10, has been used as the host for a low-temperature protein production system, in which the target proteins are constitutively expressed. To increase its utility, I introduced a repressible promoter of the *trp* operon of this bacterium into this system. When the gene coding for ß-lactamase was expressed under the control of this promoter, the yields of ß-lactamase were 33 mg/L-culture and 75 mg/L-culture at 4 °C and 18 °C, respectively, in the absence of L-Trp and were significantly decreased in the presence of L-Trp. I also found that 3-indoleacrylic acid, a competitive inhibitor of the *Escherichia coli trp* repressor, increased the expression of the reporter gene. This repressible gene expression system would be useful for regulatable recombinant protein production at low temperatures.

CHAPTER II

Isolation and characterization of a novel psychrotrophic bacterium, *Pseudoalteromonas nigrifaciens* Sq02, as a prospective host for secretory protein production at low temperatures

I isolated a novel psychrotrophic bacterium, *Pseudoalteromonas nigrifaciens* Sq02, as a prospective host for a heterologous protein secretion system operating at low temperatures. This strain secreted a protein named P320 to the culture supernatant with a large quantity and high purity. Deletion of the P320 gene demonstrated that P320 functions as an adhesion factor and facilitates biofilm formation of this strain. Whole genome sequence of this strain showed that the gene coding for P320 is located at the downstream of a gene cluster including the genes coding for homologs of several components of a canonical type II secretion system (T2SS) and proteins of unknown function. Deletion of the genes in the vicinity of the P320 gene suggested that P320 is selectively secreted by the function of a T2SS-like machinery consisting of homologs of canonical T2SS components and proteins of unknown function. *P. nigrifaciens* Sq02 and its selective protein secretion system are expected to be useful to construct a recombinant protein secretion system working at low temperatures.

CHAPTER III

Construction and application of a low-temperature protein production system by using the psychrotrophic bacterium, *Pseudoalteromonas nigrifaciens* Sq02

In this chapter, I first assessed whether the promoter of the P320 gene, named P_{p320}, is useful for construction of a low-temperature protein production system by using *P. nigrifaciens* Sq02 as the host. When the gene coding for ß-lactamase was used as a reporter, the yield of ß-lactamase was higher than that obtained from the protein production system constructed previously by using *S. livingstonensis* Ac10 as the host. Next, I constructed a protein secretion system by using P_{p320} as the promoter and *P. nigrifaciens* Sq02 as the host, in which foreign proteins were produced as C-terminal fusion proteins of P320. To evaluate the utility of this system, four enzymes of a psychrophilic bacterium, *Desulfotalea psychrophila* DSM12343, were produced as fusion proteins. As the result, each of the fusion proteins was produced as a single major protein in the culture supernatant when a P320-depleted mutant was used as the host. The amounts of the secreted fusion proteins were comparable to that of P320 produced by the wild-type strain. Thus, P320 functions as a carrier to secrete foreign proteins to the culture supernatant, and this system is useful for production of recombinant proteins with high purity at low temperatures.