Trap identification of the constitutive promoter-like sequences from the bacterial fish pathogen, as exemplified by Edwardsiella tarda

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A trap identification system for isolating functional sequences to allow the constitutive expression of foreign protein from Edwardsiella tarda was developed. Using the green fluorescent protein (GFP) reporter-based trap system, various functional sequences to drive heterologous expression of the GFP were selectable in Escherichia coli host. However, from the bioinformatic sequence analysis, all the segments predicted as regulatory regions were not native promoters actually existing upstream of endogenous E. tarda genes. Instead, a number of non-authentic sequences, possibly resulted from the random shuffling and/or intermolecular ligation were also proven to be able to display a potent GFP expression in the recombinant E. coli. Further analysis with selected clones showed that both authentic and non-authentic sequences could function in as a constitutive promoter, leading quite a consistent and stable GFP expression after repetitive subcultures. Microscopic examination also confirmed the uniform pattern of GFP expression in every host bacterium. Semi-quantitative assay of GFP showed that there was no clear relationship between expression levels and organizational features of the promoters trapped. Functional promoter-like elements achieved in the present study could be a good starting material for multivalent genetic engineering of E. tarda in order to produce recombinant vaccines in a cost-effective fashion.

Key words: Constitutive expression, Edwardsiella tarda, Promoter trap

Vaccination-based prevention against a specific disease has already been indispensable with the health control of many farmed fish species (Lorenzen, 1999; Adams and Thompson, 2006). Compared to the vaccination of terrestrial animals, administration of the vaccines to aquaculture-relevant species should often include a greater number of individuals. Hence, the efficient expression system allowing the cost-effective production of the recombinant vaccines is one of pivotal prerequisites for successful adoption of the vaccines in the farming practice of the aquacultured fishes (Secombes, 2008).

Edwardsiella septicaemia, caused by infection with a Gram negative bacterium Edwardsiella tarda, has been one of the major causes of mass mortalities in cultured fish (Thune et al., 1993; Plumb, 1999). Recently, recombinantly inactivated vaccine types of E. tarda, such as ghost and cadaver bacteria, have been reported as an alternative to classical heat-or formalin-inactivated vaccines (Kwon et al., 2005, 2006, 2007 and 2009; Lee et al., 2008). In addition, the utilization of the ghost or cadaver E. tarda as a delivery tool for heterologous antigens was reported recently (Choi et al., 2010).
Most post-mortem approaches for fish vaccination have been based on the use of tightly inducible promoters, in which the transcription of the antigen-coding gene was activated by the addition of a chemical inducer (e.g., isopropyl β-D-1-thiogalactopyranoside; for lac or tac promoters) (Plant et al., 2009; Qin et al., 2010) or the thermal elevation of bacterial culture (e.g., for lambda P_R/CI regulatory system) (Kwon et al., 2006; Ningqiu et al., 2008). Although these expression systems have been widely successful in laboratory applications, the use of such systems in a commercial scale may often cause the increase of the production cost for the vaccines due to their needs of a priced inducer compound or a specific apparatus for the programmed thermal control of the bacterial culture.

For this reason, the development of alternative expression system with novel regulatory element(s), which is able to drive the expression of recombinant proteins during the normal growth phase of the host cells (i.e., a constitutive expression), might also be required in certain circumstances, although the constitutively expressed recombinant proteins should have no adverse effect on the viability and growth of the hosts (Gat et al., 2003; Brondyk, 2009). However, the functional sequences to drive constitutive expression have been little exploited for developing the recombinant fish vaccines. In line with our long-term goal to generate efficient expression systems to produce fish vaccines in a cost-effective fashion, the objective of this study was to generate the trap identification system for isolating and characterizing the functional sequences capable of driving the constitutive expression of foreign protein from a known bacterial fish pathogen, Edwardsiella tarda.

Materials and Methods

Bacterial species, culture conditions, nucleic acid preparation and enzymes

A donor bacterial species, Edwardsiella tarda (Gram-) used in this study was the FSW910410 strain (Kwon et al., 2005), while an Escherichia coli strain (XL1-blue MRF'; Stratagene, La Jolla, CA, USA) was used as a recipient platform for the trap identification of promoter-like sequences. Bacterial cells were grown in Luria Broth (LB; Difco Laboratories, Detroit, USA) at 28 (for E. tarda) to 37 °C (for E. coli). Transformed E. coli cells were grown in LB supplemented with 100 μg/ml of ampicillin (Sigma-Aldrich, St. Louis, MO, USA). Genomic DNA from the E. tarda was prepared using the LaboPass™ Mini Kit (Cosmo Genetech, Seoul, Korea). Purification of plasmid DNA from recombinant E. coli was carried out using the AccuPrep® Plasmid Extraction Kit (Bioneer, Daejeon, Korea). All the procedures for nucleic acid preparations were conducted according to the manufacturers’ instructions. Polymerase chain reactions in this study were carried out using the Expand High Fidelity PCR System (Roche Applied Science, Mannheim, Germany), and restriction endonucleases used in this study were purchased from New England Biolabs (NEB; Ipswich, MA, USA).

Construction of promoter trap vector

The trap vector was constructed with the pUC18 (Stratagene) as a backbone plasmid (Fig. 1). From the pUC18 backbone, lac promoter and lacZ' fragment including multiple-cloning site (MCS) were eliminated by site-directed PCR of the selective region using a