Development of genetic manipulation in *Flavobacterium columnare*

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Abstract

Flavobacterium columnare, an important pathogen of freshwater fish, can infect wild, farmed and ornamental fish species worldwide and cause heavy mortality and economic loss. However, reports about protective antigens and virulence factors of *F. columnare* are rare. Although chondroitin AC lyase has been reported as a probable virulence factor for its degradation of chondroitin sulphates, it has not yet been experimentally proven. Other probable virulence factors, such as collagenases have been facing the same problem. Previous researches attributed it to the lack of a suitable molecular manipulation system for *F. columnare*, especially a plasmid-based gene transferring system. Recent reports revealed the establishment of a genetic manipulation system for the bacterium [1], but there has been no actual success in the establishment of in-frame deletion in *F. columnare*. Other members of the genus *Flavobacterium* have been proven amenable to genetic manipulation. For example, a successful in-frame mutagenesis of *F. johnsoniae* by using rpsl gene as a counter selectable marker has been achieved [2]. Our purposes of this study were to construct a transposon mutant library of *F. columnare* and to construct an in-frame deletion strain of *F. columnare* by developing a knockout-rescue system.

Based on previous work, the plasmid pEP4351 containing Tn4351 was introduced into *F. columnare* G_4 strain by conjugation. The positive clone was screened by antibiotic selection, PCR, and phenotypic tests. As a result, more than 3000 clones were obtained and there was no background growth when the erythromycin level was increased to 2–3 μg/ml. The insertion sites of Tn4351 in positive clones were identified using random primer PCR.

The main procedure of the development of a knockout-rescue system and construction of an inframe deletion strain of *F. columnare* could be described as follows. A *sacB* gene and *rpsl* gene was cloned into modified pCP23 [3] in which replicase was removed, respectively. The resultant plasmids were pMS75 and pCP-rpsl. About a 4 kb fragment, which had the deletion of the functional domain of chondroitin AC lyase gene, was cloned into pMS75 and pCP-rpsl, respectively. The two resultant plasmids were transformed into G_4 and G_4 streptomycin mutant by conjugation. After two rounds of recombination, the mutant of deleted *chl* gene was obtained. The ability of the mutant to degrade chondroitin AC was decreased. To complement the phenotype of the mutant, the fragment containing the complemented ORF of *chl* and its probable promoter region was cloned into the shuttle plasmid pCP23 and transformed into the mutant by conjugation. As a result, the ability of the complementary strain to degrade chondroitin AC improved. The result was verified by SDS-PAGE of extracellular proteins. After inframe deletion of the *chl* gene, the encoded protein band could not be found in the analysis.

In conclusion, a higher efficiency of transposon-based mutagenesis was obtained and a suicide vector based on a gene deletion strategy was successfully constructed.

References

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