H-3

Comparison of *Lactococcus garvieae* antigenic proteome

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Introduction

Lactococcus garvieae is an emerging pathogen at mariculture farms and was isolated from a variety of fish species. In Korea, diseases caused by Gram positive cocci may be one of major problems in the field. The isolation, identification, and differentiation of these Gram-positive bacteria are not simple and are time-consuming.

This study was aimed to identify and characterise strain specific proteome, in addition, to find antigenic proteins of a *L. garvieae* strain isolated from Korea. On basis of the proteome study, it would be able to find and develope diagnositic marker. Two-Dimensional Gel Electrophoresis (2-DE) and its immunoblotting was emplpyed to compare and identify with two type strains and one isolate from Yosu area.

Materials and Methods

Strains used in this study was ATCC 49156 (YT3), NCINB 702155, and an isolate from Yosu showed similar reaction both on biochemical and serological examination. The bacteria were cultivated using Tryptic Soy Broth (TSB) supplemented with 2% NaCl and incubated at 25℃. Cells were harvested by centrifugation, washed twice with PBS (20 mM Phosphate, 150 mM NaCl, pH 7.2). The pellet was resuspended in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 65 mM DTT, 40 mM Tris and 0.5% IPG buffer pH 3-10NL) and then sonicated the bacteria until clarification, and the lysate was centrifuged at 13,000rpm for 30min to remove cell debris. The supernatant was diluted with lysis buffer and stored -70 ℃ until further use.

For 2-DE, the protein sample was added to the rehydration buffer (9.5 M urea, 4%

CHAPS, 65 mM DTT, 40 mM Tris, and 0.5% IPG buffer pH 4-7L) and then loaded onto IPG (Immonilised pH Gradient) strips to allow rehydratation for 12 hours at 22 ℃. Focusing was initiated at 200V, and was gradually increased to 8000V. After equilibration of the focused IPGstrips, SDS-PAGE was run by loading the strip onto the polyacrylamide gels. The gel was stained with silver nitrate, or it was transferred to nitrocellulose membrane to perform immunobortting using rabbit anti-YT3 serum. 2-DE gel and immunoblotted membrane was analyzed using phoretix™ 2D program. Sample preparation for MALDI-TOF MS analysis was followed by Fountoulakis's method with some modification. Measured peptide masses were identified by searching a protein sequence database, such as MS-Fit on the Internet.

Result and Summary

Gram positive cocci bacteria, including type strains and isolates from diseased fishes, was classified according to biochemical and serological examination. A representive isolate (Yosu0109Nubchi9) was identified highly similar strain with the type strain (ATCC49156(YT3)). 2-DE was able to evaluate different proteome composition and its immunoblotting was showed common and specific antigens between *L. garvieae* strains. MALDI-TOF MS was also performed to identify some of these antigenic proteins.

Reference

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