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Proteome analysis of *Streptococcus iniae* ATCC 29178

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Introduction

Strpetococcus iniae is a hemolytic, gram-positive coccus first isolated in 1976 from a subcutaneous abscess of a captive freshwater dolphin. It cause meningoencephalitis and death in aquicultural fish species and has recently been identified as an emerging human pathogen. Depending on environmental condition, numerous sreptococcal protein was showed much differences in expression type. It was, however hard to see many difference with traditional protein seperation method, such as SDS-PAGE.

At present study, proteomics, two-dimensional gel electrophosis (2-DE) and MALDI-TOF MS analysis, was employed to separate individual proteins and to know proteome composition of *S. iniae*.

Materials and method

Type strain of *S. iniae*, ATCC29178, was used in this study. The bacteria were grown in Tryptic Soy Broth (TSB) supplemented with 2% NaCl and incubated for three days at 25 ℃. Cells 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).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, 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

2-DE gel of *S. iniae* was stained with coomassie blue and silver nitrate. Through 2-DE gel image analysis, most of *S. iniae* proteins were located between 4 to 7 pI range, especially 4.5 - 5.5 pI, and between 70 to 30 kDa molecular weight range. To confirm the accuracy of MALDI-TOF MS, bovine serum alubumin was employed and identified it from protein database at low level of mass tolerance $\leq$50ppm. On the basis of the result, a variety of proteins spots on 2-DE gel of *S. iniae* were identified by searching database, such as heat shock protein (pI 4.92, M.W. 75 kDa), enolase (pI 4.74, M.W. 47 kDa), ATPase (pI 4.83, M.W. 51 kDa), and DNA-binding (pI 4.90, M.W. 43 kDa) .

Reference

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