PROTEOMIC STUDIES OF *VIBRIO SALMONICIDA*

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SUMMARY

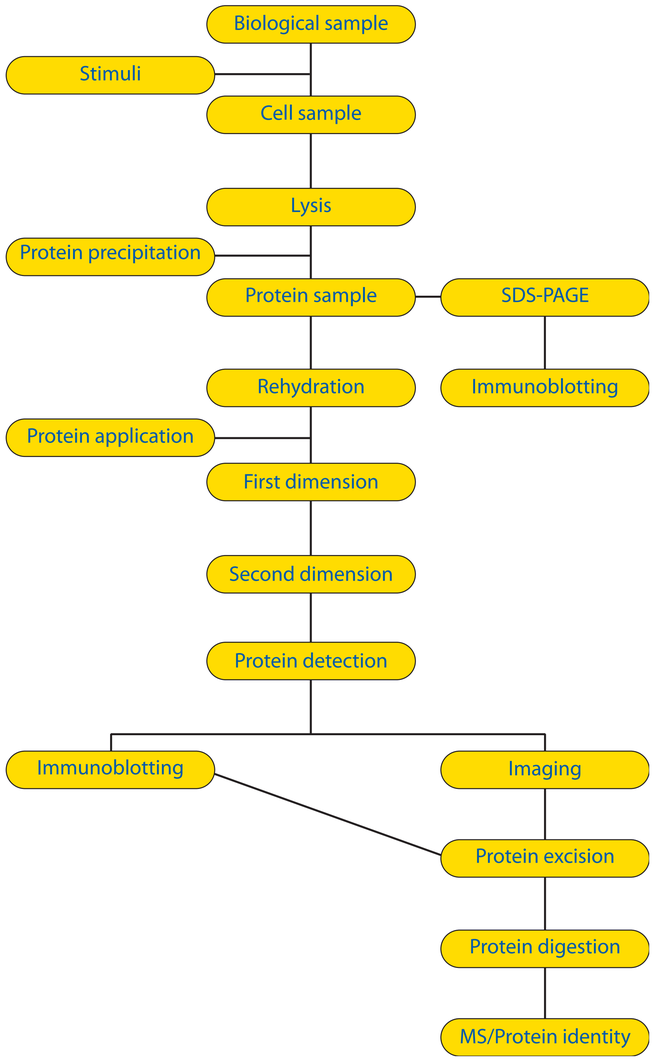
Effects of different cultivation conditions on the expression pattern of *Vibrio salmonicida* proteins were investigated by western blot and 2D gel electrophoresis. With different growth stimulants, altered expression patterns of unstimulated bacteria cells have been discovered. Currently, stimulants like salmon mucus, hydrogen peroxide, iron chelator and different temperatures have been used. Also, immunoblotting of the gels with immunsera from salmon infected with *V. salmonicida* strain LFI315 was performed to identify proteins potentially involved in virulence.

INTRODUCTION

V. salmonicida is a psycrophilic/psycrotroph gram-negative bacterium. This bacterium is the causative agent of cold-water vibriosis in Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadhus morhua*).

To understand more about the pathogenesis of this bacterium, the project aims at detecting and exploring virulence factors responsible for host specificity and adaptation: e.g. host-bacterial interaction and oxidative stress.

METHODS



Flowscheme of methods in use in the proteomic studies of virulence and adaptation in *V. salmonicida*.

RESULTS AND DISCUSSION

Figure 1A shows differences in protein expression due to temperature and mucus addition to the growth medium. In addition, different ways of preparing the protein samples revealed variation in protein expression. Immunoblot analysis of the parallel gel (fig. 1B) demonstrated a binding pattern dependent on culturing temperature, mucus addition and protein extraction method.

V. salmonicida cytoplasmic proteins were separated on a S76 gel filtration column (fig. 2A). Dot-blot analysis of the separated fractions (fig. 2B) showed binding of immunsera in all the different protein fractions.

Two dimensional gel electrophoresis analysis (fig. 3) of cytoplasmic proteins revealed differences in expression pattern consisting of a number of proteins. Also, differences in expression patterns have been observed with altered growth temperatures, and with hydrogen peroxide and iron chelator supplemented to the media (data not shown).

Differentially expressed spots from 2-D gels have been excised, digested with trypsin and used to do MALD-TOF (MS) and MS/MS (data not shown). Peptide mass fingerprints and MS/MS data obtained have to be further investigated in aim of protein identification.

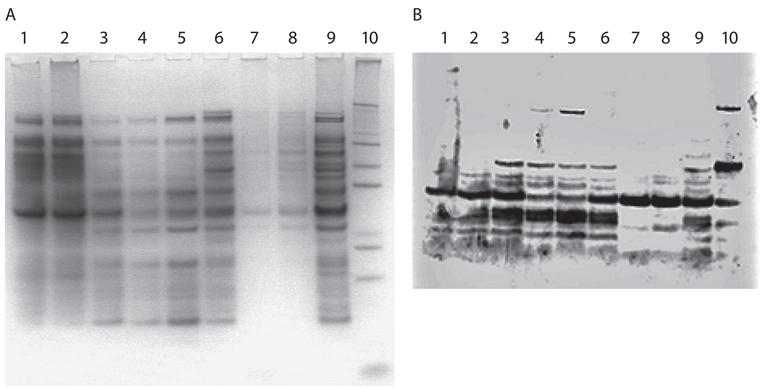


Figure 1:
SDS-PAGE **(A)** and immunoblot analysis **(B)** of proteins from *V.salmonicida* strain LFI315. Cells were grown in marine broth with and without supplements. Different growth conditions and methods for protein extraction were used: lane1-2; cells grown at 8 °C and 16°C, lane 3-5; cells grown at 12°C with mucus added: 0%, 1% and 4%, respectively, lane 6-8; cells grown at 12°C showing: cytosolic proteins, proteins of intermediate solubility and less soluble proteins, respectively, lane 9; mix of sample 1-8, lane 10; ladder (200 Da-2.5 kDa). Coomassie blue was used as protein stain.

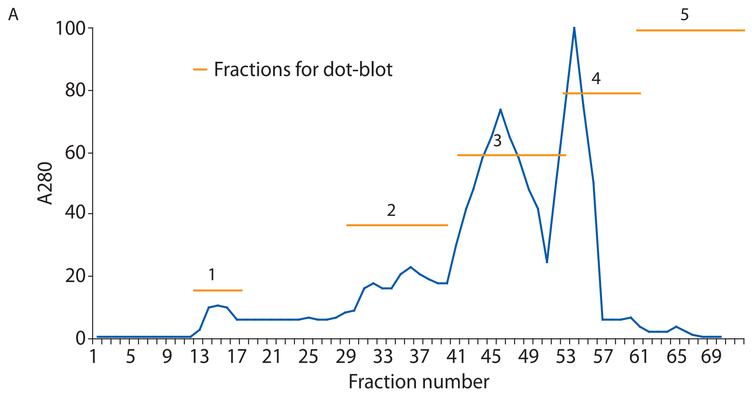


Figure 2:
(A) *V. salmonicida* cytoplasmic proteins separated on a S75 gel filtration column.
(B) Dot-blot analysis using immunsera raised against live *V. salmonicida* in Atlantic salmon. Protein fractions are numbered 1-5. Control samples are named C.

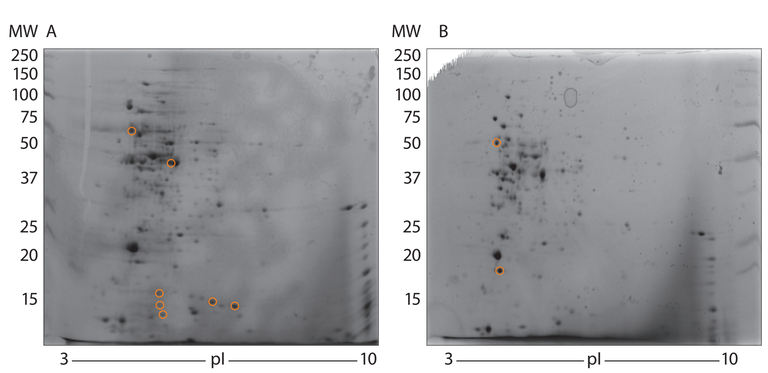


Figure 3:
2D- gel analysis of proteins from *V. salmonicida* grown in **(A)** marine broth and **(B)** marine broth with 1% mucus added. Immobiline dry strips, pH 3-10, were used for isoelectric focusing. Circles indicate differences in expression pattern as effect of mucus in culturing medium. Culturing temperature was 12°C.

CONCLUSION

Proteins were separated by SDS-PAGE and gel filtration, and identified by binding of immunsera. Different growth stimuli revealed different expression patterns on 2D gels. The expression pattern as an effect of stimuli in culturing medium, showed higher expression of proteins when mucus was added to the medium. Also, variation in protein expression was observed with different methods of preparing protein samples. However, more studies have to be performed in order to identify proteins from MS and MS/MS analysis. Studies involving a number of different growth stimuli should be performed and analysed on 2D gels to reveal proteins involved in adaptation and virulence.