# Use of *in vivo*-induced antigen technology to identify bacterial genes expressed during S*olea senegalensis* infection with *Photobacterium damselae* subsp. *piscicida*

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## Abstract

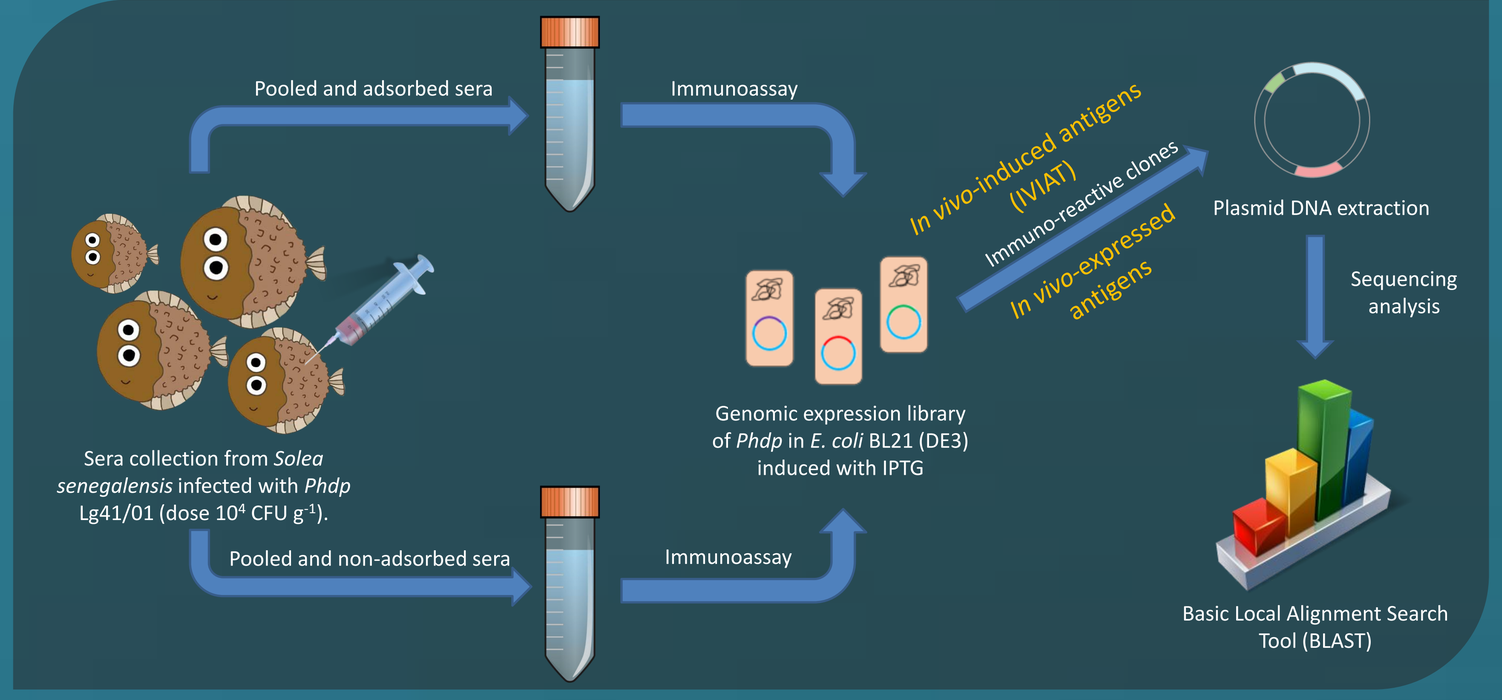
The marine fish pathogen *Photobacterium damselae* subsp. *piscicida* (*Phdp*) is responsible for important outbreaks affecting several fish species including flatfish *Solea senegalensis*. The aim of this work was to identify *in vivo*-induced expressed immunogenic proteins using pooled sera from fish that have experienced photobacteriosis. *In vivo* induced immunogenic proteins included inosine-5'-monophosphate dehydrogenase (Impdh) and alkyl hydroperoxide reductase (AhpC), two proteins involved in peptide synthesis: serine hydroxymethyl transferase (Shmt) and alanyl-tRNA synthetase (AlaRS) and the non-ribosomal peptide synthetase involved in the synthesis of the siderophore piscibactin (Irp2).

## Introduction

*Photobacterium damselae* subsp. *pisicicida* (*Phdp*) is an opportunist pathogen in marine fish responsible for important economic losses. Several virulence factors have been identified in Phd; however, most studies have been carried out *in vitro* and bacterial activities are modulated by their environment. Genes expressed during pathogen infection are important for pathogenicity. In vivo-induced antigen technology (IVIAT) has been used to identify in vivo-induced genes using pooled sera from fish that have experienced photobacteriosis.

## Materials and Methods

Sera were obtained from *S. senegalensis* specimens after sublethal infection with *Phdp* and subsequently pooled and adsorbed against *in vitro* grown *Phdp* Lg41/01 and *Escherichia coli* BL21 (DE3) cells and lysates to remove antibodies against *in vitro* expressed antigens according to Handfield et al. [1]. The efficiency of sera adsorption was evaluated by ELISA, based on the immuno-reactivity with whole and lysed *Phdp* cells, grown *in vitro,* as immobilized antigens. A genomic expression library of *Phdp* Lg41/01 was generated in *E. coli* BL21 (DE3) using pET-30 expression system (Novagen, San Diego, CA, USA). The expression library was probed with adsorbed (for *in vivo*-induced antigens, IVIAT) and non-absorbed sera (for *in vivo*–expressed antigens) using immunoblot technique. Inserted DNA from reactive clones was sequenced (Macrogen Europe, Amsterdam, The Netherlands). Nucleotide sequences were compared against the NCBI protein database using BLASTx.




## Results

Specific antibody titers against *Phdp* were determined in the sera from each surviving *S. senegalensis* specimen. All fish showed significantly higher antibody titers compared to control fish (non-infected fish). After sera adsorption rounds, a progressive reduction in sera immuno-reactivity against *in vitro* grown *Phdp* cells was detected, especially after the first adsorption step. Thus, following adsorption steps substantially resulted in relative enrichment in antibodies recognizing *in vivo* expressed antigens. The library from *Phdp* Lg14/01 constructed in *E. coli* BL21 (DE3) consisted of approximately 6500 recombinants.

| Functional category | Identification | Accession | Predicted cellular location |
|---|---|---|---|
| Replication, recombination and repair | DNA gyrase subunit B | WP_044180341.1 | Cytoplasm |
| | Recombination associated protein RdgC | WP_044176475.1 | Cytoplasm |
| Iron acquisition | Non-ribosomal peptide synthetase | AKQ52531 | Membrane |
| Transport and metabolism | Inosine-5'-monophosphate dehydrogenase | EEZ41661.1 | Cytoplasm |
| | Arginine decarboxylase catabolic | AEU10010.1 | Cytoplasm |
| | Glutamine amidotransferases class-II (GATase) | WP_044179637.1 | Cytoplasm |
| | Putative amidotransferase | AEU09986.1 | Cytoplasm |
| | Diguanylate phosphodiesterase | WP_044178285.1 | Membrane/ Cytoplasm |
| Antioxidant activity | Alkyl hydroperoxide reductase | WP_005298372.1 | Cytoplasm |
| | Superoxide dismutase | WP_005298367.1 | Periplasm |
| Cell envelope and wall metabolism | Lytic transglycosylase | WP_044174705.1 | Outer cell membrane |
| | Murein transglycosylase | WP_044178572.1 | Outer cell membrane |
| Translation, ribosomal structure and biogenesis | 23S rRNA methyltransferase | WP_044175535.1 | Cytoplasm |
| | Serine hydroxymethyltransferase | WP_005300693.1 | Cytoplasm |
| | Alanyl-tRNA synthetase | WP_044174517.1 | Cytoplasm |
| Cell cycle control, cell division, chromosome partitioning | Chromosome partitioning protein ParA | WP_014386679.1 | Cytoplasm |
| Mobile and extrachromosomal element functions | Putative transposase | AEU10011.1 | Cytoplasm |
| Other | Intramembrane serine protease GlpG | WP_044173910.1 | Membrane |

A total of 118 clones were selected for their reactivity with pooled adsorbed and non-adsorbed sera from convalescent *S. senegalensis* specimens after a first round of screening. In a second screening, 18 out of 118 candidate clones showed positive reaction, indicating the immunogenic character of proteins expressed during *Phdp* infection. Predicted proteins codified by inserted sequences have intracellular and membrane cell location and are involved in virulence, synthesis of intermediary products, energy metabolism and DNA replication. *In vivo* induced immunogenic proteins included inosine-5'-monophosphate dehydrogenase (Impdh) and alkyl hydroperoxide reductase (AhpC), two proteins involved in peptide synthesis: serine hydroxymethyl transferase (Shmt) and alanyl-tRNA synthetase (AlaRS) and the non-ribosomal peptide synthetase, involved in the synthesis of the siderophore piscibactin (Irp2).

## Discussion

Environmental stimuli determine the gene expression of organisms. Thus, bacteria invading a host sense *in vivo* environment and adapt by inducing or repressing the expression of specific genes. In the case of pathogens, identification of genes with up-regulated expression *in vivo* compared to *in vitro* conditions might give an insight into the genes relevant to the bacterial virulence [2]. AhpC peroxidase activity has a protective role by reducing hydrogen peroxide, peroxynitrite and organic hydroperoxides. Immunization with AhpC conferred protection against *Helicobacter pylori* infection [3]. Impdh catalyzes the conversion of products essential in *de novo* synthesis of guanine nucleotides. Adequate levels of purine nucleotides are critical for cell proliferation, nucleic acid replication, cell signaling and as a biochemical energy source. This gene is an important therapeutic target against bacterial diseases [4]. On the other hand, the non-ribosomal peptide synthetase involved in the synthesis of the siderophore piscibactin is considered a virulence factor in *Phdp*. In the present work, the induction in *S. senegalensis* and its immunogenic character have been determined.

## Conclusion

Different proteins expressed during *Phdp* infection in *S. senegalensis* have been identified. Among them, Impdh, AhpC, Shmt, AlaRS and Irp2 have been identified as *in vivo* induced antigens expressed during *S. senegalensis* infection with *Phdp*. They are likely to play a role in the virulence of *Phdp*. The antigenic character of these proteins makes them potential targets for the development of new vaccines.

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### References

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