

BOLSA DE INVESTIGAÇÃO (M/F)

Referência: PTDC/MAR/101143/2008

Título do Projecto: “Rational design of an AIP56-based anti-pasteurellosis vaccine”

Código interno: PR062603

Está aberto concurso para recrutamento de um(a) bolseiro(a) de Investigação para colaborar no projecto acima referido, financiado pelo programa COMPETE - Programa Operacional Factores de Competitividade na sua componente FEDER e pelo orçamento da Fundação para a Ciência e a Tecnologia na sua componente OE.

A bolsa, em regime de exclusividade, terá a duração de 1 ano eventualmente renovável, com início previsto em 1 de Maio de 2010.

O valor mensal da bolsa será de € 745,00, pago por transferência bancária (preferencialmente).

Local de trabalho: IBMC - Instituto de Biologia Molecular e Celular (Grupo de Imunologia e Vacinologia de Peixes), Porto, Portugal

Programa de trabalho: ver anexo.

Perfil pretendido:

Os candidatos devem pelo menos ter o grau de licenciatura nas áreas de Bioquímica, Biologia, Microbiologia ou área afim, dando-se preferência a quem tiver média de licenciatura igual ou superior a 14 valores e manifesta experiência com técnicas necessárias à boa execução do referido projecto.

O prazo para recepção de candidaturas decorre de 7 a 21 de Abril de 2010.

As propostas deverão incluir carta de motivação, CV, e duas cartas de recomendação e ser enviadas por correio electrónico para candidaturas@ibmc.up.pt com indicação do código interno (PR062603).

A contratação será regida pelo estipulado na legislação em vigor relativamente ao Estatuto de Bolsheiro de Investigação Científica, nomeadamente a Lei 40/2004, de 18 Agosto, e o Regulamento de Bolsas de Investigação Científica do IBMC (www.ibmc.up.pt/fellowships.php).

“Rational design of an AIP56-based anti-pasteurellosis vaccine”

Supervisor:

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Project Summary:

Pasteurellosis, a bacterial infection caused by *Photobacterium damsela* subsp. *piscicida* is one of the most serious threats to the success of aquaculture of several economically important marine fish species [1].

Using an experimental model of pasteurellosis, we showed that virulent strains of *Phdp* induce extensive systemic apoptosis of sea bass neutrophils and macrophages through the secretion of the AIP56 exotoxin [5, 6].

Passive immunization of sea bass with anti-AIP56 rabbit antibodies confers protection against *Phdp* infection [6], indicating that AIP56 is a key virulence factor of *Phdp* and suggesting that an AIP56-based vaccine would protect against *Phdp* infection. We performed preliminary vaccination experiments using as antigens: inclusion bodies of AIP56 expressed in *E. coli* (that would maintain only limited AIP56 conformational structure), and also formalin-inactivated AIP56 enriched bacterial culture supernatants. Despite the presence of high levels of anti-AIP56 antibodies in the immunized fish, the relative protection conferred by these vaccines was not satisfactory (the levels of protection ranged from 20 % to 70 % relative percent survival and varied between experiments) (unpublished results). These results indicate that the quality and amount of key-epitope specific antibodies may be crucial to induce protection, since in the case of inclusion bodies many antibodies generated would be specific to irrelevant epitopes, and in the case of culture supernatants the production of antibodies to proteins other than AIP56 could detract from the anti-AIP56 response. Therefore, studies are needed to obtain an AIP56-based vaccine formulation that preserves the natural protective epitopes of the toxin, but lacks apoptogenic activity to consistently elicit the production of antibodies conferring protection against fish pasteurellosis.

A gene coding for an AIP56 homologue was found in *Vibrio campbellii*, another widely distributed pathogen of marine organisms [7]. Moreover, AIP56 N-terminal part has a high degree of identity to a conserved type III effector of unknown function of enterohemorrhagic *E. coli* (EHEC), *Salmonella enterica* and *Citrobacter rodentium* while the C-terminal of AIP56 has high degree of identity to an hypothetical protein of *Acyrtosiphon pisum* bacteriophage APSE-2 [6, 8], suggesting the existence of two different domains in AIP56.

Limited proteolysis experiments performed at our laboratory support the existence of two structural domains in AIP56. Thus, we hypothesize that AIP56 is an AB-toxin (similarly to shiga, cholera and anthrax toxins, among others). The AB-toxins have an A subunit which is responsible for the enzymatic activity and a B subunit that mediates toxin binding/internalization [9]. Considering that a conserved putative metalloprotease signature is located in the AIP56 N-terminal region and that this

region has a high identity with type III effectors from pathogenic enteric bacteria [6], we hypothesize that this region is responsible for the toxin's apoptogenic activity and the C-terminal is responsible for AIP56 binding/entry into the cells.

An effective vaccine must contain epitopes that induce the production of neutralizing antibodies. Chemically detoxified toxins (toxoids) have been successfully used for a long time as vaccines. In most instances the toxins were detoxified using high concentrations of formalin and this can eliminate immunodominant, protective B-cell epitopes [10]. Another problem is that neutralizing epitopes are usually not immunodominant (a strategy used by the pathogen to evade the attack by host's immune system) and only induce very weak humoral responses or even keep silent, when the target protein is used as immunogen.

The progress in biotechnology and molecular biology has allowed the genetic attenuation of several toxins [10, 11]. In contrast to chemically inactivated toxins, genetically detoxified toxins maintain their shape, their B-cell epitopes and the ability for an active interaction with antigen-presenting cells [10] and have been replacing the classical toxoids as they are safer and more immunogenic.

In this project, we will apply the knowledge available regarding the AIP56 structure and function to the design of an AIP56-based vaccine.

Truncates corresponding to the putative A and B subunits of AIP56, and toxin mutated in the zinc-binding consensus sequence are first class candidates for testing in vaccination experiments. Strategies allowing the identification of neutralizing epitopes of AIP56, such as the peptide scanning approach, will also be developed.

This project will lead to a better understanding of the mechanisms involved in the generation of a protective immunity against *Phdp* and may allow the design of a vaccine capable of eliciting protective immune responses against fish pasteurellosis.

Our team is well qualified to undertake this project and our technical and scientific skills (see for exemple [1, 12-19]) together with the excellent conditions of the Proponent Institution will allow this project to be carried out successfully.