

POSTER PRESENTATION

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Cloning of a synthetic chimeric gene containing recombinant *Mycoplasma hyopneumoniae* antigens for expression in *Pichia pastoris*

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From 5th Congress of the Brazilian Biotechnology Society (SBBIOTEC)
Florianópolis, Brazil. 10-14 November 2013

Background

Mycoplasma hyopneumoniae is the primary etiologic agent of Swine Enzootic Pneumonia (EP), one of the most common respiratory disease that affects swine worldwide, causing considerable economic losses. Vaccination constitute one of the main practices to control EP. The production of recombinant experimental vaccines against EP has been considered an important approach towards the development of an improved vaccine [1]. This study aimed to clone a synthetic gene composed by the fusion of *M. hyopneumoniae* antigens R1 (P97), P42 and NrdF to *Escherichia coli* fragment B of the heat labile enterotoxin (LTB) into the vectors pPICZB (intracellular expression) and pPICZαB (secreted expression), in order to produce this chimeric recombinant protein in *Pichia pastoris* and to test it as a vaccine candidate. The *M. hyopneumoniae* antigens were selected due to their capacity to confer partial protection in pigs when evaluated individually [2-4]. These antigens were associated to LTB, a potent mucosal and parenteral adjuvant.

Methods

The DNA fragment coding for the recombinant chimeric protein was designed in silico [5]. The synthetic gene and the vectors pPICZαB and pPICZB were digested with the enzymes *Bam*HI and *Eco*RI and ligated. The ligation product was transformed into *E. coli* TOP10 strain, plated on Luria - Bertani (LB) culture medium containing the antibiotic Zeocin™ (Invitrogen). The resulting colonies were selected through a rapid screening method using a protocol with phenol - chloroform. Recombinant clones were

expanded in LB liquid medium with antibiotic, the plasmid was extracted and then characterized with restriction enzymes *Bam*HI and *Eco*RI.

Results and conclusions

The synthetic gene coding for the chimeric protein, composed by the fusion of *M. hyopneumoniae* antigens R1 (P97), P42, NrdF and to LTB were efficiently cloned into the two expression vectors in *P. pastoris*, pPICZB and pPICZαB. Digestion of the recombinant clones resulted in the release of a fragment of 1500 pb, corresponding to the size of the insert. Future tests will be performed with these clones in order to optimize expression of this chimeric protein in *P. pastoris*.

Acknowledgements

MAPA, CNPq, CAPES and FAPERGS.

Published: 1 October 2014

References

1. Reynolds SC, St Aubin LB, Sabbadini LG, Kula J, Vogelaar J, Runnels P, Peters AR: Reduced lung lesions in pigs challenged 25 weeks after the administration of a single dose of *Mycoplasma hyopneumoniae* vaccine at approximately 1 week of age. *The Veterinary Journal* 2009, **181**:312-320.
2. Shimoji Y, Oishi E, Muneta Y, Nosaka H, Mori Y: Vaccine efficacy of the attenuated *Erysipelothrix rhusiopathiae* YS-19 expressing a recombinant protein of *Mycoplasma hyopneumoniae* P97 adhesin against mycoplasmal pneumonia of swine. *Vaccine* 2003, **21**:532-537.
3. Chen YL, Wang SN, Yang WJ, Chen YJ, Lin HH, Shiuan D: Expression and immunogenicity of *Mycoplasma hyopneumoniae* heat shock protein antigen P42 by DNA vaccination. *Infection and Immunity* 2003, **71**:1155-1160.
4. Fagan PK, Djordjevic SP, Eamens GJ, Chin J, Walker MJ: Molecular characterization of a ribonucleotide reductase (nrdF) gene fragment of *Mycoplasma hyopneumoniae* and assessment of the recombinant product as an experimental vaccine for enzootic pneumonia. *Infection and Immunity* 1996, **64**:1060-1064.

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5. Tavares LA, Gomes CK, Fisch A, Marchioro S, Jorge S, Oliveira NR, Peiter AC, Conceição FR, Dellagostin OA: **Design of synthetic gene for expression of a recombinant chimeric protein containing *Mycoplasma hyopneumoniae* antigens.** *Proceedings of the 59^o Congresso Brasileiro de Genética: 16-19 September 2013; Águas de Lindóia* .

doi:10.1186/1753-6561-8-S4-P247

Cite this article as: Gomes *et al.*: Cloning of a synthetic chimeric gene containing recombinant *Mycoplasma hyopneumoniae* antigens for expression in *Pichia pastoris*. *BMC Proceedings* 2014 **8**(Suppl 4):P247.

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