

Poster Presentation

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## Direct and indirect approaches for the improvement of heterologous proteins secretion levels in *Zygosaccharomyces bailii*

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from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology  
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

*Microbial Cell Factories* 2006, **5**(Suppl 1):P68 doi:10.1186/1475-2859-5-S1-P68

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### Background

In the market of biochemical products a very important role is played by heterologous proteins production, and despite recent advances in mammalian cells exploitation, yeasts can still present advantages as host systems. Among them, the spoilage yeasts belonging to the *Zygosaccharomyces* genus have become, due to some peculiar properties, significantly attractive. In particular, *Z. bailii* is characterized by acid resistance, osmotolerance to high sugar and ethanol concentration combined with high biomass yield. Despite still little is known about its genetics and cellular biology, our group is working on its development and exploitation for recombinant productions with an integrated approach coupling physiological study with the creation of molecular tools for heterologous proteins production. We previously described and developed two patent applications regarding the first techniques necessary to transform this yeast and to express and secrete different proteins derived from different sources.

### Results

Here we present and discuss the last data related to host optimisation. Two parallel strategies were followed, one exploiting the reproducible strategy for target gene deletion we developed, one exploiting a screening selection method. On one side, we obtained the *Zbgs1* mutant strain, thanks to the cloning and the subsequent disruption of the gene *ZbGAS1* (homologous to the *S. cerevisiae* *GAS1*) involved in cell wall biosynthesis. With this strain the production of a model heterologous protein results to be slightly but significantly improved. Also the indirect strategy, implying the selection of clones resistant to

orthovanadate, allowed to isolate mutants where heterologous protein production resulted improved in respect to the wild type strain.

### Conclusion

Here we showed that direct and/or indirect strain manipulation allowed to improve heterologous protein production in the yeast *Z. bailii*. Although confirms once more the potentiality of *Z. bailii*, further improvements and development of new molecular tools are necessary to assess if this yeast could be consider in the array of hosts for industrial productions.