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Characterization of the *Bgs13* protein's role in the super-secretion of recombinant peptides in the yeast Pichia pastoris

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Statement of the Problem: The yeast *Pichia pastoris* is a popular host for expressing and exporting recombinant proteins, such as human insulin and a hepatitis B vaccine protein, out of its cell. Secreted proteins are easier to purify and therefore are more useful than non-secreted proteins. However, *Pichia pastoris* has been known to secrete certain proteins efficiently while struggling to secrete others.

Approach & Methodology: Our lab has created a strain with a mutated *Bgs13* gene that is a super-secreter of multiple recombinant peptides. To understand why the Bgs13 strain displays enhanced secretion, cell wall assays were first performed using Congo red and Calcoflour white to determine if super-secretion is a result of defective cell walls. In addition, *Bgs13* appears to be a homolog of the Saccharomyces cerevisiae protein kinase C (PKC1). Thus, we tested if super-secretion in our *Bgs13* strain is a result of elevated or decreased protein kinase C activity compared to the wild type parent. Lastly, the localization of wild type *Bgs13* and mutant *Bgs13* proteins was compared by fusing each protein to EGFP and examining them with fluorescence microscopy analysis.

Results & Significance: The mutant *Bgs13* strain had a cell wall with apparent structural defects. Not only did the mutant *Bgs13* protein have lower protein kinase C activity, but it also was localized to different parts of the P. pastoris cell compared to the wild type *Bgs13* protein. By characterizing mutant and wild type *Bgs13* proteins, the results will help us create strains with optimized secretion of many different recombinant proteins.

Biography

Geoff Lin-Cereghino supervise a lab of undergraduates and Masters students at the University of the Pacific in Stockton, California. His work focusses on investigating the secretory mechanism of the yeast Pichia pastoris in order to improve the production of recombinant proteins in this host. Most recently, his research has concentrated on characterizing a group of super-secreting mutant strains isolated in his lab as well as optimizing the secretion efficiency of the α -mating factor prepro-peptide, which is the most commonly used secretion signal for heterologous protein expression in P.pastoris. He have been recipients of NIH AREA and NIH RUI grants.

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