Characterization of the \textit{Bgs13} protein’s role in the super-secretion of recombinant peptides in the yeast \textit{Pichia pastoris}

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\textbf{Statement of the Problem:} The yeast \textit{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, \textit{Pichia pastoris} has been known to secrete certain proteins efficiently while struggling to secrete others.

\textbf{Approach & Methodology:} Our lab has created a strain with a mutated \textit{Bgs13} gene that is a super-secretor of multiple recombinant peptides. To understand why the \textit{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, \textit{Bgs13} appears to be a homolog of the \textit{Saccharomyces cerevisiae} protein kinase C (PKC1). Thus, we tested if super-secretion in our \textit{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 \textit{Bgs13} and mutant \textit{Bgs13} proteins was compared by fusing each protein to EGFP and examining them with fluorescence microscopy analysis.

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

\textbf{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 \textit{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-secretating mutant strains isolated in his lab as well as optimizing the secretion efficiency of the \text{ alpha} mating factor prepro-peptide, which is the most commonly used secretion signal for heterologous protein expression in \textit{P. pastoris}. He have been recipients of NIH AREA and NIH RUI grants.  
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