CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
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Project Number
S1314

Project Title
The Effect of EtOH and Antimicrobial Peptide RP-1 on Apoptosis in Various Pathogenic Microbes

Abstract

Objectives/Goals
The purpose of this project was to elucidate mechanisms and stages of microbial apoptosis/programmed cell death, as well as to evaluate the effects of the antimicrobial agents ethanol and RP-1 on apoptosis on three prototypic microoorganisms: Staphylococcus aureus (gram + bacteria), Escherichia coli (Gram - bacteria), and Candida albicans (fungus).

Methods/Materials
A homology search for microbial homologues of human apoptotic proteins was performed using NCBI's BLAST engine. S. aureus, E. coli, and C. albicans were grown overnight to stationary phase, then subcultured to mid-logarithmic phase. Cells were then harvested, washed, and resuspended in buffer. The inoculum was quantified by spectrophotometry (420 nm and 600 nm) and diluted to 5 x 106 colony-forming units. Cells were then incubated with DiOC5, treated with either buffer, 70% ethanol, or 50 ug/ml RP-1 and were incubated at 37º with agitation for varying time periods of 0, 30, and 60 minutes. The fluorophores AnnexinV and propidium iodide were then added to the samples and samples were incubated for 30 minutes. Samples were then put through flow cytometry; fluorescence data was gathered on channels FL-1 through FL-4 using a Becton-Dickinson FACSCalibur cytometer and CellQuest software.

Results
The homology search on BLAST failed to find any significant microbial homologues (E values were all above 1). C. albicans showed a strong signal for cell membrane energy, even while a strong apoptotic signal was also being expressed. Overall, changes in permeability were rare; most change occured in terms of cell energy as cells lost their membrane energy. However, RP-1 actually hyperpolarized some cells.

Conclusions/Discussion
The homologue search's failure to find significant results does not mean that microbial homologues of human apoptotic proteins do not exist; a different method or search algorithm may discover such homologues. Also, contrary to expectations, ethanol induced a higher level of apoptosis than did RP-1 in all three organisms, and RP-1 induced a higher level of permeability than did ethanol in all three organisms. This may be due to the fact that EtOH permeabilized cells to the point of total lysis, causing the fluorphore signal to become less concentrated and appear weaker. Also, RP-1, which is slower-acting than EtOH, may not have expressed its full effect in just 30 minutes of incubation with the microorganisms.

Summary Statement
My project focuses on studying different aspects of apoptosis in microorganisms, using both bioinformatics and experimentation.

Help Received
Dad helped with board; worked in lab at Harbor-UCLA under the supervision of Dr. Yeaman