



CALIFORNIA STATE SCIENCE FAIR 2002 PROJECT SUMMARY

Name(s) Stephanie H. Lee	Project Number S1399
Project Title Genetic Manipulation of Rhizopus oryzae	
<p style="text-align: center;">Abstract</p> <p>Objectives/Goals Rhizopus oryzae is the most common organism isolated from patients infected with mucormycosis. The primary goal of this research project is to determine the genetic tools, needed to study the molecular mechanisms and virulence factors of R. oryzae infections by 1) establishing a growth curve and 2) developing a transformation system.</p> <p>Methods/Materials We first executed two experiments involving this organism's general development patterns. The first involved fungal growth on different medias and temperatures. The second involved comparing the inoculum size and the resulting diameter. We then used dry weight measurements of R. oryzae hyphae to establish a growth curve. We then tried to isolate auxotrophic or spontaneous URA- clones of R. oryzae with the use of 5-FOA but ultimately used chemical mutagenesis. The final part of this research involved transforming R. oryzae via electroporation and then extracting DNA from the transformants for a Southern Blot.</p> <p>Results R. oryzae had optimal growth at 30°C and grew best on YPD and least on YNB. The diameter of the growth was directly proportional to the inoculum size but reached a plateau around the concentration of 105 spores/ml. For the growth curve, R. oryzae developed at a steady rate until attaining a plateau at 24 hours. The doubling time is approximately 4.53 hours. Electroporation is a successful method of transformation. The Southern Blot, however, failed to provide us with any information because no signal appeared on the film.</p> <p>Conclusions/Discussion R. oryzae grew best at 30°C, the closest to human body temperature. YPD, the most nutritious medium, provided the most growth while YNB, the least nutritive, led to the least growth. The growth curve probably reached a plateau because of competition for space and food among the fungi. A spontaneous URA- clone was difficult to obtain because some clones were probably 5-FOA resistant. While, electroporation is a method of transformation, we do not know if it is the most efficient. The Southern Blot experiment probably failed due to the lack of selectivity of PDA. Some other possibilities are the spores' loss of the plasmid containing the cassette or a mutation restoring the URA gene. In the future, we will test the efficiency of electroporation, redo the Southern Blot experiment using a different media, and try an experiment involving enzyme digestion to pinpoint the exact location of cassette integration.</p>	
Summary Statement Due to the current dearth of studies on Rhizopus oryzae's pathogenesis, this project focused on developing genetic tools that are critical for the study of this fungus by specifically establishing a growth curve and transformation system.	
Help Received Used lab equipment at Harbor-UCLA REI under the supervision of Dr. Ibrahim	