APPENDIX

B

Sample Proposals

AN OUTSTANDING PROPOSAL

Isolation of Antibiotic-Producing *Streptomyces* from Soil

Streptomyces is a bacterial genus represented by over 500 species, a number of which are antibiotic producers. Some studies have reported that 50 percent of streptomycetes isolated are antibiotic producers. This genus is responsible for the manufacture of over 500 antibiotic substances (2), including chloramphenicol, erythromycin, neomycin, nystatin, streptomycin, and tetracycline (1, 3, 4), which play a major role in the treatment, control, and cure of human and animal diseases and which are used extensively for research (selectivity) in microbiological laboratories.

We have obtained a specimen isolated from pine soil that we believe is *Streptomyces* and have cross streaked it with *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* with excellent inhibition. This technique involves streaking one-third trypticase soy agar plate with the test organism and placing cross streaks of known bacterial species in close proximity to it. A clearing on the agar between the test and known bacteria indicates antibiotic production by the test organism.

The colony morphology, cell morphology, and earthy smell are all consistent with this organism. Also, a pathologist at Oregon State University examined the specimen under the microscope and stated that he believed it was *Streptomyces*.

Precise identification by DNA sequence analysis would tell us which strain we have isolated and consequently what antibiotics the strain generally produces. Chemical analysis is probably necessary to definitely identify these antibiotics. We request that funds be allotted for only the DNA sequence analysis at this time to help us narrow the range of possible antibiotics this organism is generating.

References

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A POOR PROPOSAL

Polution: Man made? Natural? A combination of both?

Who's t say that polution in todays world is a result of mankind. The possibility of naturally occuring polution I believe is out there. As can be seen with the human population, species population explossion is possible for any species. With the great increase of a population, be it humans, plants, or microorganisms, such as bacteria, the ecosystem in which they reside in is put to the limits of support. So, is todays polution problem strickly due to mankind? Or could it be a result of nature and mankind working together? If they are working together to create the polution, who's to say that they can not work together to clean it up.

The two environmental samples that were taken may in a way help to answer these questions, by way of a polymerase chain reaction (PCR) of the 16S rRNA of both samples. If any environmental influence, e it natural or man made, have caused chemical alterations, of any sort, it will be detecable at the variable region or V2 site. If the chemical alterations are found to have some degree of homology in bothe V2 sites then some factor that is the same for both samples has influance a mutation in the RNA sequance. The samples were taken from similar ecological nitches, aquatic, which contained very different ecosystems. The first sample was taken from a rock pit pool. The pool is located in solid rock and is stagnent unless it is raining, then naturally occring springs and rain water disterb it, as do animals that use it. With the spring water silt, clay is added along with organic matter from surrounding grass, trees, and brush. The pool was selected on the bases that humans were in no way involved in the ecosystem that it support or that the pool is apart of. The second sample was taken from a man made pond in an industrial park area. The only water source for the pond is rain water and rain run off from surrounding areas. The pond is located on top of a mud, sand, and silt bottom and has various plant forms growing within along with fish. The ecosystem that the pond supports is in constent interaction with industrial by-products and humans as is the ecosystem that the pond is a apart of.

Perliminary tests, such as TNA plate cultivation and gram stain analysis has identified some unique characteristics of the microbes that were collected. Both plates cultured presented with very few and isolated colonies when incubated at 37 degrees celsius. Both sets of colonies showed small pin point colonies to about 2 mm in diameter. The colonies that developed from the rock pit pool were visibly a cloudy white in color with some clear spots contain within. The colonies from the pond showed a yellowish colored colonies with a slight pink tint to them. These colonies also presented a distinc odor when the plate lid was lifted. Microscopic analysis of the the gram stained cultures showd great similarity between to two cultures. Both cultures were gram positive bacillus with dipolar red spots at each end. The cells from the pond were in collections forming chains and also individual cells. The rock pit cells were only presented as individual cells. For pure culture isolation the two samples were inoculated to nutrient broth and incubated. The culture from the pond was placed at 37 degrees celsius and the rock pit culture was placed at 30 degrees celsius. The rock pit sample was initialy grown at 37 degree celsius and due to decreased growth and the cool environment that it was isolated from it was decided to

place this culture at a lower temperature for further incubation in hopes of collecting a greater quantity of cells.

Because the samples were taken from two completely different ecosystems, with different human interaction levels, any homology in the V2 site on the 16S rRNA could be due to polution. With the source of any polution being different for both samples, the homology would suggest that yes polution could be man made, natural, or even a combination of both.

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