Identification of Actinomycetes, Antibiotic Producing Bacteria, Using rRNA Gene Restriction Fragment Patterns

Background and Significance

Many of the antibiotics used today are originally chemicals produced by bacteria to kill competing bacterial species in natural environments. Misuse and overuse of antibiotics has led to a rapid evolution of antibiotic resistance of many pathogens. This evolution of resistance has also led to older antibiotics becoming ineffective.

The genus of bacteria in question produces approximately 85% of the antibiotics used in the medical field today. These bacteria are widely distributed in terrestrial environments and have long been a source of commercially useful medicinal molecules. Due to the fact this genus encompasses a large number of species, the task of classifying the bacteria becomes increasingly difficult and furthermore makes it difficult to pinpoint the more important species. The ability to identify these species will provide a pathway for further research as well as potential sources for new antibiotic production.

Methods

The identification of new bacterial species that produce antibiotics can be separated into a few different pathways. It is first important to identify the unknown candidates that have been obtained from terrestrial environments in order to discern if they are known bacterial species which produce antibacterial compounds or if they are novel candidates awaiting discovery.

The methodology behind our approach to identification of these novel candidates is molecular genetic analysis. The 16S rRNA gene is common within this species of bacteria being examined. The restriction sequence will be compared to the DNA of a known species. Comparison of the DNA will allow us to directly identify antibiotics that are produced and to then determine if the antibiotics that are identified in the lab are of any significance. This specific gene that is common to all actinobacteria is then amplified using polymerase chain reaction (PCR). PCR requires gene specific primers (commercially synthesized DNA molecule) and an enzyme to amplify the gene of interest (Taq DNA polymerase). The process produces billions of copies of the target gene for further analysis. Restriction enzymes and gel electrophoresis are used to then identify fragment patterns in the gene.

The expected results from this analysis will allow for a more rapid identification of unknown actinomycetes to the genus level. The results are then compared to published databases containing gene sequences of actinomycetes. The comparison will provide the information needed to determine if the candidate is already identified.

Results

It is difficult to differentiate these genera (and species) of bacteria based on morphological differences; other classification methods require several tests which are time consuming and produce several discrepancies. The expected results from this molecular genetic analysis will allow for a rapid method for identifying unknown actinomycetes to the genus level.

PCR was performed on 10 different species of streptomycetes (Streptomyces griseus) as well as on Escherichia coli. F1 and R5 primers (modified from F1 and R2 respectively, of Weisburg, et al.) were used to cleave the 1500 base pair sequence which is a common product of a gene that is highly conserved. Gel electrophoresis was then run to show the anticipated 1500 base pair fragment. A 1500 base pair fragment was produced from S. griseus and E. coli. Furthermore, this sequence is highly conserved. Restriction enzymes Sau3AI, Sph I, and Pst I were then used to differentiate between candidate #28, S. griseus, E. coli and pUC19, which will produce different sized base pair fragments.

The results illustrate that the restriction endonucleases cleaved fragments of each of the four different sequences respectively. The gel electrophoresis was successful in producing bands where they were presumed to be, and the controls were effective in exhibiting proficiency of techniques utilized. Gel #1 and part of Gel #2 shows different concentrations of PCR product used to obtain the best results when performing the Sau3AI digest. pUC19 was used to determine whether the PCR product was interfering with the digest. The remaining portion of Gel #2 displays the sequences being digested with Pst I as well as PCR controls. Finally, Gel #3 shows the Sph I digest, where a "partial digest" can be observed (due to perhaps not enough enzyme or an inadequate digestion time).

Future Plans

Now we can do further experimentation on the oxidative negative candidates. Currently in process are the following experiments: Glucose Fermentation, Urease Test and Motility Test. P. fluorescens and P. aeruginosa should come back as negative for the glucose fermentation and the urease test. Both should show motility because both have polar flagella.

Experiments in the future depend on the conclusion of the glucose and the urease test. If the results are negative, there is a very good chance that the bacteria are not P. aeruginosa or P. fluorescens. However, if the results are positive then there is a very good chance that the bacteria are P. aeruginosa or P. fluorescens. Future plans are in the works and hopefully we can find one that is negative bacteria that can someday produce an antibiotic to improve human infectious disease chemotherapy.