A NEW APPROACH TO CREATING PRADIMICIN-TYPE ANTIFUNGAL/ANTIVIRAL COMPOUNDS

Thomas Anderson, Whitney Morgan
Utah State University

Dr. Jon Takemoto, Dr. Jixun Zhan
Utah State University

I. Introduction
There is currently a rising demand for antifungal therapeutics with clinical efficacy against systemic fungal infections, especially in patients with compromised immune systems. Pradimicins A, B, and C, compounds produced by the soil bacterium Actinomadura hibisca, have been shown to have broad spectrum fungicidal activity and represent promising candidates for new anti-fungal antibiotics. Furthermore, pradimicin A has been shown to have antiviral properties, inhibiting both influenza and HIV. This is significant because AIDS patients infected with HIV frequently suffer from fungal infections as a result of immuno-deficiency. Pradimicin could potentially be effective in treating both conditions.

II. Methods
Several enzymes, and their corresponding genes, have been identified in the biosynthetic pathway of pradimicin. In this study, we sought to determine the function of one of these enzymes, PdmS.

Five plasmids were constructed which contained non-characterized genes, pdmS, pdmO, pdmT, pdmO, and pdmF. A targeted gene knockout of the pdmS gene was performed by PCR with primers designed to flank the gene. An intergeneric conjugation using E. coli was carried out to introduce the recombinant plasmid into A. hibisca. Both strains were co-cultivated to trigger a crossover recombination and incorporation of the recombinant DNA, with the partially deleted gene, into the genomic DNA of A. hibisca. The products produced by the mutant A. hibisca cultures were analyzed by liquid chromatography (HPLC) and mass spectroscopy, and the recombinant pdmS gene was amplified and sequenced.

III. Results
Mutant strains of A. hibisca in antibiotic free media produced a compound with a different HPLC retention time than regular pradimicin. Mass spectroscopy of the new molecule showed that it had a molecular weight of 549.48, lower than pradimicin (838), and NMR analysis revealed it to be the pradimicin aglycon, a version of the molecule lacking the normal sugar attachments. This demonstrated that PdmS works as a glycosyltransferase. The extraction of pdmS gene from A. hibisca mutant strains confirmed the deletion of the glycosyltransferase.

Gel electrophoresis results showing the extraction of pdmS gene from A. hibisca mutant strain genomic DNA compared to the wild type.

IV. Discussion
The solubility and antifungal properties of pradimicin are both related to its sugar attachments. These results demonstrate that Pdms is a glycosyltransferase, and the enzyme which attaches the first sugar in pradimicin biosynthesis. With this information, the aglycon form of pradimicin can be produced and used as a start molecule for structural modifications to improve solubility and activity. Furthermore, exogenous enzymes similar to Pdms might be used to change the type of sugar which is attached and potentially enhance the properties of pradimicin.

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