ABSTRACT

Polyketide synthase (PKS) pathway modifications offer a limitless diversity of products. Soil microbes such as the Acidobacteria phylum are physiologically diverse; however, their vast metabolic potential does not allow them to be easily cultured in vitro. These bacteria have been found to be rich in polyketide production and are a desired focus for polyketide discovery, specifically antibiotics compounds. Environmental DNA (eDNA) offers a new route to examine these PKS pathways and assay their antibiotic activity, as well as their potential for antitumor and antiviral properties. eDNA transformed into Escherichia coli contain a sequence of genes to be tested for antibiotic activity. Those samples are plated against the gram-positive Bacillus subtilis, in both liquid and solid media, to determine their antibiotic activity.

The efficacy of these natural products decreases when the target bacteria develop resistance to their effects. Chimeric compounds, utilizing the deoxysugars, loading domains, and PKS pathways of other compound production mechanisms offer an untapped source of novel antibiotics, for which no bacterial resistance has yet to be seen. Erythromycin, a highly-effective antibiotic, has faced resistance from a multitude of prevalent bacteria today. Its synthesis consists of iterative dehydrating reactions from the erythromycin precursor, 6-deoxyerythronolide B (6-DeE). This then undergoes post-translational modification with the addition of two-deoxysugar groups and methyl-hydroxylation. Through the genomic modification of the loading domain and diversifying which molecules the synthesis begins with as well as the sugar groups that are added onto the molecule, novel compounds are formed that can exhibit greater antibiotic activity than erythromycin.

INTRODUCTION

The goal of this study is dual-fold; to determine the protocol necessary to produce a successful titre of 6-DeE with structural modifications, and to develop a screening protocol for complex new natural product discovery. The primary specific aim of the study is the discovery of a novel class of antibiotics (A), while the secondary specific project of the aim is the discovery of a novel antibiotic from an existing class (B). In its current state, novel antibiotics are being discovered at a rate of approximately 2-3 per decade, while bacterial species are becoming resistant to existing antibiotics at an even faster rate. With the exponential increase in antibiotic resistant bacteria, it is crucial to develop a method of rapid novel drug discovery through high-throughput eDNA scanning, as well as through genetic modifications. eDNA scanning results in the discovery of not only novel forms of antibiotics, but perhaps a novel class of antibiotics, of which none have been discovered since 1987 (ansamycins). Our supplier of eDNA, Lucigen, has spiked the collection of eDNA from soil microbes into bacterial artificial chromosomes (BACs) that were each identified as having a PKS pathway, into E. coli, resulting in 53 samples. E. coli cells transformed with the BACs were tested on their antibiotic activity against Bacillus subtilis in solid and liquid media, as well as extraction of cellular proteins onto a filter disk assay. E. coli is utilized due to its expansive manipulability as a heterologous host for those genes (as compared to the native fastidious host of the genes); as a gram-negative bacteria, it is generally more resistant to antibiotics, and is therefore a suitable host for their production, while B. subtilis is a gram-positive bacteria, and is more susceptible to infection and therefore more likely to react to an antibiotic produced by the E. coli sample. The filter disk assay is crucial if the E. coli are killed by the antibiotic they produce, thus resulting in a low efficacy against the B. subtilis, while maintaining a high native potency. The eDNA screening results of 53 samples in 4 different hosts, each with a slightly altered genetic makeup to facilitate the production of the different forms of PKS pathways. As the current study is proceeding, 6 out of the available 31 BACs show promise towards antibiotic activity.

ERYTHROMYCIN SYNTHESIS

PIKROMYCIN SYNTHESIS

DEOXYSUGAR VARIANCE

SCREENING PROCESS

CONTINUED RESEARCH

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