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Extended Abstract

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Comparative interactomic analysis to decipher the role of genes involved in rifamycin production in genetically modified Amycolatopsis mediterranei S699

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The development of rifampicin-resistance strains of Mycobacterium tuberculosis (Mtb) has resulted in imperative need for development of analogs of rifamycin. The endless efforts by genetic manipulation of rifamycin biosynthetic gene cluster of Amycolatopsis mediterranei S699 has led to discovery of a new anti-tuberculosis drug, 24-desmethylrifamycin B, more effective against rifampicinresistance strains of Mtb. The mutant strain A. mediterranei DCO#34 had undergone substitution of acyltransferase domain of module 6 of rifamycin polyketide synthase with that of module 2 of rapamycin polyketide synthase. Genetic manipulation resulted in reduced yield of analog ~20mg/litre as compared to 50mg/l yield by the wild type strain. In order to decipher the impact of domain swapping on rifamycin/analog production, and the intricate make-up and clinical importance of rifamycin brought up the idea to study the regulation of rifamycin biosynthesis, stateof- the-art protein expression methodologies were carried out. Whole cell proteins were extracted and digested with trypsin. The resulting peptides were analysed by nLC-MS/ MS (Thermo Scientific Q Exactive HF Mass Spectrometer in conjunction with Dionex Ultimate 3000 UPLC). The emerging technique of protein-protein interaction approach was employed to determine the relation and interaction between various structural and regulatory genes involved in Rifamycin biosynthetic gene cluster. Comparing the relative abundance expression values for the wild type and mutant strain revealed the altered expression of structural genes, rifC-I (down-regulated), rifR, rifZ and other regulatory genes (up-regulated), that might have resulted from modified rifamycin polyketide backbone by domain swapping. Reduced yield of analog by the mutant strain is the outcome of subsequent down-regulation of structural genes due to absence of rifP transport gene. The repressed rifamycin transportation outside the cell further activated negative feedback mechanism. Complete protein profile of the rifamycin B producer A. mediterranei S699 revealed intricate mechanisms of rifamycin biosynthesis and its regulation. Rifamycin B is produced by Amycolatopsis mediterranei S699 as a secondary metabolite. Its semi-synthetic derivatives have been used for curing tuberculosis caused by Mycobacterium tuberculosis. But the emergence of rifampicin-resistant strains required analogs of rifamycin B to be developed by rifamycin biosynthetic gene cluster manipulation. In 2014 genetic engineering of the rifamycin polyketide synthase gene cluster in S699 led to a mutant, A. mediterranei DCO#34, that produced 24-desmethylrifamycin B. Unfortunately, the productivity was strongly reduced to 20 mgL-1 as compared to 50 mgL-1 of rifamycin B. To understand the mechanisms leading to reduced productivity and rifamycin biosynthesis by A. mediterranei S699 during the early and late growth phase we performed a proteome study for wild type strain S699, mutant DCO#34, and the non-producer strain SCO2-2. Proteins identification and relative label-free quantification were performed by nLC-MS/MS. Data are available via ProteomeXchange with identifier PXD016416. Also, in-silico protein-protein interaction approach was used to determine the relationship between different structural and regulatory proteins involved in rifamycin biosynthesis. Our studies revealed RifA, RifK, RifL, Rif-Orf19 as the major regulatory hubs. Relative abundance expression values revealed that genes encoding RifC-RifI and the transporter RifP, down-regulated in DCO#34 and genes encoding RifR, RifZ, other regulatory proteins up-regulated. SIGNIFICANCE: The study is designed mainly to understand the underlying mechanisms of rifamycin biosynthesis in Amycolatopsis mediterranei. This resulted in the identification of regulatory hubs which play a crucial role in regulating secondary metabolism. It elucidates the complex mechanism of secondary metabolite biosynthesis and their conversion and extracellular transportation in temporal correlation with the different growth phases. The study also elucidated the mechanisms leading to reduced production of analog, 24-desmethylrifamycin B by the genetically modified strain DCO#34, derivatives of which have been found effective against rifampicin-resistant strains of Mycobacterium tuberculosis. These results can be useful while carrying out genetic manipulations to improve the strains of Amycolatopsis to produce better analogs/drugs and promote the eradication of TB. Thus, this study is contributing significantly to the growing knowledge in the field of the crucial drug, rifamycin B biosynthesis by an economically important bacterium Amycolatopsis mediterranei. The ansamycin class of antibiotics are produced by various Actinomycetes. Their carbon framework arises from the polyketide pathway via a polyketide synthase (PKS) that uses an unusual starter unit. Rifamycin (rif), produced by Amycolatopsis mediterranei, is the archetype ansamycin and it is medically important. Although its basic precursors (3-amino-5-hydroxy benzoic acid AHBA, and acetic and propionic acids) had been established, and several biosynthetic intermediates had been identified, very little was known about the origin of AHBA nor had the PKS and the various genes and enzymes that modify the initial intermediate been characterized.

Bottom Note: *This work is partly presented at* International Conference on BIOINFORMATICS & SYSTEM BIOLOGY
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