

# 3<sup>rd</sup> Global Microbiologists Annual Meeting

August 15-17, 2016 Portland, Oregon, USA

## Manipulation of rifamycin polyketide synthase gene cluster of *Amycolatopsis mediterranei* to produce a novel analog, 24-desmethylrifamycin B effective against MDR strains of *Mycobacterium tuberculosis*

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Rifamycin B is produced by an actinobacterium *Amycolatopsis mediterranei* S699. Semisynthetic derivatives of rifamycin B are in use for curing tuberculosis caused by *Mycobacterium tuberculosis*. Almost all these semi-synthetic derivatives have been produced by modification of naphthaquinone ring of rifamycin B. However further modifications of rifamycin by using chemical methods to produce clinically effective analogs were not possible. The other alternative available was to modify the polyketide ansa chain of rifamycin B. This was achieved by manipulating rifamycin polyketide synthase gene cluster (*rifPKS*) to produce analogues of rifamycin B. However this was also not very feasible due to the non-availability of transformation system and cloning vectors for the producer strain *Amycolatopsis mediterranei* as well as the recalcitrance of the organism to genetic manipulations. Overcoming these difficulties stepwise that includes developing cloning vectors for the genus *Amycolatopsis*, standardization of transformation techniques led to the development of a proof of concept and a rifamycin B analogue: 24-desmethylrifamycin B after a concerted effort of 25. The efforts led to the swapping of acyltransferase (AT) domain of the sixth module (AT6) of rifamycin polyketide synthase (which adds propionate unit to the growing polyketide chain) with that of AT domain of the second module (AT2) of rapamycin PKS (*rapPKS*) (which adds acetate unit) in *Amycolatopsis mediterranei* S699. The resulting mutant produced rifamycin derivative 24-desmethylrifamycin B which lacked a pendant methyl group at C-33 of the rifamycin skeletal structure, as predicted. It was confirmed using NMR and LC-MS studies. The novel analog was further converted to 24-desmethylrifamycin S & 24-desmethylrifampicin, which were found to have a better antibacterial activity than rifamycin B against *Staphylococcus aureus*, *Mycobacterium smegmatis*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*. These findings eventually required testing of 24-desmethylrifamycin S and 24-desmethylrifampicin against MDR strains of *Mycobacterium tuberculosis*. Therefore, for this study, the activity of 24-desmethylrifamycin was tested against three rifampicin resistant strains. Here it is pertinent to mention that rifamycin resistance is associated with genetic alterations in an 81-bp region of the *rpoB* gene encoding the DNA-dependent RNAP  $\beta$ -subunit. The rifampicin resistant strains were obtained from OSDD. Thus we used rifampicin-resistant *M. tuberculosis* strains, OSDD 321 and OSDD 206 (S531L) and OSDD 55 (H526T) mutation in their RNAP  $\beta$ -subunit. The results indicated that the two derivatives of 24-desmethylrifamycin B; 24-desmethylrifamycin S and 24-desmethylrifampicin were 10 to 50 times more effective than the already available rifampicin against MDR strains of *M. tuberculosis*. This study has been taken as a cornerstone for further genetic manipulations and producing large number of rifamycin analogs for biological and pharmaceutical applications.

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