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Title: **Screening of cyanobacterial strains for production of biofuel candidates and genetic characterization of its metabolic pathway**

The increasing demand for energy driven by a rapid increase in the population and economic growth has resulted in excessive use of the available fuel resources coupled with drastic climate changes. In order to address these concerns various inventive approaches have been explored for the production of renewable and sustainable fuels. Numerous efforts towards the application and development of next generations of biofuels are in progress. Bioethanol and biodiesel are the two major biofuels which are currently being generated for use in transportation at a commercial level. However various shortcomings associated with the use of these fuels like difficulties in transport with the current infrastructure due to the tendency of biodiesel to form wax at low temperatures and the low energy content of ethanol, renders technical limitations to the use of these fuels. In this context the concept of 'drop-in' biofuels having a high energy content and which can be readily dropped into the existing infrastructure without the need for any engine modifications, has gained tremendous attention. The discovery of native pathways for alkane biosynthesis has paved the way for microbial engineering of various organisms to produce these drop in biofuels. Alkanes are known to be produced in various prokaryotic and eukaryotic organisms, with the most consistent reports being from cyanobacteria and natural habitats dominated by cyanobacteria. With the advent of various synthetic biology tools involving rational design and engineering of different microbes, rapid progress has been made in the microbial production of a wide range of biofuel compounds including alkanes. Alk(a/e)nes are known to be produced in various prokaryotic and eukaryotic organisms, with the most consistent reports being from cyanobacteria. Two pathways for alkane biosynthesis are known to exist in cyanobacteria, AAR/ADO pathway and PKS pathway. This study focuses on the AAR/ADO pathway which involves the production of alkanes from fatty acid intermediates by the action of two enzymes AAR and ADO. Various analogs of the AAR enzyme are known to exist which produce aldehydes through different routes. The aldehydes produced by these routes can be coupled to ADO of the AAR/ADO pathway to produce alkanes thus rendering the reaction catalysed by the ADO enzyme a highly unique process. Considering the potential applications of alk(a/e)nes as biofuel candidates this study involves the screening of different cyanobacterial

strains for the production of alk(a/e)nes and the identification of the pathways involved in the formation of these products. Further, the ADO enzyme of the AAR/ADO pathway identified in the alkane producers was explored for its operational stability at increased temperatures. To improve the stability of the ADO enzymes reported to be comparatively more efficient, different computational and screening approaches were employed for generation of thermostable ADOs. In this study firstly, 50 cyanobacterial strains from diverse habitats (21 freshwater and 29 marine isolates) were screened for the production of alkanes. Of these, *Oscillatoria* strains were found to be the highest producers of alkanes in both the freshwater and marine categories. It was observed that the fresh water strains primarily produced heptadecane while the marine strains produced pentadecane, a unique observation that has never been reported. In order to investigate the impact of different factors on the alkane chain length, a cross media test was performed and GC-MS/MS analysis revealed that the freshwater strains predominantly continued to produce heptadecane while the marine strains produced pentadecane, akin to the trend observed with standard cultivation medium. Analysis of the fatty acid content of the alkane-producing strains showed that the marine strains consistently produced higher amount of C16 chain length fatty acid, while the relative proportion of C18 chain length fatty acids increased significantly in freshwater strains as compared to marine. Phylogenetic analysis of the alkane producers was performed which again indicated differences in the branching pattern linked to the strain habitat and alkane chain length produced. Genome sequencing of the strains, *Oscillatoria* CCC305, *Oscillatoria* BDU50131, *Phormidium* CCC495, *Phormidium* sp. BDUN661 was carried out. Analysis of sequenced genomes indicated the presence of AAR/ADO pathway in all of the sequenced genomes. Owing to the unique nature and the irreplaceable reaction mechanism catalysed by the ADO enzyme, this enzyme was taken up for further analysis. The de-novo functionality of all the ADOs (from *Oscillatoria* CCC305, *Oscillatoria* BDU50131, *Phormidium* CCC495, and *Phormidium* sp. BDUN661) was tested in *E. coli* DH5α. In the quest to identify thermostable ADO enzymes functional at temperature beyond 50 °C, we designed a pipeline to construct a consensus thermostable ADO using hot spring metagenome libraries of cyanobacterial strains whose activity was tested and the role of different residues contributing to the increased activity was analysed using computational methods and further validated by experimental studies. Thus from the above findings it can be concluded that the thermostable ADO generated might prove to be highly useful in biorefineries where enzymes having longer operational stability offer robust catalyst alternatives capable of withstanding comparatively stringent environments of industrial processing.