World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:10, No:02, 2016

De Novo Assembly and Characterization of the Transcriptome from the Fluoroacetate Producing Plant, Dichapetalum Cymosum

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Abstract : Organically bound fluorine (C-F bond) is extremely rare in nature. Despite this, the first fluorinated secondary metabolite, fluoroacetate, was isolated from the plant Dichapetalum cymosum (commonly known as Gifblaar). However, the enzyme responsible for fluorination (fluorinase) in Gifblaar was never isolated and very little progress has been achieved in understanding this process in higher plants. Fluorinated compounds have vast applications in the pharmaceutical, agrochemical and fine chemicals industries. Consequently, an enzyme capable of catalysing a C-F bond has great potential as a biocatalyst in the industry considering that the field of fluorination is virtually synthetic. As with any biocatalyst, a range of these enzymes are required. Therefore, it is imperative to expand the exploration for novel fluorinases. This study aimed to gain molecular insights into secondary metabolite biosynthesis in Gifblaar using a high-throughput sequencing-based approach. Mechanical wounding studies were performed using Gifblaar leaf tissue in order to induce expression of the fluorinase. The transcriptome of the wounded and unwounded plant was then sequenced on the Illumina HiSeq platform. A total of 26.4 million short sequence reads were assembled into 77 845 transcripts using Trinity. Overall, 68.6 % of transcripts were annotated with gene identities using public databases (SwissProt, TrEMBL, GO, COG, Pfam, EC) with an E-value threshold of 1E-05. Sequences exhibited the greatest homology to the model plant, Arabidopsis thaliana (27 %). A total of 244 annotated transcripts were found to be differentially expressed between the wounded and unwounded plant. In addition, secondary metabolic pathways present in Gifblaar were successfully reconstructed using Pathway tools. Due to lack of genetic information for plant fluorinases, a transcript failed to be annotated as a fluorinating enzyme. Thus, a local database containing the 5 existing bacterial fluorinases was created. Fifteen transcripts having homology to partial regions of existing fluorinases were found. In efforts to obtain the full coding sequence of the Gifblaar fluorinase, primers were designed targeting the regions of homology and genome walking will be performed to amplify the unknown regions. This is the first genetic data available for Gifblaar. It has provided novel insights into the mechanisms of metabolite biosynthesis and will allow for the discovery of the first eukaryotic fluorinase.

Keywords : biocatalyst, fluorinase, gifblaar, transcriptome
Conference Title : ICB 2016 : International Conference on Biocatalysis
Conference Location : Barcelona, Spain
Conference Dates : February 15-16, 2016