DNA sequencing and partial characterization of a metagenomic clone producing a brown pigment.

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Metagenomics allows direct access to the environmental microbiota genetic diversity without using cultivation media. A metagenomic library was constructed using DNA soil samples from Brazilian Atlantic Forest, and screened for lipase/esterase activity in 1% tributyrin containing LB medium allowing isolation of the clone MAF1125F06 which produces a brown pigment. To characterize the MAF1125F06 clone, fosmidial DNA was purified, fragmented (3 and 8 kb) and subcloned into pUC19 vector producing a sub-library for shotgun DNA sequencing. By using the Phred-Phrap-Consed package, 576 reads were assembled in 5 contigs. The MAF1125F06 clone has a 27 kb insert which codes for 32 potential ORFs as identified using Artemis software. To cover DNA sequencing gaps, transposon reaction was performed in four clones from the pUC19 DNA library. Two genes coding for a fumarylacetoacetate and a homogentisate 1,2-dioxygenase, involved in the tyrosin catabolic pathway, are present in the MAF1125F06, that could be related to the production of the brown colored product. This compound was partially purified and preliminary infrared analysis indicated that the brown colored could be indeed related to homogentisate metabolism.

Word Keys: Metagenomics, bioprospecting, brown compound

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