CLONING AND EXPRESSION OF LIPASE AS A BIOCATALYST FOR BIODIESEL PRODUCTION

Project Reference No.: *45S_BE_1534*

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Keywords:

Lipase, catalyst, purification, molecular characterization, biodiesel, sequencing, cloning.

Introduction:

Biocatalysts-based biotechnological applications are receiving immense attention, Lipases are the hydrolases group of enzymes which catalyzes the hydrolysis of triglycerides to glycerol and free fatty acids. Enzymatic production of biodiesel is more attractive than chemical synthesis of biodiesel because, of its ability to yield high-quality products, simplify the separation of products in the process, mild reaction conditions, the reuse of the catalyst and resist environmental impacts (Kareem *et al.,2017*). For these reasons, researchers have attempted to locate and identify other more suitable lipase enzymes. The most suitable enzyme in this regard must possess not only the ability to produce biodiesel efficiently using oil (K S Yang *et al.,2021*) but also the ability to utilize all mono, di, and triglycerides, both esterification and transesterification, low product inhibition, high activity and yield in nonaqueous media, low reaction time, temperature and alcohol stability, reusability of the immobilized enzyme and so forth. The main industrial application of lipases is in the hydrolysis of fats and oils, although their use in the transesterification of oils for the synthesis of biodiesel is increasing. Lipases are produced from different bacterial species of plants, animals, and microorganisms. They usually exist in different environments like a compost heap, decaying food, dairies, and oil waste (Bharathi *et al.,)*. The oil-rich environment with organic substrates furnished a suitable habitat for the isolation of lipase-producing bacteria. Commercially useful lipases are usually obtained from microorganisms that produce a wide variety of extracellular lipase. Previously the lipase was isolated from *Lysinibacillus macroides* FS1 from the oil-rich samples, screening of lipases producing bacteria and its media optimization to facilitate enhanced lipase production (Shilpa jigajinni *et al.,)* the major drawback for lipase as biocatalyst in biodiesel production for high activity lipase. The current research is intended to enhance the activity of lipase by expressing the gene responsible for the transesterification identifying the gene sequence of the enzyme lipase responsible for the transesterification, cloning the gene and expressing it into a suitable vector and producing biodiesel of high yield.

Objectives:

1. *Molecular characterization of lipase:* Identifying the molecular weight of the protein responsible for the production of biodiesel by using SDS-PAGE (polyacrylamide gel electrophoresis).
2. *To identify the gene sequence responsible for lipase from Lysinibacillus macroides FS1 by BLAST (Basic Local Alignment Search tool):* Based on phenotypic characteristics the particular gene sequence is identified.
3. *Cloning and expression of the lipase-producing gene in a suitable host:* The gene of interest is inserted into a plasmid vector that contains protein expression and the gene is expressed.
4. *Biodiesel production using lipase:* Biodiesel production from cloned and expressed lipase-producing genes.

Materials and methodology:

Materials:

1. TBA media
2. Inoculum of *Lysinibacillus macroides FS1*
3. Production media
4. Ammonium sulphate salt
5. SDS-PAGE (polyacrylamide gel electrophoresis): Resolving gel (12%), stacking gel (12%), Running buffer (pH 8), Staining buffer (Stain used Coomassie Brilliant Blue), Destaining buffer (1:1 ratio of water and methanol and 10ml acetone)

Methods:

1. Production of lipase from *Lysinibacillus macroides* FS1 (from previously isolated) in optimized media.

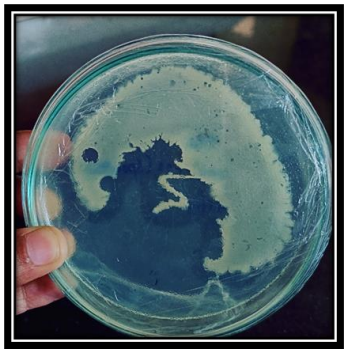

Figure 1: Culture plate containing Lysinibacillus macroides FS1 showing zone clearance in optimized TBA media.

Figure: 2) submerged Fermented production media consists of bacteria (*Lysinibacillus macroides FS)* producing lipase. 3) crude lipase obtained after centrifugation

2. Fermented media is centrifuged at 10,000 rpm and a temperature of about 4° C and the supernatant is collected and stored for protein purification.
3. Purification of produced lipase (collected supernatant) by ammonium sulphate fractionation.

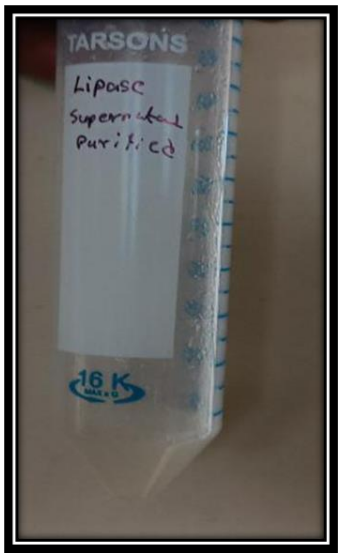

Figure 4: Ammonium salt precipitated purified lipase

4. Identification of Molecular weight of lipase by SDS page using standard markers.
5. Identification of a gene, based on phenotypic characteristics which are responsible for lipase production in bacteria by using BLAST.
6. Cloning and expression of an identified gene sequence in a suitable host where the gene of interest is inserted into a plasmid vector and the activity of the protein are identified.
7. Immobilization of the recombinant gene expressed protein which in turn is used for biodiesel production.

Results and conclusion:

Production of lipase from *Lysinibacillus macroides* FS1 was carried out in optimized media by submerged fermentation as mentioned in the methodology Qualitative analysis (lipase assay): Lipase activity of all the samples was measured by a titrimetric method using olive as substrate, this method gives satisfactory results. Culture supernatant was collected and lipase activity of 14.1U/ml was obtained. The lipase produced was subjected to partial purification using ammonium sulphate fractionation, the lipase was precipitated at 50% of saturation at pH 7.

Table 1: Partial purification of Lipase from Lysinibacillus macroides FS1

Purification steps	Total protein (mg)	Total activity (U/ml)	Specific activity (U/mg)	Purification Fold	Yield (%)
Crude lipase	0.6 mg	27.91 U/ml	2.31 U/mg	1.6	166.66
Ammonium sulphate (50%)	20 mg	14.16 U/ml	35.41 U/mg	25.46	84.56

The purification fold of purified Lipase is 25.46, the specific activity of 35.41U/mg and the yield of 85.56%.

Characterization of Lipase:

1. The purified lipase was subjected to qualitative analysis by Ninhydrin and Biuret test wherein, the test resulted positive which confirms the presence of Lipoproteins.
2. Quantitative estimation of protein was done by Lowry's method using BSA as a standard, protein content of purified lipase was 107.65mg/ml whereas, partially purified lipase resulted in 90.465mg/ml.
3. SDS-PAGE (molecular weight identification): There are different active proteins of Lipase shown up in polyacrylamide gel.

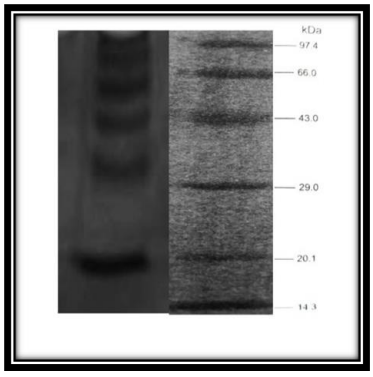

Figure 5: Bands which represents the molecular weight of the various active protein of lipase

Scope for future work:

Lipase being the extreme superior catalyst in the enzymatic production of biodiesel, lipase produced from the bacteria has many advantages because they participate in catalyzing transesterification of honge oil with ease and in the least time possible.

Immobilisation of the enzyme isolated from the bacteria *Lysinibacillus macroides FS1,* and optimisation of the production media were standardised, the current research was intended to analyse and isolate the active proteins present in the enzyme lipase which is actively responsible for the biodiesel production. The research is proceeding in a way where the active proteins are analysed and sequenced to study the compatibility of the proteins with a suitable host by rDNA and in silico studies. Future work can be expected to over-express the enzymes in a cloned vector and to use it in the production of biodiesel, to enhance the activity of the enzyme by immobilization and optimising the parameters that are crucially considered in the process of biodiesel production. Currently, the free lipase enzyme that is isolated from the bacterial *Lysinibacillus macroides* FS1 is methanol tolerant to some extent. Research has to be done on the stability of the cloned lipase. And to enhance its recovery rate, and reusability, and activate it in different environmental conditions to make efficient utilisation of the enzyme in overall bioprocesses which ultimately battle the problems associated with free enzyme immobilisation. In future innovation of enzyme technology strategies like enhancing the enzyme activity, selectivity, stability, recovery and multiple uses in bioprocesses are expected which may help improve the economy.