INTRODUCTION

Lipases are groups of hydrolase which catalyze the hydrolysis of triglycerides to glycerol and free fatty acids over an oil water interface in addition lipases catalyze the hydrolysis and trans-esterification of other esters as well as the synthesis of esters and exhibit enantio-selective properties. The ability of lipases to perform very specific chemical transformation has made them increasingly popular in the food, detergent, cosmetic, organic synthesis and pharmaceuticals industries. Lipases have emerged as one of the leading biocatalysts with proven potential for contributing to the bio-industry and have used in lipid metabolism and multifaceted industrial applications.

Lipase is a physiologically necessary enzyme. It occurs in many plants and animals as well as in microorganisms. However, its richest source is bacteria, fungi and yeast. Microbiological lipases, especially those originating from bacteria, are more stable than those from plants or animals. They possess unique qualities and, because of them, are used more often for industrial purposes.

Heavy consumption of fossil fuel resources, their effect on climate change and concern over energy security are the main drivers for the increased interest in biofuel. India is emerging fast in using non edible oils for the production of biodiesel. The trees borne oil yielding seeds like Jatropha curcas, Ricinus communis, Shorearobusta, mixed seed cake could grow well on waste land and could withstand draught and dry conditions producing non-traditional oil seeds.

The technology of biodiesel production consumed only extracted vegetable oil from nonedible seeds and left large amount of unutilized biomass as seed cake. The disposal of generated cakes as waste can led to environmental problems and indirectly effects cost for biodiesel production. Pongamiaand Jatropha has topped the biodiesel market in India and as superior sources of biodiesel production. They are rich in nitrogen phosphorous potassium content and are used as organic manure. The seed cakes have been exploited in
the field of fermentation technique which has resulted in the production of bulk chemicals
and value added products such as amino acids and enzymes.

OBJECTIVES
The following objective has been carried out for the industrial, economic importance of
lipase.

- Collection of soil samples.
- Isolation of lipase producing microorganism.
- Screening of lipase producing microorganism.
- Enzyme assay: lipase assay extracted from screened isolates.
- Characterization of lipase producing microbes.

METHODOLOGY

Isolation of lipase producing microorganism: Twenty one Lipase producing microorganisms were isolated from different natural sources using microbiological techniques like serial dilution and other aseptic techniques. Further axenic cultures will be obtained by sub-culturing the isolates.

Screening of lipase producing microbes: The obtained organisms were being screened by qualitative plate assay, based on the hydrolytic activity on tributyrin agar plates.

Enzyme assay: lipase assay extracted from screened isolates: The crude and partially purified zextract from the screened isolates were studied for catalytic activity and kinetics.

Characterization of lipase producing microbes: The characterization is in progress for species identification and biochemical tests as per the Bergey's manual of systematic bacteriology and further species identification by 16SrRNA sequencing.

Results

Collection of Samples: The soil samples were collected from different sites of Gulbarga. The seed cakes were obtained from Biofuel Information and Demonstration Centre, Gulbarga University, Kalaburagi.

Isolation of organisms: Based on colony morphology 25 isolates were obtained on tributyrin medium supplemented with 1% (V/V) tributyrin. These isolates were sub cultured and maintained as axenic cultures.

Screening of lipase producing organisms

Primary screening: Screenings of organisms were carried out by using qualitative plate assay. The isolate KAR15 and KAR21 have shown larger zone of hydrolysis than the other isolates i.e, 44mm and 56 mm and they were selected for further secondary screening.

Secondary screening: The isolates KAR15 & KAR21 showed potentially in primary screening and were further screened for the ability to utilize the de-oiled seed cake as a substrate for the production of lipases. The lipase activity was analyzed for both crude extract and partially purified extract and titrated against 0.1 N NaOH.

After the analysis of enzyme activity using Pongamia pinnata seed cake as substrate, the value of KAR 15 in crude extract is 14000 units /ml and in partially purified the value is
13600 units /ml, whereas the value of KAR21 is 14400 units/ml in crude extract and 14000 units/ml in partially purified extract.

The value of enzyme activity using Jatropha curcas seed cake as substrate KAR 15 in crude extract and in partially purified extract is same 13800 units/ml whereas the value of KAR21 in crude extract and partially purified extract is 14200 units/ml.

**Specific activity**

Specific activity of enzyme was determined by estimation of protein content of enzyme fraction using Lowry’s method. The total protein content fraction produced using deoiled seed cake as substrate was determined by comparing with the standard BSA Curve. The specific activity of Lipase was calculated using the formula.

\[ \text{Specific Activity} = \frac{\text{Enzyme activity in units per ml}}{\text{concentration of total protein content}} \]

The specific activity of KAR15 in crude extract using Pongamia pinnata seed cake is 18.42 units/mg and in partially purified the value is 21.25 units/mg while using Jatropha seed cake the value in crude extract is 15.33 units/mg and in partially purified the value is 19.43 units/mg.

The specific activity of KAR21 in crude extract using Pongamia pinnata seed cake is 16.94 units/mg and in partially purified the value is 19.71 units/mg and in using Jatropha curcas seed cake the value in crude extract is 16.90 units/mg and in partially purified the value is 18.20 units/mg.

**Characterization:** The samples KAR 15 and KAR21 have been send for 16S rRNA sequencing.

**Summary and Conclusion:**

In present project work Bacterial strain was isolated by employing standard techniques from oil contaminated soil and further investigated for its lipase producing ability. The clear zone indicates the Lipase producing microorganism on Tributryin agar medium. We have screened twenty one microorganisms for lipase activity. Among which two strains have shown maximum activity on Pongamia pinnata seed cake that is 14 units/ml and 14.4 units/ml and Jatropha curcus seed cake is 13.8 units/ml and 14.2 units/ml. It is concluded that Pongamia seed cake, which is available locally in large quantities can serve as rich source for the production of lipase, with strains like KAR15 and KAR21. Further work required to be carried for establishing new applications of lipases.