A-Review on the isolation of lipolytic strains from different sources.

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Abstract – A class of specialized proteins called an enzymes, which perform various catalytic activities. Microbial isolation of enzymes for the industrial application is widely applicable in various procedures, out of those lipase are the class of enzyme esterase are two main classes of hydrolase enzymes , these perform the hydrolysis of long chain fatty acids or triacylglycerol substrates. Due to hudrolysis activity these enzymes are widely used various industries such as starch, paper, pulp, food, leather , fats and oils , organic synthesizers , used in environmental polluted sites, oil spills etc. various microbial strains having capacity to produce lipase enzymes some of the examples are Some microbial species reported to produce these enzymes include Bacillus sp., Pseudomonas sp., Burkholderia sp., Candida rugosa, Candida antarctica, Galactomyces geotricum, Saccharomyces cerevisiae, Yarrowia lipolytica, Trichosporon fermantans, Cryptococcus albidus, Aspergillus flavus, Thermomyces lanuginosus and Rhizopus oryzae etc, this article reviews about the production of lipases by various strains and its applications.

Key words – Lipolytic, lipases, esterase, hydrolase enzymes, triacylglycerol, long chain fatty acids.

Introduction- The microbial enzymes are used in various applications, out of lipase enzymes are the considered one, these share great effort in the in the industrial application , lipase belongs to esterase (EC 3.1.1.3), a major group of biocatalysts catalyse hydrolysis of fatty acids and triglycerols to glycerol (Sharma et al.,2001). These enzymes are isolated by fungi, yeast, bacteria, plant and animal sources , out of these strains bacterial strains are stable (snellman et al.,2002). Bacterial enzymes were used in industries of food industries like cheese ripening, enhancement of flavors (Falch., 1991), also they are widely used in detergent industries, biodegradable polymers , textile industries , biodiesel industries , in pharamaceutical etc (Noureddini et al.,2005; Bajpai et al., 1999; Hasan et al.,2006) . The pulp and paper industries , these enzyme helps as woods extraction role, also in pulping , pitch particles contains triglycerides, fatty acid esters, glycosides, free and conjugated sterols and acts as cleaning agents , the residual pulps , coalesce to form black pitch deposits, which makes problems to machines, there is chance of mix-up these remaining pulps in other process, in this situation lipase play a vital role cleaning residues, especially in Sulphite pulps which are acidic these enzymes acts on those makes alkaline by hydrolysis , these are helpful in the degradation of celluloses pulp generated . The tradition methods leads to high amount acidic molecule generation and various chemicals were used , for these alternative solution is lipases (Gurung et al.,2013; Nigam., 2013; Back et al., 2000; Gutiérrez et al.,2010). Nonylphenol ethoxylates (NPEs) are used in the

removing of pitch compounds but these chemical makes estrogen mimicking effects also banned in various countries America and Europe . these are also used in the degradation of conjugated sterols, fatty acids and resin acids. The enzyme Laccases are widely used as in lignin degradation . lipases shows a very stable activity in pH levels, temperature and in various environmental stress, by considering all ,lipases are the suitable for the industrial as well as environmental uses.(Robl et al.,2016; Paice.,2005; Dube et al.,2009; Sithole et al., 2010). Various researchers used a different techniques in the islation of lipase by different environment samples .

Isolation and procedure for the lipolytic microorganisms.

As per Ramnath et al.,2017 reported a procedure for the isolation lipolytic microorganisms from Eucalyptus wood, the procedures as follows – a five grams of Eucalyptus wood chips are washed thoroughly by vortexing with 5 ml of phosphate buffer (pH 8.0) for 5 min. using serial dilutions procedure the sample spread on the nutrient agar (NA) and potato dextrose agar (PDA) (Merck, South Africa) and incubated at 37 °C and 40 °C for 1 and 5 days, for the growth of bacteria and fungi, respectively. Those colonies selected by based on morphological features; size, shape, pigmentation, margin, consistency and elevation and sub-cultured till pure isolates were obtained for the DNA was extracted from isolates and 16S rRNA and 18S rRNA for bacteria and fungi, respectively, identified as the respected strains genetic construction and Basic Alignment Search Tool (BLAST) algorithm . Further for the isolates Phenol red agar plates supplemented with 1% olive oil or tributyrin was ascertained to be the most favourable method of screening for lipolytic activity. Lipolytic activity of the various enzymes were highest at 45–61 U/ml at the optimum temperature and pH of between at 30–35 °C and pH 4–5, respectively. The majority of enzymes tested displayed a propensity for longer aliphatic acyl chains such as dodecanoate (C12), myristate (C14), palmitate (C16) and stearate (C18) indicating that they could be characterised as potential lipases and these Enzymes maintained up to 95% activity at the optimal pH and temperature for 2–3 h. The stability of the enzymes at acidic pH and moderate temperatures makes them excellent candidates for application in the treatment of pitch during acid bi-sulphite pulping, which would greatly benefit the pulp and paper industry.

Kaori L. Fonseca et al .,2020. isolated the Lipolytic bacteria prospected from polluted portuary sites, they have done marine bacteria capable to produce extracellular lipases and then test the lipolytic activity of their extracts. For this purpose, TBT(Tributyltin) resistant bacterial islaoted from Portuguese harbors were collected isolated and then REP-PCR characterized.

Their extracellular lipase activity was assayed by the method of Rhodamine B in solid culture medium. Rhodamine is a dye which together with fatty acids released by the hydrolysis of triacylglycerols, forms a fluorescent complex when exposed to ultraviolet light. The use of this test was due to its sensitivity in detecting lipase activity even in organisms with low production of extracellular lipases.

Further Lipolytic extracts activities were estimated using p-nitrophenyl palmitate method for optimization of activity conditions. This highly sensitive spectrophotometric method estimates the amount of p-nitrophenol (p-NP) released during the hydrolysis of the substrate p-nitrophenyl palmitate (p- NPP). Isolates producing

extracellular lipases were then identified by MALDI-TOF-MS. From a total of 111 different isolates, 10 were able to produce extracellular lipases - belonging to *Serratia* and *Pseudomonas* genus and higher potential in the production of lipolytic enzymes with the operational conditions (temperature and pH) were studied to optimize lipase activity for these isolates from temperature was varied between 8.9ºC and 51.2ºC isolates showed tolerance.

Jair Carrazco-Palafox et al.,2018 studied the Bacterial strains were isolated from environments located in northern Mexico city, the procedure as follows-

These isolates were exposed to soils contaminated with burnt oils. A total of 98 isolates were collected and then analyzed for the lipolytic activity. Strains were maintained on tripticasein soy agar (Bioxon, Becton, Dickinson and Company, Franklin Lakes, NJ) at room temperature. They were sub-cultured in tripticasein soy broth (BD) at 37 °C for 20–24 h before use. *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, and *Proteus mirabilis* ATCC 12453 were used as control bacteria strains. Control bacterial strains were maintained under the same conditions. Culture agar was TBA, containing 15 g bacteriological agar (BD), 3 g yeast extract (BBL, Mexico), 5 g peptone (BBL, Mexico), tributyrin 1% v/v (Sigma-Aldrich, St. Louis, MO), modified by adding Ca (CaCl) and/or Mg (MgSO). A full factorial design, with concentrations of calcium and magnesium ions (0.0 mM, 2.5 mM, 5.0 mM, and 10.0 mM in distilled water) was used to test the efficiency of metallic ions on mTBA performance. Lipolytic activity was carried out on mTBA in 0.6 cm diameter wells; the wells were cut-off using sterilised glass Pasteur pipettes. The strains were subsequently inoculated in the wells by placing a 20 µL aliquot of a 24 h bacterial culture (1 − 2 × 10 cells approximately). All plates were incubated at 28 °C and lipolytic hydrolysis was measured as a clear zone around the inoculated wells. Lipolytic activity data was measured in 22 replicates for each treatment and control; the diameter of the clear zone or halo was measured as a lipolysis indicator. The halos were measured at 12, 24, and 48 h. Small variations of TBA consistency was observed during preparation of plates that can be attributed to the concentration of the metallic ions present. Overall, the firmer the medium consistency, the better was the observation of lipolytic activity. All data were analysed using the SAS software by ANOVA for factorial design.

Priyanka Patel Binita Desai., 2018 isolated a lypolyic bacteria procedure as follows For the present study 11 Samples were collected from oil and fat contaminated soils from Area of Adajan, Jahangirpura and Olpad Of Surat,Gujarat. The soil samples were taken in appropriately labelled pre-sterilized bottles with the help of sterile spatula from the depth of 0.5 to 1.0 cm surface and subsurface and Samples of soil were diluted serially from 10-1 up to 10-6 in sterile distilled water, each dilution were cultured on nutrient agar plates by Spread
plate method to obtain isolated colonies after 24 hours of incubation Pure bacterial isolates were screened for lipase production by Bacterial colonies were streaked on Tributyrin agar Plate and Phenol red agar , Plateand Tween 80 agar Plate and incubated at 37˚C for 48 hours..

The Morphological and Colony characteristics were studied using Nutrient agar Plate. The Physiological characteristics of all the obtained isolates were studied. The Biochemical characteristics (Indole, Catalase,

Voges-Proskauer, Methyl Red, Citrate, Hydrogen sulphide production, nitrate Reduction, Oxidase, Gelatine hydrolysis test,) and sugar .fermentation tests were also carried out using standard reference Biochemical tests for identification of medical bacteria .The isolates capable of lipase production were further screened to isolate the best possible lipase producing bacteria based on agar well diffusion (Cup well method). Sterile Phenol Red Agar Plate with olive oil as a substrate use for the Agar well diffusion assay. These sterile agar plates were punched aseptically with sterile cup borer to obtain well of 4mm diameter. The isolates were grown in 10ml sterile nutrient broth for 24hrs and loaded with 50µl in each well separately and incubated at 37˚C in the incubator for 48 hrs. The developed yellow zones around the wells were measured (mm) and the data was used for further production of enzyme. The isolate with higher zone production on phenol red agar plate was inoculated in the 15 ml of inoculum media (20 gm glucose, 10 gm yeast extract, 10 gm peptone, 10 gm CH3COONa.3H2O, 0.09 gm MgSO4, 0.03 gm MnSo4, 1.5 mg CuSO4.5H2O, 0.5 gm KCL, 5 ml Olive oil, 1000 ml Distilled water and pH 10.8). The inoculum flasks were then incubated at 37°C temperature for overnight on rotary shaker at 120 rpm. The composition of production medium used in this study was Peptone 0.2gm %,NH4 H2 PO4 0.1gm%, NaCl 0.25gm%,MgSO4.7H2O 0.04gm%, CaCl2.2H2O 0.04gm%, Olive oil 2ml, Tween 20- 2-3 drops. Submerged microbial cultures were incubated in 250 ml Erlenmeyer flasks containing 100 ml of liquid medium on a rotary shaker (120 rpm) and incubated at 37°C. After 96 hours of incubation, the culture was centrifuged at 10,000 rpm for 20 min at 4ºC and the cell free culture supernatant fluid was used as the sources of extracellular enzyme. The lipase activity in the supernatant was determined by the titrimetric method.

Lipase activity was measured by titrimetric using olive oil as a substrate. One ml of the culture supernatant was added to the reaction mixture containing 2ml of Phosphate buffer with pH 7.0 and 1ml of olive oil and incubated at 37˚C for 60 min. The reaction was stopped and fatty acids were extracted by addition of 1.0 ml of acetone: ethanol solution (1:1). The amount of fatty acid liberated was estimated by titrating with 0.05M NaOH until pH 10.5 using phenophathelin as indicator. One unit of enzyme activity is defined as the amount of enzyme required to liberate 1 µmol of equivalent fatty acid under the standard assay conditions.

Lipase activity (Units/ml) = Volume of alkali consumed × Strength of alkali × 1000/Volume of sample × Time in min.

Kalpana Sagar et al.,2013 isolated Lipolytic Bacteria From Waste Contaminated Soil, the isolation procedure as follows Study site .The study site for the current investigation was the domestic waste dumping site of TezpurUniversity, Assam, India.the procedure as follows Replicate soil samples were collected from a depth of 5-10 cm with the help of a sterile spatula and stored in sterile plastic bags. Following collection, the soil samples were immediately transferred to the laboratory for examination and subsequent

analysis. For the isolation of strains A total of eighteen bacterial isolates were isolated from the study site by serial dilution. For serial dilution, 1 gm of soil sample was dissolved in 10 ml of sterile distilled water in a 50 ml Erlenmeyer flask, and agitated at 120 rpm for 30 min at 37°C on a rotary shaker. The sample (aqueous slurry) was serially diluted up to 10-6 dilution using 0.8% saline. 100 µl of each dilution was spread on tributyrin agar plates containing 0.5% (w/v) peptone, 0.3 % (w/v) yeast extract, 1% tributyrin and 2% agar by spread plate

technique. The plates were incubated at 37°C for 24-72 hours following which the lipolytic activity was determined (visual observation by the formation of zone of hydrolysis around the bacterial colonies). Out of eighteen isolates, two accessions viz., TU-L1 and TU-L2 were found prominent with regard to lipolytic activity. The criteria for selection of the above accessions were on the basis of utilization of different substrates like tributyrin, olive oil, and anionic detergents (tween-20 and tween 80). Further the Morphological and biochemical characterization of lipase producing bacterial strains were studied by Bergey's manual of systematic bacteriology. Finally the lipase production assayed The liquid culture medium (Tryptic soy broth) containing (g/L) pancreatic casein, 17; enzymatic digest soybean, 3; NaCl, 5; dipotassium phosphate, 2.5; glucose, 2.5; 1% olive oil; pH 7.5 was used for lipase production. For the production of lipase, an Erlenmeyer flask (250 ml) containing 100 ml of medium was inoculated with an aliquot of approximately 1% of the pre culture prepared in LB broth (g/L); Tryptone, 10; Yeast extract, 5: NaCl, 10; pH 7.5. The inoculated flasks were thenincubated for a period of 16 hours at 37ºC with constant shaking at 200 rpm. Cell-free supernatant was recovered by centrifugation at 5,000 rpm for 15 min at 4ºC and the clear supernatant was used to determine the lipolytic activity by universal titrimetric method.

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