Optimization of Lipase Production from Bacillus thuringiensis (TS11BP), Achromobacter xylosoxidans J2 (TS2MCN)–Isolated from Soil sediments near oilseed farm

Mohammad Badrud Duza#1, Dr. S Mastan2
1Research Scholar, Dept. of Biotechnology, Acharya Nagarjuna University, Guntur - 522510
2Post-Graduate Department of Biotechnology, PG Courses and Research Centre, DNR College, Bhimavaram – 534202, West Godavari Dist., A.P.

Abstract:
Aim: The aim of the present study is to estimate the optimum growth conditions of bacterial isolates TS11BP and TS2MCN having lipolytic activity.
Materials and methods: Oil degrading bacteria were isolated from soil sample using serial dilution and pour plate method. Bacteria were further identified by morphological and biochemical tests and subjected to lipase production in 250 ml Erlenmeyer flask using Rhodamine olive oil medium and Tween 80 medium for 48h of fermentation period at 37°C with agitation speed of 150rpm in rotator shaker.
Results: Different bacterial strains were isolated and screened for lipase production in submerged fermentation process. Among these two bacterial strains gave positive test for lipase production. The two strains were further characterized by biochemical and morphological tests and identified as Bacillus thuringiensis (TS11BP) and Achromobacter xylosoxidans (TS2MCN). Supplementation of various bacterial growth components such as Carbon, Nitrogen, Vitamin and Amino acids increased enzyme production. The optimum pH and temperature for the lipase activity of crude enzyme was 8 and 45°C for both the bacterial isolates. Maximum lipase production was recorded during 96hr of incubation period and 14% of inoculums level.

1. Introduction

Lipases (E.C.3.1.1.3) are a group of hydrolytic enzymes that catalyze the degradation of triacylglycerols to diacylglycerol, mono-acylglycerol, fatty acids and glycerol’s at the interface between aqueous and the lipid phase. They are widely spread in nature, they were isolated from different source such as plants, animals and microorganisms; Lipase have immense potential application in various industries like cosmetic, food, detergent, paper and pharmaceutical industries.

Enantiomers of lipase are used for resolution of chiral drugs. Biofuels products and flavor enhancers. Bacterial lipases are extensively used in foodindustry for quality improvement, dairy industry for hydrolysis of milk fat, cheese ripening, in beverages improve aroma and in health foods for transesterification. In detergent industry for removal of oil stains from fabrics by hydrolysis of fats, in textile industry to increase fabric absorbency.

In addition the enzyme is used in chemical industry for enantio selectivity and synthesis of different products. In pharmaceuticals monoglycerides are used as binders in tablets enabling slow drug release, for the synthesisof biodegradable polymers or compounds in pulp and paper industry to improve quality; degreasing leather in leather industry and for the production of various products in cosmetic industry. Microbial lipases have received much more attention in industry mainly because of the availability of a wide range of hydrolytic and synthetic activities, the high yields possible, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and easy cultivation of microbes on inexpensive media.

Lipases of fungal and bacterial origin are widely used in industrial applications. Some important lipase producing bacterial genera include Bacillus, Pseudomonas and Burkholderiaetc. Lipase/esterase-producing bacteria have been found in diverse habitats such as soil contaminated with oil, dairy waste, industrial wastes, oil seeds and decaying food, compost heaps, coal tips and hot springs. The demand for the biocatalysts with novel and specific properties such as specificity, stability, pH, and temperature is increasing day by day. Currently bacterial lipases are of great demand because of potential industrial applications. The present paper focused on screening and isolation of microorganisms and optimization of different growth parameters for maximal lipase activity.

www.iosrjournals.org
II. Materials And Methods

2.1 Sample collection
Bacteria were isolated from soil collected from different sources such as soil near to oil refineries, some samples are collected from soil exposed to different oils i.e. rice bran oil, vegetable oil, palm oil, engine oil for long periods, some samples were collected from Jatropha based bio-diesel processing plant, soil samples were also collected from garbage sites comprising waste from kitchen, restaurant, market and diaries.

2.2 Isolation of lipase producing bacteria
Soil samples were serially diluted with sterile distilled water and spread on the nutrient agar plates followed by incubation for 24-48 h at 37°C for the growth of microorganisms. Microbial colonies, which appeared on nutrient agar plates, were purified and subjected to qualitative screening for identification of lipase/esterase producing microorganisms on Rhodamine olive oil (ROA) and tween 80 agar plates.

2.3 Identification of lipase producing bacteria
The bacteria were characterized morphologically based on Bergey’s manual of systematic bacteriology[19] and 16S rDNA analysis.

2.3.1 Biochemical characterization of bacterial isolates
Different biochemical tests have been performed on bacterial isolates for classifying them according to their biochemical features. The tests include Citrate test, Methyl red test, Urease activity test, Indole test, Oxidase test, Catalase test, Vogue-Proskauer test, Gelatin test, Casein hydrolysis test, Congo red test, Motility test, Amylase test, Nitrate reduction test, Carbohydrate fermentation test by standard methods [20].

2.3.2 16S rRNA sequencing and data analysis:
Sequencing of RNA was performed by 1500 bp PCR product. For the sequence analysis ABI automated sequencer was used. The two 16S rRNA sequences were aligned and compared with other 16S rRNA genes in the GenBank by using the NCBI Basic Local Alignment Search Tools (BLAST).

2.4 Screening of lipase producing Bacteria
A sensitive and specific plate assay for detection of lipase producing bacteria makes use of rhodamine-oil-agar medium. The growth medium contained (g/L): nutrient broth, 8.0; NaCl, 4.0 and agar-agar 20. The medium was adjusted to pH 7.0, autoclaved and cooled to about 60 °C. Then, 31.25 ml of olive oil and 10 ml of rhodamine B solution (1.0 mg/ml distilled water and sterilized by filtration) was added with vigorous stirring. It was then poured into petri plates under aseptic conditions and allowed to solidify. The bacterial culture was inoculated on to the medium in these plates. Lipase producing strains were identified on spread plates after incubation for 48 h at 37 °C. The hydrolysis of substrate causes the formation of orange fluorescent halos around bacterial colonies visible upon UV irradiation. Secondary screening of lipolytic bacterial strains were carried out using Tween 80 hydrolysis, medium composed of (g/l): peptone, 10; NaCl, 5; CaCl₂-2H₂O, 0.1; agar-agar, 20; tween 80,10ml(v/v).

2.5 Lipase activity assay
Lipase activity was assayed quantitatively by using para-nitro phenyl palmitate (pNPP) as the substrate[21]. Tenmilliliter of isopropanol containing 30 mg pNPP (Sigma) was mixed with 90 ml 0.05 M sodium phosphate buffer(pH 8) containing 207 mg sodium deoxycholate and 100 mg gum arabic. A total volume of 2.4 ml freshly prepared substrate solution was prewarmed at 37°C and mixed with 0.1 ml enzyme solution. After 15 min incubation at 37°C, absorbance at 410 nm was measured against the blank. One enzyme unit was defined as amount of enzyme required to release 1 μmol of p-nitrophenol from the substrate in milliliters per minute.

2.6 Optimization of growth parameters for lipase production
Eight independent parameters carbon sources, nitrogen sources, pH, temperature, Incubation time, inoculum level, vitamins and amino acids supplement have significant influence on Lipase activity.

Different carbon sources such as Glucose, Fructose, Sucrose, Xylose, Mannitol, Mannose, Arabinose and Dextrose were added to the basal medium in 0.50% and 1.0% concentration. Different Nitrogen sources such as peptone, yeast extract, tryptone, Soyabean Meal, Beef extract and Gelatin were added to the basal medium in 0.50% and 1.0% concentration.

For optimizing pH, temperature, incubation time and inoculums level different values of pH ranging from 3 to 11 has been maintained and tested, temperature optimization studies are carried out in 20°C to 60 °C range, incubation time was maintained in the range of 24,48,72,96,120,144 and 168, in order to optimize
Optimization of Lipase Production from Bacillus thuringiensis (TS11BP), Achromobacter

inoculums level different % of inoculums was added to the media. Not only the conventional parameters pH, temperature and above mentioned growth parameters but also micro nutrients are also optimized for lipase production. In this regard Vitamins such as Riboflavin, Ascorbic acid, Biotin, PyrodoxineHCl, Folic acid, Nicotinic acid and ThamineHCl, Amino acids such as L-Lysine, L-Leucine, L-Histidine, Tyrosine, Tryptophan, Aspargine, Glycine, D-Alanine, Cysteine, Arginine and Phenylalanine were added in 0.01% concentration to the media.

III. Results And Discussions

3.1 Isolation of Lipase producing bacteria:

Two bacteria out of 17 isolates (TS2MCN and TS11BP) from different soil samples were screened as Lipase producing bacteria and showing clear zone on Rhodamine agar plate.

Table 1: Screening of different enzymes producing bacterial strains

<table>
<thead>
<tr>
<th>S.No</th>
<th>Organism tested</th>
<th>Amylase</th>
<th>Protease</th>
<th>Celulase</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TS1MCP</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>2</td>
<td>TS2MCN</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>TS3BP</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>4</td>
<td>TS4BP</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>TS5SRP</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>6</td>
<td>TS6MCN</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>7</td>
<td>TS7BN</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>8</td>
<td>TS8MCN</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>9</td>
<td>TS9MCN</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>10</td>
<td>TS10MCN</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>11</td>
<td>TS11BP</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>12</td>
<td>TS12BN</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>13</td>
<td>TS13SP</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>14</td>
<td>TS14SN</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>15</td>
<td>TS15MCN</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>16</td>
<td>TS16MCN</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>17</td>
<td>TS17BP</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

3.2 Identification of Isolated bacterial strains:

These bacterial isolates were characterized on the basis of colony characteristics, microscopic appearance and biochemical tests. Molecular characterization of these strains was done by 16S rRNA analysis. Further these amplified 16S rRNA sequences of the bacterial strains was blasted using online tool (MEGA 4). The taxonomical identification was done by the phylogenetic tree construction and the comparison of these bacterial strain sequences with other homologous bacterial sequences. After morphological, physiological, biochemical and taxonomical identification, these Two bacterial isolates were identified as Achromobacter xylosoxidans(TS2MCN) and Bacillus thuringiensis (TS11BP).
Table 2: Morphological and biochemical characters of strains isolated from soil sample

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of the Test</th>
<th>TSI M C P</th>
<th>TSI S2 M C P</th>
<th>TSI S3 M C P</th>
<th>TSI S4 R P</th>
<th>TSI S5 R P</th>
<th>TSI S6 B P</th>
<th>TSI S7 B N</th>
<th>TSI S8 M C P</th>
<th>TSI S9 M C P</th>
<th>TSI S10 M C P</th>
<th>TSI S11 B P</th>
<th>TSI S12 B N</th>
<th>TSI S14 S N</th>
<th>TSI S15 M C P</th>
<th>TSI S16 M C P</th>
<th>TSI S17 B P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram Staining</td>
<td>+</td>
<td>(G P)</td>
<td>-</td>
<td>+</td>
<td>(G P)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Citrate utilization</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Urease</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>H₂S test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Oxidation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Methyl Red</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Indole test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Vogues-Proskaeur(VP) test:</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Fermentation test</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>G P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>G P</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Nitrate reductase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Methyl red, Catalase and nitrate reductase tests confirms Monococcus strains. And Urease test, Vogues-Proskaeur (VP) test, Fermentation Tests confirms Bacillus strains.

3.2.1 16S rRNA sequencing and analysis data:

TS2MCN is Identified as Achromobacter xylosoxidans strain using 16S ribosomal RNA gene sequence studies

GenBank: KF525350.1 GenBank Graphics PopSet

>gi|530549974|gb|KF525350.1| Achromobacter xylosoxidans TS24B 16S ribosomal RNA gene, partial sequence

ACATGCACGTCCACCGCTGGCCGAATCTTGCTGGTGCAAGTGCCGCGGTTGAGTGTGATGTATC
GGAACT
GTGCCCTTGGTCCGGGGAACCTCAATCCTGACCGTACTACGACTGAGACATCGCTCCCTCTCC
CTCC
TACGGGAGACTGCCTCTTGGGAATTGGGCAATAGGGGAAACCTTGAGCCAGACATCCCGCGGTA
GCAA
GACGGCCTCTGATTAAGCTTTTCTTTTGGCAGAATCATCAATTTGCACGGACTATTACCTCCCAAAT
CTGA
CGGCTTCCTGCTTAACAGCACCTGATCATTACGTGACGCTACCTCGGTGCTACCTACCGTGCAAG
CGTT

www.iosrjournals.org 69 | Page
Optimization of Lipase Production from Bacillus thuringiensis (TS11BP), Achromobacter

TATCTGAATTACTGGGCGTAAAGTTTGCCGACCTGATTCCGCAAAGCAGGTATGTTCTATCGTATAACGTAA
CTTTTGAACCTGCCTTCTTAACCTGGGGCTAGAGAGTGTCAGACGGAGGTGCAAATCCGCGTATTGC
AGTT
ACATGTGTAGATATGAAGAGGAACAACGTGTTCTACAAAGCACCCTCTGCGGAGGATGACACTGACGCTT
CTGCA
CCAGAGCTCGGGAGCAACCGGAGTGGAGCCGCCCCTGAGGAGGACTGAGGACTGCTCAGGACTGATG
ACCTG
CTGGAGTCTCTCAGTGGATAGCCTACCCCTCAGTGGACTGACTGAGGACTGACCTCAGGACTGATG
TCC
GCCAGATTAAATCACTACACAGCAATCTACAGCTACCCCAGCTTGGGAGTACGCAATTACGTGACAC
TGCC
ATCGGTCGCTTT,AACCTGTAAGTACCTACCCTCGGACTGCTCAGGAGGTGACTGACTGACTCAGT
GCAA

TS11BP is identified as Bacillus thuringiensis using 16S ribosomal RNA gene, partial sequence
GenBank: KF525352.1
GenBank Graphics PopSet

>gi|530549976|gb|KF525352.1| Bacillus thuringiensis strain SSME 16S ribosomal RNA gene, partial sequence

TGGATTTGGGACCTGCTCTTATATGAAGATTAGCCGGCCGACGGGATGATACACGTGGGTAACCTGCCC
ATAAG
ACTGGGATAACTCCGGGAACCCGGGCTATACACGGGATAAACCTTGAAACCGCATGTTGCAAT
TAAAA
GCCGCTTCTGGCTTACCTATGAGTGACCCGCACTTGGCAGCGATGCTAGTGTTGAGGAAACCGTGCTC
ACCA
AGCGAAGATCGCCGCACTGGACGAGGTGATCAGCACGCACTGGGACTTACCGGCGCTAATACTGATG
GTGAT
AACGGCTTTCCGGGCTTAAAACTCTGGTTGTTAGGGAAGAAAATGCTGATTTGAAATAAGCTGGCAC
CTTGA
CGTGACCTACAGGAAGCACCAGCTACTACGTGCCAGCAGCCGTGTAATACGTAGGTGCGGAA
CGGT
ATCCGGAAATTATGGCGCGTAAAGCAGCGGCAGGTGTGTCTTTTATCTGCTAGTGTAAGGGCACAGCC
TCAA
CCCGTGGAGGGCTATGGGAAACTCTGGAGACTTAGTGCAAGAGAGGAAGATTCAATCTGTAAGC
GCGT
GAAAATCGTATAGATTAGGAAACACACTTGGCGGGAAGGCACCTTTCTGTCTGTAAACCTG
GAGGC
GCCAAGCGTGAGGGGACCAAACAGGATTAGATACCTCATTGATCCAGCCGTAATAACGATGAGTGT
AAAGTG
TTAGGGGTCTCCGCCCCCTTTATGTGCTAGAAGTAAAACGCTAATTACGACACTCCGTCCTGGGAGTG
CGCC
GCTGAAACTCAAAGGAAATGGCCGGGCCCGCAGCGCAGGTGAGCCTTGTTTAATTCGGAAACACGCG
AGAAC
CTACGAGTCTGACATCATCTGACACCCCTAGAGATAGGGCTGCTCCTCGGGAGCAGAGATGACAGT
CTGCA
TGTTGTCGCTAGCTCGTCATGACATCGTGAATAGGATTAGTGATCTGCTACGATCGACATCGACATG
ACCAT
TAGTGTCACCTACGGATCAGCGTACTACGAGACGTGGAGATGACGCTAATCTCATGCTATGACACTG
GCTA
ACGT
3.3 Assay of Lipase activity

Estimation of enzyme activity for the positive isolates has conducted. Concentration of enzyme in crude sample was calculated by reacting with the enzyme with substrate comparing the resultant O.D with standard graph. The enzyme activity was expressed in International Unit (IU). 1U/ML = Amount of enzyme which releases 1micro mole glucose under assay conditions. The results of amylase assay are shown in Table 3.

### Table 3: Determination of Lipase activity from Lipase producing Bacteria

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Organism tested</th>
<th>Type of Strain</th>
<th>Lipase activity in the culture supernatant IU/mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TS2MCN</td>
<td>Monococcus sp.</td>
<td>3.9</td>
</tr>
<tr>
<td>2</td>
<td>TS11BP</td>
<td>Bacillus sp.</td>
<td>1.6</td>
</tr>
</tbody>
</table>

3.4 Optimization Studies

3.4.1 Effect of different Carbon Sources on lipase production

To determine the effect of carbon sources on lipase production by Bacillus thuringensis(TS11BP), and Achromobacter xylosoxidans(TS2MCN) carbon sources mainly carbohydrates were screened for their efficiency to support lipase production like Glucose, Fructose, Sucrose, Xylose, Mannitol, Mannose, Arabinose and Dextrose were used as the basal carbon sources in basal medium and was assayed to check for the lipase activity.

### Table 4: Effect of different Carbon sources on Lipase production by Two Strains

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Achromobacter xylosoxidans (TS2MCN)</th>
<th>Bacillus thuringiensis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.NO</td>
<td>Carbon Source</td>
<td>0.50% IU units</td>
</tr>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>Glucose</td>
<td>3.81</td>
</tr>
<tr>
<td>2</td>
<td>Fructose</td>
<td>3.28</td>
</tr>
<tr>
<td>3</td>
<td>Sucrose</td>
<td>1.69</td>
</tr>
<tr>
<td>4</td>
<td>Xylose</td>
<td>1.52</td>
</tr>
<tr>
<td>5</td>
<td>Mannitol</td>
<td>2.02</td>
</tr>
<tr>
<td>6</td>
<td>Mannose</td>
<td>2.55</td>
</tr>
<tr>
<td>7</td>
<td>Arabinose</td>
<td>3.63</td>
</tr>
<tr>
<td>8</td>
<td>Dextrose</td>
<td>4.12</td>
</tr>
</tbody>
</table>

In the present study, it has been observed that both the bacterial isolates produced maximum enzyme yield in presence of Dextrose in the medium, comparatively Achromobacter xylosoxidans (TS2MCN) produced (4.12IU/ml/min,4.49IU/ml/min in 0.05%,1.0% Dextrose concentrations respectively) more lipase enzyme than Bacillus thuringensis (TS11BP) which produced 1.89IU/ml/min, 2.28IU/ml/min in 0.05%,1.0% Dextrose concentrations, respectively.

![Fig.2 Effect of different carbon Sources on Lipase production by Two Strains](image)

3.4.2 Effect of different Nitrogen Sources on lipase production

Optimization was carried out by using different organic nitrogen as nitrogen sources. Different nitrogen source used were peptone, yeast extract, tryptone, Soyabean Meal, Beef extract and Gelatin were added to the medium and incubated at 37°C for 24 hrs in a rotary shaker.
Table 5: Effect of different Nitrogen sources on Lipase production by Two Strains

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Achromobacter xylosoxidans (TS2MCN)</th>
<th>Bacillus thuringiensis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen Source</td>
<td>0.50% units</td>
<td>% Assay</td>
</tr>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>Peptone</td>
<td>4.29</td>
</tr>
<tr>
<td>2</td>
<td>Yeast Extract</td>
<td>3.19</td>
</tr>
<tr>
<td>3</td>
<td>Tryptone</td>
<td>2.56</td>
</tr>
<tr>
<td>4</td>
<td>Soyabean Meal</td>
<td>2.11</td>
</tr>
<tr>
<td>5</td>
<td>Beef extract</td>
<td>4.01</td>
</tr>
<tr>
<td>6</td>
<td>Gelatin</td>
<td>3.96</td>
</tr>
</tbody>
</table>

In the present study, Achromobacter xylosoxidans (TS2MCN) produced 4.29 U/ml/min, 4.57 U/ml/min in 0.05%, 1.0% peptone concentrations respectively and Bacillus thuringenesis (TS11BP) yielded 2.15 IU/ml/min, 2.59 IU/ml/min in 0.05%, 1.0% Beef extract concentrations. Thus for Achromobacter xylosoxidans (TS2MCN) best carbon source was Peptone whereas Beef extract was best for Bacillus thuringenesis (TS11BP) yielding maximum enzyme production.

Figure 3: Effect of nitrogen sources on Lipase production by Two Strains

3.4.3 Effect of pH and Temperature on Lipase Activity

The optimum pH for enzyme production was selected by varying the pH of the medium from 3 to 11. For selection of optimum temperature for the production of lipases, the temperature varying from 20 to 60°C were maintained and tested for optimization studies.

Table 6: Effect of pH on Lipase production by Two Strains

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Achromobacter xylosoxidans (TS2MCN)</th>
<th>Bacillus thuringiensis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.NO</td>
<td>pH</td>
<td>IU units</td>
</tr>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.045</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.23</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.66</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>1.26</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>4.33</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>2.07</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>0.59</td>
</tr>
<tr>
<td>9</td>
<td>11</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table 7: Effect of Temperature on Lipase production by Two Strains

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Achromobacterxylosoxidans J2 (TS2MCN)</th>
<th>Bacillus thuringiensis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.NO</td>
<td>Temperature (in °C)</td>
<td>IU units</td>
</tr>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.37</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>2.24</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>3.56</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>4.69</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>2.69</td>
</tr>
<tr>
<td>8</td>
<td>55</td>
<td>1.24</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>1.11</td>
</tr>
</tbody>
</table>
In the present study, maximum lipase production was obtained around pH 8 for both of the bacterial isolates, Achromobacter xylosoxidans (TS2MCN) showed enzyme activity of 4.33 IU/ml/min whereas Bacillus thuringenesis (TS11BP) produced 1.88 IU/ml/min. Optimum temperature for maximum lipase production was 45 ºC illustrated by enzyme assay in temperature optimization studies shown in Table 7.

3.4.4 Effect of incubation time and inoculums level on lipase production:
The effect of incubation time on lipase production was determined by incubating the medium for 24, 48, 72, 96, 120, 144, 168 hours and the enzyme activity was assayed at different time intervals (0-168 hr). Different inoculums size in range of 4, 6, 8, 10, 12, 14 and 16 % were added to the basal medium and optimum inoculums level was tested and determined.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Achromobacter xylosoxidans (TS2MCN)</th>
<th>Bacillus thuringenesis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.NO</td>
<td>Incubation time(in hr)</td>
<td>IU units</td>
</tr>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>1.53</td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td>2.35</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>3.89</td>
</tr>
<tr>
<td>4</td>
<td>96</td>
<td>4.35</td>
</tr>
<tr>
<td>5</td>
<td>120</td>
<td>2.22</td>
</tr>
<tr>
<td>6</td>
<td>144</td>
<td>1.03</td>
</tr>
<tr>
<td>7</td>
<td>168</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>Achromobacter xylosoxidans (TS2MCN)</th>
<th>Bacillus thuringenesis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.NO</td>
<td>Inoculums level</td>
<td>IU units</td>
</tr>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>1.22</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>2.55</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3.22</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>4.23</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>4.79</td>
</tr>
<tr>
<td>6</td>
<td>14</td>
<td>3.99</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>3.55</td>
</tr>
</tbody>
</table>

In the present study, maximum lipase production was recorded during incubation period of 96 hr by the Achromobacter xylosoxidans (TS2MCN) and Bacillus thuringenesis (TS11BP) as 4.35 IU/ml/min and 2.04
IU/ml/min. In case of inoculums level 12% is considered as best for maximum growth of both the bacterial isolates and facilitate maximum production of lipase enzyme.

Figure 6: Effect of Incubation time on Lipase production by Two Strains

Figure 7: Effect of Inoculums level on Lipase production by Two Strains

3.4.5 Effect of micro nutrients on lipase production:

Micro nutrients include Vitamins and Amino acids, these two parameters were also termed as additional nourishing agents added to the medium for efficient growth of bacterial isolates. Different Vitamins such as Riboflavin, Ascorbic acid, Biotin, PyrodoxineHCl, Folic acid, Nicotinic acid and ThamineHCl were added to the medium in 0.01% concentration and assayed for selecting the optimum vitamin source. Amino acids such as L-Lysine, L-Lucine, L-Histidine, Tyrosine, Tryptophan, Aspargine, Glycine, D-Alanine, Cysteine, Arginine and Phenylalanine were added in the concentration of 0.01% and best amino acid giving maximal production of lipase was selected through optimization studies by comparing assay values.

Table 10: Effect of Vitamins on bacterial isolates

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Vitamins</th>
<th>Achromobacter xylosidans (TS2MCN)</th>
<th>Bacillus thuringiensis (TS11BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90 % Assay</td>
<td>1.60 % Assay</td>
</tr>
<tr>
<td>1</td>
<td>Riboflavin</td>
<td>2.50 % Assay</td>
<td>0.56 % Assay</td>
</tr>
<tr>
<td>2</td>
<td>Ascorbic acid</td>
<td>3.99 % Assay</td>
<td>1.99 % Assay</td>
</tr>
<tr>
<td>3</td>
<td>Biotin</td>
<td>3.01 % Assay</td>
<td>1.05 % Assay</td>
</tr>
<tr>
<td>4</td>
<td>PyrodoxineHCl</td>
<td>4.88 % Assay</td>
<td>1.22 % Assay</td>
</tr>
<tr>
<td>5</td>
<td>Folic acid</td>
<td>3.89 % Assay</td>
<td>1.88 % Assay</td>
</tr>
<tr>
<td>6</td>
<td>Nicotinic acid</td>
<td>2.00 % Assay</td>
<td>1.23 % Assay</td>
</tr>
<tr>
<td>7</td>
<td>ThamineHCl</td>
<td>1.55 % Assay</td>
<td>0.24 % Assay</td>
</tr>
</tbody>
</table>

www.iosrjournals.org 74 | Page
Optimization of Lipase Production from *Bacillus thuringiensis* (TS11BP), Achromobacter

Table 11: Effect of Amino acids on bacterial isolates

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Amino acids</th>
<th>IU units (TS2MCN)</th>
<th>% Assay</th>
<th>IU units (TS11BP)</th>
<th>% Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Standard</td>
<td>3.90</td>
<td>………</td>
<td>1.60</td>
<td>………</td>
</tr>
<tr>
<td>1</td>
<td>L-Lysine</td>
<td>3.99</td>
<td>102.30</td>
<td>1.99</td>
<td>124.37</td>
</tr>
<tr>
<td>2</td>
<td>L-Lucine</td>
<td>4.88</td>
<td>125.26</td>
<td>0.49</td>
<td>30.62</td>
</tr>
<tr>
<td>3</td>
<td>L-Histidine</td>
<td>2.88</td>
<td>73.84</td>
<td>1.55</td>
<td>96.87</td>
</tr>
<tr>
<td>4</td>
<td>Tyrosine</td>
<td>4.66</td>
<td>119.48</td>
<td>2.50</td>
<td>156.25</td>
</tr>
<tr>
<td>5</td>
<td>Tryptophan</td>
<td>4.51</td>
<td>115.64</td>
<td>2.31</td>
<td>144.37</td>
</tr>
<tr>
<td>6</td>
<td>Asparagine</td>
<td>0.53</td>
<td>13.58</td>
<td>0.53</td>
<td>33.12</td>
</tr>
<tr>
<td>7</td>
<td>Glycine</td>
<td>2.77</td>
<td>71.02</td>
<td>1.51</td>
<td>94.70</td>
</tr>
<tr>
<td>8</td>
<td>D-Alanine</td>
<td>2.01</td>
<td>51.53</td>
<td>1.44</td>
<td>90.00</td>
</tr>
<tr>
<td>9</td>
<td>Cystine</td>
<td>2.44</td>
<td>62.56</td>
<td>0.56</td>
<td>35.00</td>
</tr>
<tr>
<td>10</td>
<td>Arginine</td>
<td>0.56</td>
<td>14.35</td>
<td>0.23</td>
<td>14.37</td>
</tr>
<tr>
<td>11</td>
<td>Phenylalanine</td>
<td>1.55</td>
<td>39.74</td>
<td>0.55</td>
<td>34.37</td>
</tr>
</tbody>
</table>

In our study it was depicted that medium supplemented to Achromobacter xylosoxidans (TS2MCN) with PyrodoxineHCl produced higher yield of lipase 4.88 IU/ml/min compared to Bacillus thuringenesis (TS11BP) which yielded 2.22 IU/ml/min of lipase. When compared to other vitamin sources like Ascorbic Acid, Folic Acid, Biotin and other vitamin sources PyrodoxineHCl was best vitamin source for maximum lipase production. In case of Amino acids, Achromobacter xylosoxidans (TS2MCN) bacterial medium containing L-Luecineproduced 4.88 IU/ml/min of lipase which was comparatively high than Bacillus thuringenesis(TS11BP) yielding 2.50 IU/ml/min with Tyrosine supplemented medium.

**Figure 8:** Effect of Vitamins on Lipase production by Two Strains

**Figure 9:** Effect of Amino acids on Lipase production by Two Strains
IV. Conclusion:

Laboratory scale optimization of some of fermentation conditions for lipase production by Bacterial isolates in submerged process was carried out. It can be concluded from the above studies, Achromobacter xylosoxidans (TS2MCN) and Bacillus thuringiensis (TS11BP) based on the high enzyme yields it could be considered as useful source of lipase enzyme production and has the potential for industrial applications. The optimum conditions of various growth parameters were stated by optimization studies. Lipases were in great demand these days owing to their numerous biotechnological applications. Further research on production, characterization and purification of lipases through optimization of process parameters such as pH, temperature and various substrate utilizations would reveal those strains with higher lipase production potential.

References: