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A Genetic Variability Study of Lipase Producing Gene among *Aeromonas* Species Isolated From Clinical Isolates by RAPD DNA Markers

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Abstract

Lipases are most commonly used enzymes and do have lots of industrial applications. Even though these enzymes are used abundantly, little work was done in isolating the enzyme from microbes. *Aeromonas* species was the common contaminant of nosocomial infections, which do processes this enzyme. Study of such enzymes and their phylogenetic analysis could reveal loads of information of the organisms applications. We isolated and purified the enzyme from *Aeromonas* species, from clinical samples. PCR amplification of lipase gene was done to confirm the organism ability to produce lipase. Lipase enzyme was then studied for stability at varying temperatures and pH. The DNA was then used for the RAPD profiling to screen phylogenetic relation. Rhodamine plate assay was used for initial screening of bacteria. Among the 20 isolates screened all of them are lipase positive and only 5 isolates were found to be producing high lipase content. The enzyme was found to be stable at pH 6 and at temperature 27.5^0C. The phylogenetic analysis revealed that the sequences match to *Aeromonas salmonicida, Aeromonas hydrophila, Aeromonas veronii, Aeromonas enteropelogenes and Aeromonas caviae*. All the species belong to the same family. This study could pave ways to elucidate and characterize the molecular pathogenesis associated with the lipase gene whch eventually could lead to screening antibacterial targets and novel drugs against many antibiotic resistant nosocomial infections.

Keywords: *Aeromonas*; Lipase; Nosocomial infections; RAPD profiling.

1. Introduction

Lipolytic enzymes like Lipases (EC 3.1.1.3) are important classes of enzymes which carry out vital and new reactions in aqueous and non aqueous environments. They are of great importance due to their vast number of industrial applications. They are used in the generation of biodiesel, food flavours, cosmetic and laundry industries and also in pharmaceutical industries. Lipases are a triad serine hydrolases, which aids in hydrolyzing the triacyl glycerol to glycerol and also aids in the synthesis of short chain (≤10) and long chain (≥10) acylglycerols [1].

They belong to the third largest enzyme group, after proteases and glucose in terms of production and market value. Even though they are of great economical importance, study on lipases extracted from microbes are too low and limited.

Aeromonas spp. are present in wide range in both soil and water and are responsible for the cause of diseases in fishes and amphibians. They are most commonly seen in untreated and sewage waters. Not only in the water bodies but also in beef and pork, fisheris, poultry and even in raw milk [2]. As today, *Aeromonas* genus was considered to be an important disease-causing pathogen found in fishery industries and other cold-blooded species. A wide variety of *Aeromonas* species was found to be seen in the humans especially of nosocomial infections. Immunocompromised and immunosuppressed patients are most susceptible to these infections. They are infectious but the mode and pathophysiology of the infection seems to be little controversial owing to the presence of enterotoxins and hemolysisns. *Aeromonas* species is believed to cause mild abdominal cramps with diarrheal disease in healthy individuals. Studies also reported that the bacteria also causes watery diarrhea either of chronic or acute. *Aeromonas spp*. is found to cause acute diarrhea in children also [3].

Studies done so far correlate the works stating that diarrhea and other intestinal ailments are due to the infection by *Aeromonas*. And that too, most of the reported cases were found to be of contaminated drinking water. Several nosocomial infections which are reported also stated that the patients who are immunocompromised are susceptible to this infection [4]. No direct studies were done so far, to prove that the clinical samples directly harboured the pathogen. Very few studies reported the isolation of *Aeromonas* species from the human gut. And they possible suggests that these bacteria might not be normal inhabitants of the gut. And few other studies reported possible suggest that the patients are also immunosuppressed or immunocompromised. Molecular studies often confirms of

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the role of the pathogen in causing a particular disease. Isolation and purification of the strains not only allows us to identify the source of the organism but also the command on the environments the pathogen is having. Molecular characterization studies are paving new ways into finding the molecular and pathophysiologcal mechanisms which will aid in controlling the infection [5].

Epidemiological studies also aid in locating the distribution and moreover will help in determining the vastness and extent of the infection. PCR amplification and RAPD profiling will give the world a chance to find out their close relatives. This not only allows one to find out the possible cause for the spread but also to find out possible antibacterial targets. Nosocomial infections which are mostly resistant to antibiotics, need immediate attention to find out novel antibacterial targets. This will surely lead to the generation of next generation drugs. The present study was undertaken to isolate the strains from the clinical samples and to study their genetic profile by using PCR based molecular markers. In humans it is mostly enteric in nature and responsible for the diarrheal diseases. Most of the *Aeromonas* spp. which are especially associated with the human infections are found to be seen in meat and dairy products [6].

They are known to possess the virulence factors like presence of fimbria, flagella, and moreover they are encapsulated to increase their infection mode and rate. These virulence factors not only gives them a broad range of host spectrum but also aid sin anchorage to the host surfaces [7]. They mainly produce siderophores to enhance their mode of pathogenicity within the host. They try to survive within the host by releasing iron binding proteins and getting the nourishment from the host [8]. These bacteria are thought to release exotoxins and enzymes like proteases, elastases, lipases, and hemolysins which lead to extensive cellular damage and tissue destruction. Type II and III secretion systems are especially useful in this host immune response [8]. On the other hand capsule, S-layer, lipopolysaccharides, and porins are sued to enhance the pathogenic resistance towards the host defense [9].

Some of the strains of *Aeromonas* like *A. hydrophila* do contain a gene called aerocytotoxin enterotoxin (ACT) which releases a toxin called aerolysin to cause the tissue damage. This tissue damage is irreversible in nature and binds to specific glycoreceptors on the surface of eukaryotic cells before they form holes in the host surface. Most of the strains which causes loosening of the stools possess a gene called *alt* gene [10]. This gene encodes for cytotonic enterotoxin which causes severe disease in humans. Watery bowels with acute abdominal pain are the symptoms of the disease. The *LPL* gene is said to be aid in making an enzyme lipase, which is seen on the surface of epithelial cells lining tiny blood vessels (capillaries) within muscles and in fatty (adipose) tissue. Lipase also plays a critical role in breaking down the fat from triglycerides form and is carried to other organs.

Over the past decades, the development and application of molecular diagnostic techniques has initiated a mighty revolution in field of diagnosis and monitoring of infectious diseases. Microbial characteristics like protein, bacteriophage, and chromatographic profiles, as well as biotyping and susceptibility testing are most widely used in the routine laboratories. They are sued for identification and differentiation [11]. Nucleic acid techniques like plasmid profiling and some other methods for generating the DNA finger prints suing PCR and non PCR based methods are making increasing inroads into clinical laboratories. Most of the PCR-based systems are used to detect the etiologic agents of the disease which are isolated directly from the clinical samples. They do not need the culture and as such are more fast and reliable. They are most often used in the rapid detection of unculturable or fastidious microorganisms [12]. Additionally, sequence analysis of these amplified microbial DNA will allow the identification and aid in characterization of the bacteria in a better way. Such a wide range of molecular techniques need to be developed and implemented which will surely help the field of science to prevent the source and infection and also control the transmission in a better way. The studies will also throw light into the molecular pathogenesis which will surely aid in the controlling of the epidemic, endemic and pandemic diseases [13].

The present study is designed to screen for the lipase producing bacterial strains from the clinical bandages and trace their phylogenetic relation using RAPD markers. *Aeromonas* samples were screened for their lipase production. The enzyme was purified and studied for their stability under temperature and pH variations along with their relatedness using RAPD primers.

2. Materials and Methods

Sample collection and bacterial strain isolation: Cotton swabs and dressings from the wounds were collected from hospitals and dispensaries in and around Bangalore. The samples were collected aseptically and processed for the lab for isolation of the strains. For isolation of microorganisms, the swabs were suspended in about 90mL of sterile saline and incubated in a shaker at 37°C for 45min. Ten-fold serial dilution method was employed to isolate the bacterial isolates from the samples.

Isolation of bacteria: *Aeromonas* bacteria were isolated using the medium called as Rimler-Shotts (RS) medium pH at 7.0. The isolates obtained from the plates were screened for lipase activity. Rhodamine B plate assay method was used for screening the lipolytic activity. In brief, an overnight fresh colony of each isolated strain was streaked onto a rhodamine B plate (Nutrient broth 0.8%, NaCl 0.4%, 0.01% Rhodamine B solution and Agar 1%) and incubated at 37°C for about 24hr. Following incubation, the plates were exposed to UV light (350nm) for estimating the lipase activity. The presence of fluorescence on plates indicates the strain is positive for lipolytic activity. Colonies with no fluorescence have no lipase activity. The colonies with and high fluorescence were selected for further studies and subcultured.

Strains identification: Biochemical tests were done for identification of the isolated lipase positive colonies according to the Bergey's manual of systemic bacteriology. The tests done were gram staining, Vogues-Proskauer test, oxidase, catalase and citrate Consumption test.

Lipase assay: The lipase assay was determined by the pNPP (pnitrophenyl palmitate) hydrolysis method, described by Ghori, *et al.* [14]. In brief, 5ml of sample culture was added to a test tube pre soaked with 800µl of 0.25% PVA poly vinyl alcohol solution (8mmol/l pNPP solution in isopropanol). The contents are then incubated for 5min at 30°C and mixed thoroughly and centrifuged at 5000rpm for 10min. About 500µl of the supernatant was taken in a fresh tube and 1mL of 2M NaOH was added. Absorbance was measured in a spectrophotometer at 410nm.

Lipase Activity Assay: Lipase activity was assayed with ρ-nitrophenyl-palmitate (ρNPP) as substrate. ρNPP was first mixed with 0.5mL of DMSO (dimethyl sulfoxide) and then diluted to about 50mM with 50mM sodium phosphate buffer (pH 7.0). In brief, a single colony was inoculated into 20ml of LB broth and incubated at 37^0C for overnight. Following incubation, the culture was centrifuged at 8000rpm for about 10min and the supernatant was made into aliquots for further use. To 2ml of supernatant 1ml of ρ-nitrophenol (ρNP) was added and mixed thoroughly. After 5min of preincubation, 0.9mL of substrate solution (olive oil) was added and the reaction was stopped at different intervals (1 and 2min) by heat shock (90^0C, 1min). Following the treatment 1mL of saturated sodium tetraborate solution was added and the absorbance was measured at 405nm. The activity was estimated according from the standard curve using the ρ-nitrophenol (ρNP molar extinction coefficient: $1.8 \times 10^4 M^{-1}cm^{-1}$). Sample without enzyme solution was used as negative control. One unit of enzyme activity was defined as the amount of enzyme that releases 1µmol of ρNP per ml/min.

Bacterial growth curve: Each isolated colony from the isolates were inoculated separately into 15ml nutrient broth and kept for overnight incubation at 37C. Following incubation, after every 8 hours of time, 2ml of culture was removed aseptically from the tubes. OD of this culture was measured at 630nm and recorded. The samples were collected after every 30min and the experiment was done for 2 days. The same protocol was followed for each of the isolates. A standard growth curve was plotted using this OD value (Absorbance verses time).

Purification of enzyme: 20ml of overnight culture was used for the purification assay. All purification steps were carried out at room temperature. The experiment was done for all the ten lipase positive isolates.

Cell disruption: Each isolate was grown for 72hrs in the disruption medium (olive oil 1%, NaCl 10%, Tryptone 1.5%, soya peptone 0.5%). Following incubation, the cells were centrifuged at 10000g for 10min at 4^0C and the pellet obtained was washed twice in 50mM Tris-HCl buffer (pH 8.5). The cells were then homogenized in by ultrasonic treatment for 30min and spinned down at 10000g for 10min at 4^0C. The supernatant obtained was collected in a fresh centrifuge tube and used for purification.

Anion-exchange chromatography: The samples collected from the cell disruption were stored in refrigerator until further use. The supernatant was applied onto sepharose column equilibrated with 50mM Tris-HCl buffer (pH 8.5). The column was eluted with 50mM Tris-HCl buffer (pH 8.5) containing 1M NaCl. Following elution, the column was eluted with a linear gradient of sodium chloride (NaCl, 0–1M) of the same buffer. The collected fractions were assayed for the protein estimation. The fractions with more or less equal absorbance value were pooled and then dialyzed using dialysis membranes against 20mM phosphate buffer (pH 6.0). The active fractions showing lipase activity were pooled.

Effect of Initial pH on Lipase Production: The enzyme purified was studied for the characterization with the pH. pH is very critical for the overall maintenance and functioning of the enzyme. The effect of initial pH on lipase production was analyzed from 3.0 to 10.0. 2ml of enzyme solution was added to the contents and the pH was maintained in between 3 to 10. At each pH the specific activity and total activity of the enzyme was calculated using Olive oil as substrate. Lipase enzyme (100µg/ml) was used as positive control or standard. The OD values were recorded and standard graph was plotted with specific activity versus pH of the solution. 1ml of crude enzyme solution was added to 9ml of substrate and mixed thoroughly and incubated in a shaker for 30min at 28°C and 20ml of the above sample was titrated with NaOH (50mM, pH=9) solution. Specific activity is calculated by the formula: Specific Activity = amount of product formed/unit time/mg protein. Total activity = (specific activity) x (total mg protein used in reaction).

Effect of Initial temperature on Lipase Production: The effect of initial temperature on lipase production was analyzed from 20^0C to 40^0C. In brief, 2ml of enzyme solution was added to the contents and the temperature of the solution was maintained in between from 20^0C to 40^0C. At each temperature the specific activity and total activity of the enzyme was calculated. Olive oil is used as substrate and Lipase enzyme (100µg/ml) was used as positive control or standard. The OD values were recorded and standard graph was plotted with specific activity versus pH of the solution. Specific and total activity was calculated as like in the previous step.

2.1. Taxonomic Characterization of Isolated Bacteria

Extraction of DNA: Total genomic DNA from the bacteria was isolated by N- Cetyl- N, N, N-trimethyl-ammonium bromide (CTAB) method. In brief, 1ml bacterial culture was centrifuged at 10000rpm for 2min. at 4 °C and 675µl of extraction buffer was added and incubated at 37°C for about 30min. 75µl of SDS (20%) was added and incubated at 65°C for about 2 hours. The contents after mixing thoroughly was centrifuged at 10000rpm for 10min at 4°C and to the supernatant equal volume of Chloroform: Isoamyl alcohol (24:1) was added and centrifuged at 10000rpm for 10min at 4°C. To the upper aqueous layer, 0.6 volumes of isopropyl alcohol was added and incubated at room temperature for about 1hr and then centrifuged at 10000rpm for 10min. Pellet obtained was cleaned dissolved in 20µl sterile distilled water. The DNA obtained was checked for purity using spectrophotometry.

Gene amplification: The specific primers for lipase gene were designed using the Primer3 Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi/) and the designed oligonucleotides were synthesized in Sigma Corporation USA and ordered through HiMedia (Table 1).

Table-1. Table showing the characteristics of the primers designed for the lipase gene

Primer	Sequences (5'- 3')	GC %	Tm Value	Length	Product Size
FP	GACTCCCTCAAGGACAGCAG	60	59.99	20	188bp
RP	GTACCGAACCAGTCGGAGAA	55	60.11	20	

The reaction was set up to 25µl reaction with dNTPs (2.5mM), Primers 1µM; DNA polymerase (1-5 units) and template DNA (1pg to 1µg). The lip gene was amplified with denaturation at 94°C for 5min, 55°C for 1min, 72°C for 1min for 30 cycles.

RAPD PCR amplification: About 5 RAPD primers were used for amplification of the isolated *Aeromonas* species. RAPD1 (GGTGCACGTTA), RAPD2 (CCATGCCGGAG), RAPD3 (CTGCCTCGAGG), RAPD4 (TTAGGGCCGCC) and RAPD5 (TGGTGACCTGA) were used for the amplification. The reaction was set up to 25µl reaction with dNTPs (2.5mM), random primers 1µM; DNA polymerase (1-5 units) and template DNA (1pg to 1µg). The lip gene was amplified with denaturation at 94°C for 1min, 36°C for 1min, 72°C for 2min for 35 cycles [15].

Phylogenetic analysis of bacterial strain: The amplified DNA fragments were subjected to sequencing from Eurofins, Bangalore. The deduced sequences were subjected to BLAST algorithm from the National Centre of Biotechnology, Bethesda, MD, USA (http://www.ncbi.nlm.nih.gov) to retrieve for homologous sequences in GenBank and to align them for a phylogenetic analysis. The sequences aligned were run for multiple sequence alignment program using clustal omega. Phylogenetic trees were constructed by neighbor-joining analysis [16].

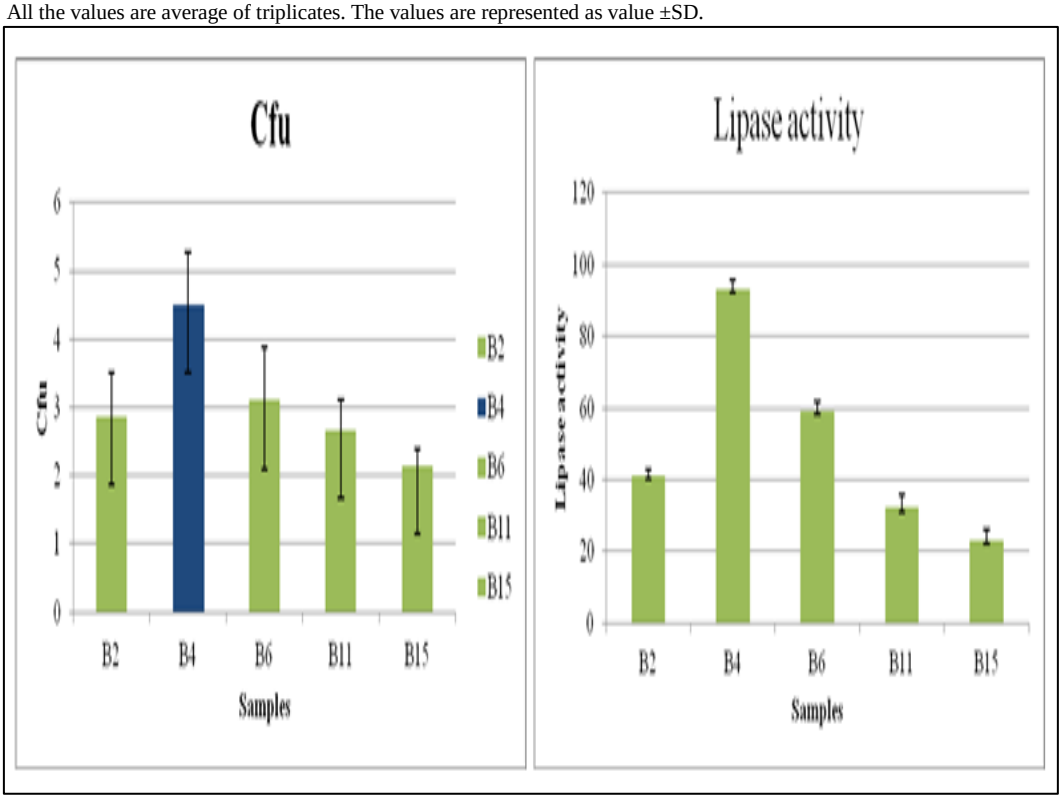
3. Results

Sample collection and bacterial strain isolation: Around 20 different colonies were isolated from the samples. All the samples were subjected to preliminary lipase activity using Rhodamine plate assay. Only 5 colonies showed highly reactiveness to the lipase assay. The lipase producing strains were isolated from different sources. From the total of 70 samples, Rhodamine negative strains were ignored and the positive strains (20 strains) were biochemically characterized using Bergey's Manual of Systemic Bacteriology.

Strains identification: Biochemical tests were done for identification of the isolated lipase positive colonies. According to the Bergey's manual of systemic bacteriology, these tests were selected and were done in triplicates. The isolates were found to be gram negative, Vogues-Proskauer positive, oxidase positive, catalase positive and citrate Consumption test positive.

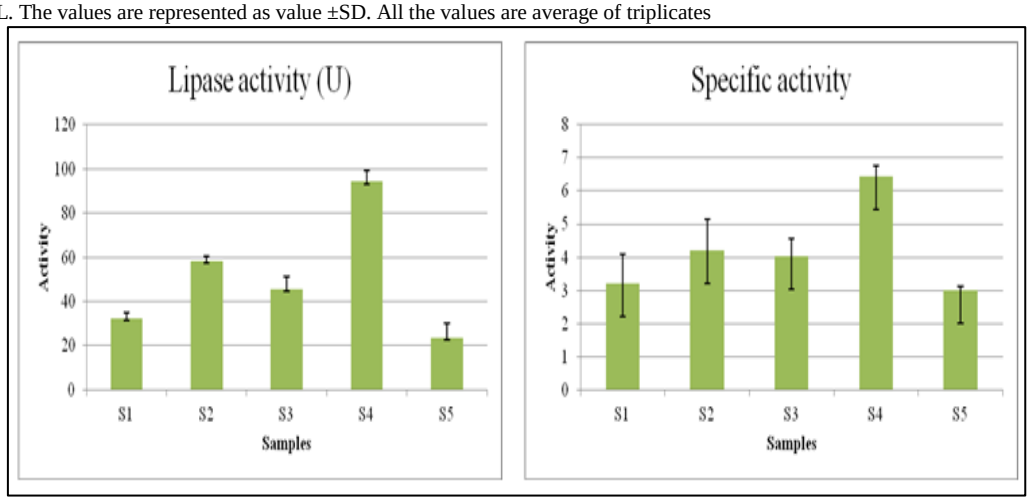
Lipase assay: Only the samples which are positive on the rhodamine plate assay were selected for further experimentation. Among the 20 isolates 5 of them were selected for further experiments. Among these 20 isolates the strains which are having high lipase activity are selected and among them 5 of them (B2, B4, B6, B11 and B15) were screened for the genetic diversity experiments. Remaining samples which are below the cutoff were discarded from the study. The samples would now on be labelled as S1, S2, S3, S4 and S5.

Figure-1. Graph showing the Colony forming units (Cfu) and lipase activity for the five isolates screened. The values are represented as value x 10^7 for cfu. All the values are average of triplicates. The values are represented as value ±SD.



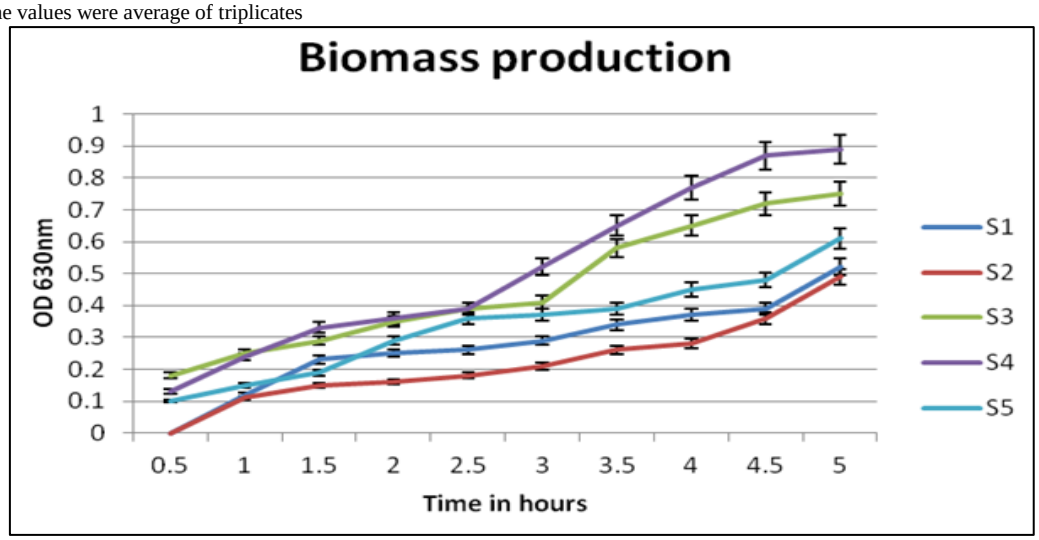
Lipase Activity Assay: Only the five samples which showed high lipase activity were selected for the remaining experiments.

Figure-2. Graph showing the lipase and specific activity of the 5 samples. Left: lipase activity; Right: Specific activity. Values are expressed in milliunits/mL. The values are represented as value ±SD. All the values are average of triplicates



Bacterial growth curve: Bacterial growth was found to be significant for the five samples. Among them the sample 4 followed by sample 3 showed high amount of growth.

Figure-3. Time growth and biomass production in the presence of olive oil; The 5 isolates which were lipase positive were selected for the growth curve. All the values were average of triplicates



3.1. Purification of Enzyme

The experiment was done for all the five lipase positive isolates.

Effect of Initial pH on Lipase Production: All the five isolates showed significant activity towards the pH. Isolate 4 was shown to have high stability in terms of pH. And the enzyme was found to be highly stable at pH 6. The specific activity was found to be 96.45, 59.65, 43.45, 33.56 and 24.56 for S4, S2, S3, S1 and S5 respectively. These results are in accordance to the lipase activity. Even the sample 4 showed highest lipase activity at pH 6.

Figure-4. Graph showing the effect of pH on specific activity of the enzyme isolated from the five isolates. S1, S2, S3, S4 and S5 are the isolates. All the values are average of triplicates. Values are expressed as activity ±s.e

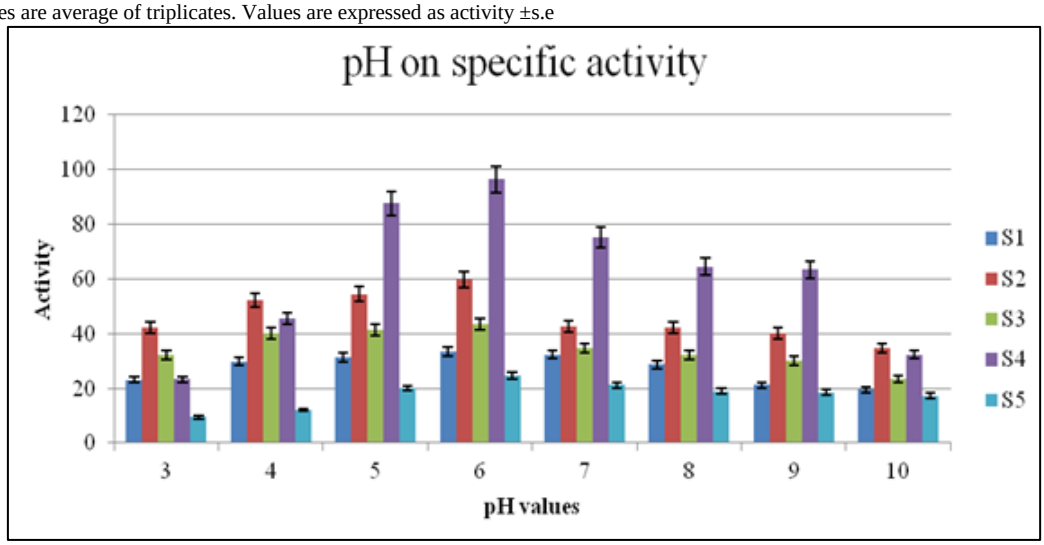
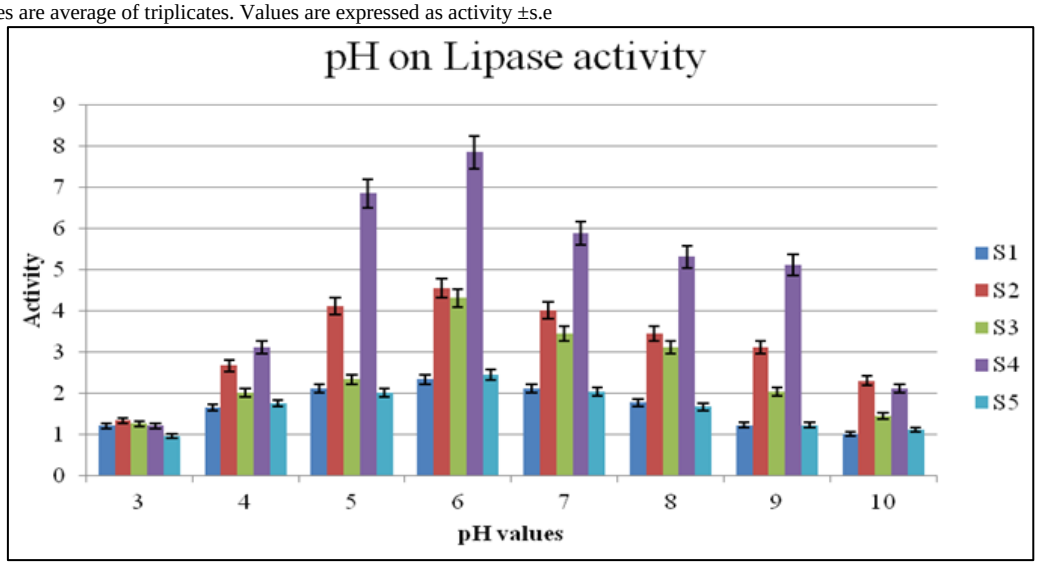


Figure-5. Graph showing the effect of pH on lipase activity of the enzyme isolated from the five isolates. S1, S2, S3, S4 and S5 are the isolates. All the values are average of triplicates. Values are expressed as activity ±s.e



Effect of Initial temperature on Lipase Production: All the five isolates showed significant activity towards the temperature. Isolate 4 was shown to have high stability in terms of temperature. And the enzyme was found to be highly stable at 27.5^{0}C. The specific activity was found to be 97.89, 62.34, 48.9, 27.5 and 23.45 for S4, S2, S3, S1 and S5 respectively.

Figure-6. Graph showing the effect of temperature on Lipase activity of the enzyme isolated from the five isolates. S1, S2, S3, S4 and S5 are the isolates. All the values are average of triplicates. Values are expressed as activity ±s.e

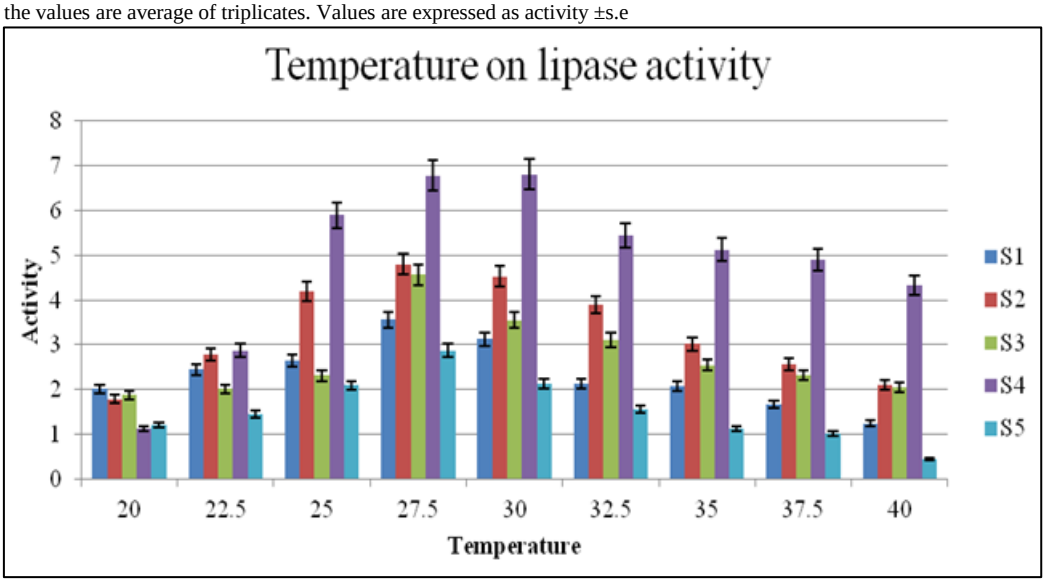
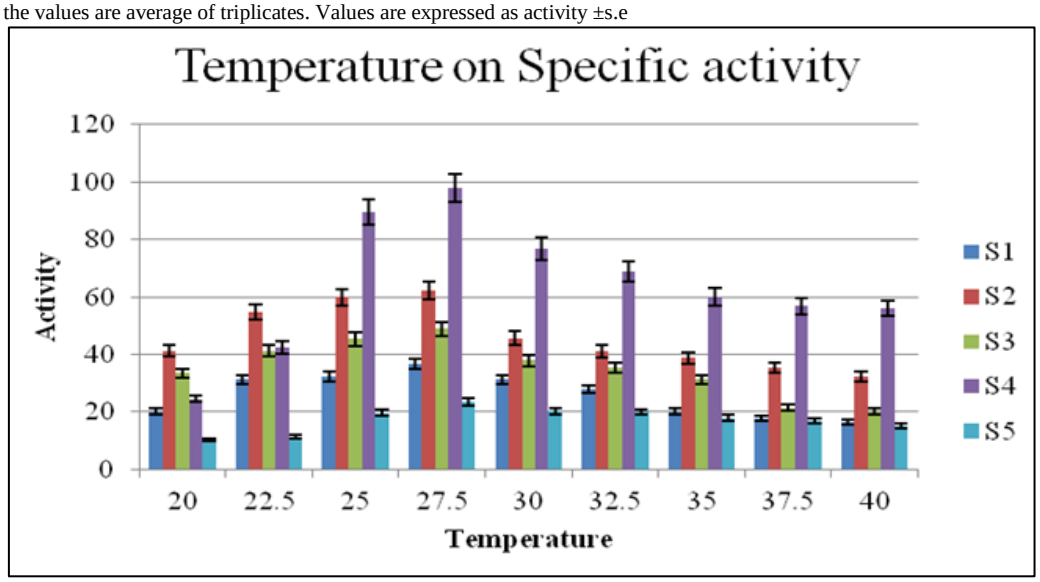


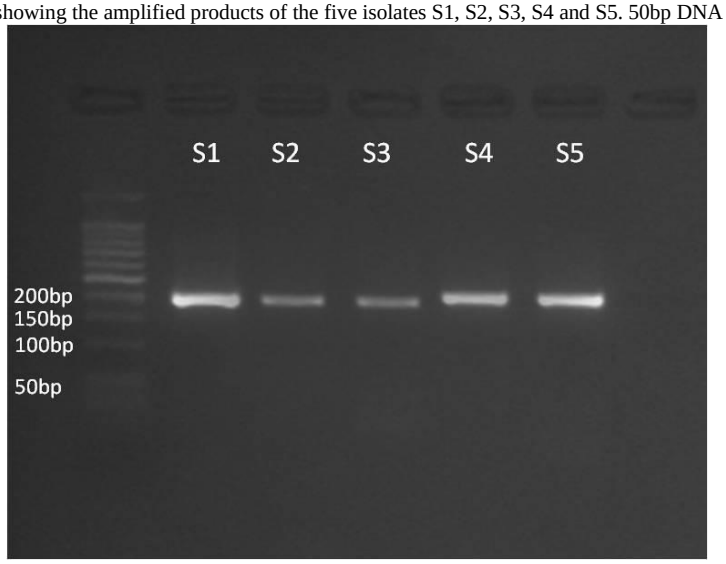
Figure-7. Graph showing the effect of temperature on specific activity of the enzyme isolated from the five isolates. S1, S2, S3, S4 and S5 are the isolates. All the values are average of triplicates. Values are expressed as activity ±s.e



3.2. Taxonomic Characterization of Isolated Bacteria

Gene amplification: The primers designed using primer3 software was used for the amplification. The product length was found to be 188bp. The five samples were amplified using the primers. The amplified product was found to be 188bp approximately.

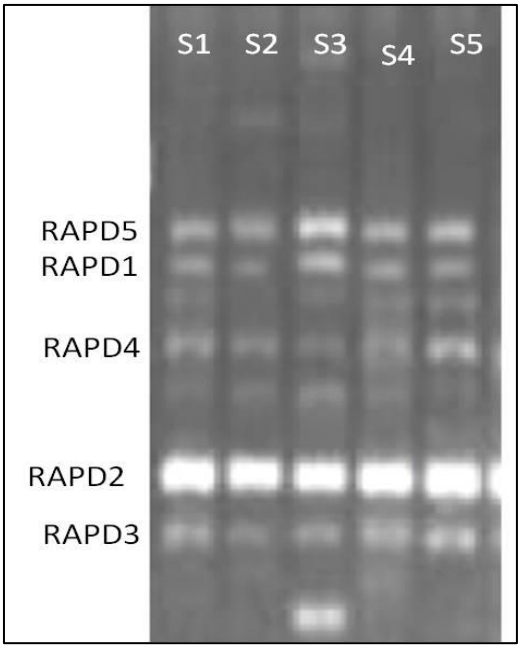
Figure-8. 1.2% agarose gel showing the amplified products of the five isolates S1, S2, S3, S4 and S5. 50bp DNA ladder was used as marker



3.3. RAPD PCR Amplification

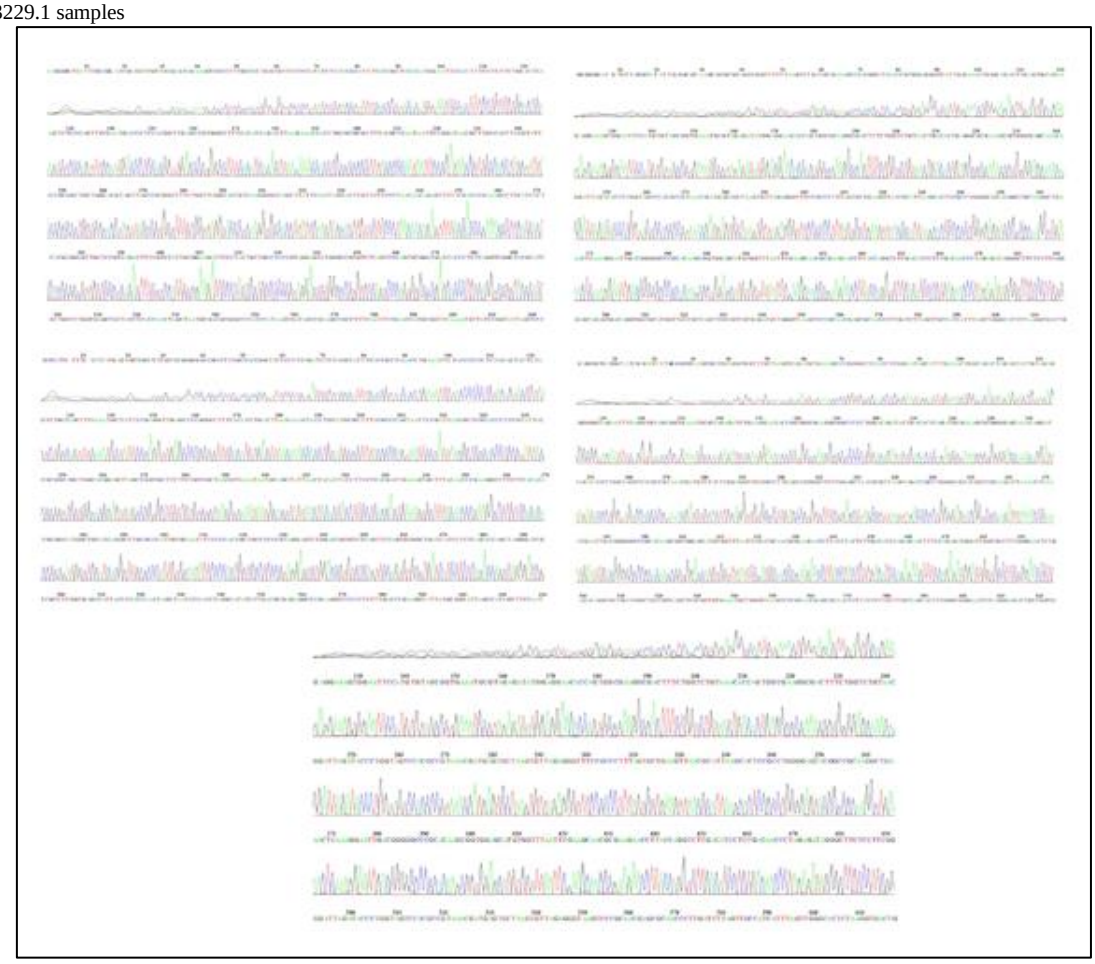
Around 7 polymorphic DNA bands were obtained using the 5 RAPD primers. All the bands amplified shows relatedness among the isolates. Although RAPD does not give authentic differentiation of closely related organisms it can be still used as preliminary screening for relatedness. All the bands amplified showed similarity among the isolates. This proves of the possible relatedness among the family. About 5 RAPD primers were used for amplification of the isolated *Aeromonas* species.

Figure-9. Gel showing the RAPD bands amplified using the five primers. The bands were run on 0.8% agarose gel. All the bands were measured suing the molecular markers



Sequencing results: The sequences obtained were retrieved in FASTA format and undergone MSA using clustal omega. A phylogenetic tree drawn by the neighbour joining method suggested of the relatedness among the species. Basing on the e score and the blast search score, the fragments were closely matching to the members of the *Aeromonas* family. SUQ143224.1(S5); SUQ143226.1 (S2); SUQ143227.1 (S3); SUQ143228.1 (S4); SUQ143229.1(S5) were found to match to *Aeromonas salmonicida, Aeromonas hydrophila, Aeromonas veronii, Aeromonas enteropelogenes and Aeromonas caviae* respectively.

Figure-10. Images of the chromatogram report of the amplified sequences of SUQ143224.1,SUQ143227.1, SUQ143226.1,SUQ143228.1 and SUQ143229.1 samples



The amplified fragments were sequenced and obtained as FASTA sequences. The sequences were aligned using MSA to find the relatedness among the members.

Figure-11. Picture showing the multiple sequence alignment using the BioEdit programme; SUQ143224.1,SUQ143227.1, SUQ143226.1,SUQ143228.1 and SUQ143229.1 are the legends for the sequences from the top to bottom

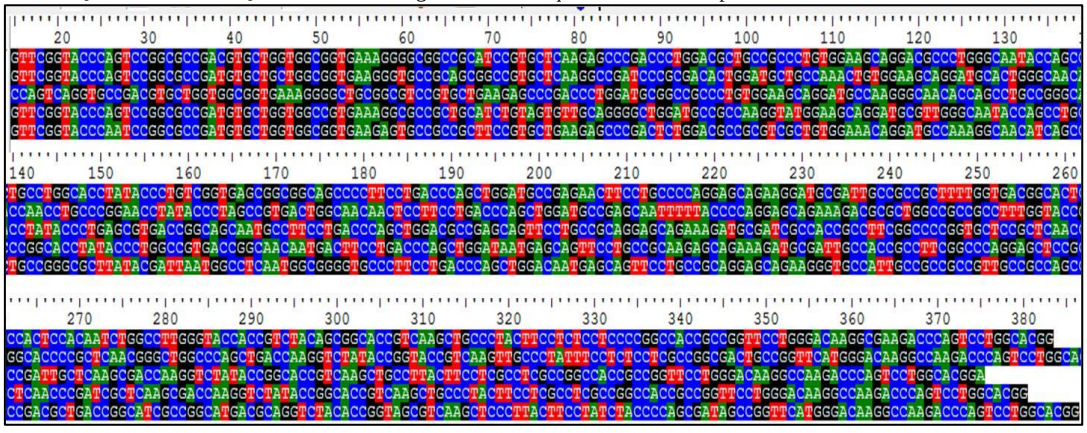
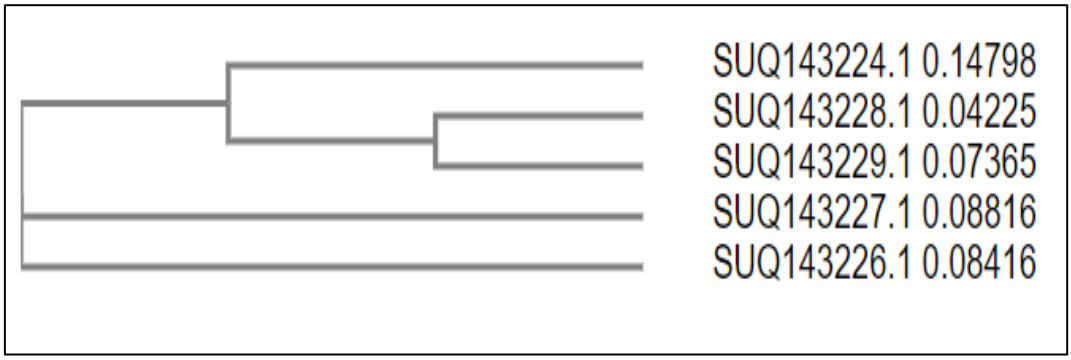


Figure-12. Dendrogram generated using clustal omega. SUQ143224.1: S5; SUQ143226.1: S2; SUQ143227.1: S3; SUQ143228.1: S4; SUQ143229.1: S5



4. Discussion

Clinical samples collected from hospital environment were screened for the lipase producing organisms. Lipase producing bacteria especially *Aeromonas* species are predominant species which live on the hospital wastes. They harbour the area with the help of the lipase protein as such the gene is very critical for its pathogenesis. Such a gene would be of great interest to study as it might lead to the discovery of novel antibacterial targets.

Samples collected were processed to the lab aseptically and screened for the lipase producing organisms. Rhodamine plate assay was used for initial screening of bacteria [17]. About 60 colonies obtained were screened and only 20 of them were found to be positive for the lipase producing organisms. Biochemical characterization done also proved of the possible confirmations of the species. They were found to be gram negative, positive for catalase, oxidase and citrate utilization test.

Among the 20 isolates screened all of them are lipase positive. This was confirmed on the rhodamine plate assay. Among these 20 only 5 isolates were found to be producing high lipase content. These 5 isolates only were further used for experimentation. The lipase enzyme was produced on shake flask and the enzyme was extracted in pure form. The purified enzyme thus separated was subjected to stability studies. The strain 4 was found to better in terms of lipase production. The enzyme was found to be stable at pH 6 and at temperature 27.5^0C. DNA was extracted from all the five isolates and the gen lipase (*lip*) was amplified with the primers specified [18].

The bands obtained was found to be at 188bp approximately and the bands were eluted from the gel and purified. The bands were further sequenced using Sanger's method of sequencing. The sequences obtained were run on Blastn and the phylogenetic analysis was determined to find the relatedness of the species. From the multiple sequence alignment and the e score obtained suggests that S5, S2, S3, S4 and S5 were found to match to *Aeromonas salmonicida, Aeromonas hydrophila, Aeromonas veronii, Aeromonas enteropelogenes and Aeromonas caviae* respectively. The RAPD fingerprint obtained 7 polymorphic DNA bands using the 5 RAPD primers. These bands confirms of the relatedness among the species with a single family [19]. All the species belong to the same family.

5. Conclusion

The present study was designed to study and isolate the enzyme lipase form the clinical wastes. The bacteria isolated were confirmed of the species *Aeromonas*. These strains produced lipase enzyme which might be of great interest for the further studies. The enzyme was isolated and purified from all the strains and was confirmed of the stability. The PCR bands obtained were sequenced and matched to *Aeromonas* species. All the 5 species isolated belong to *Aeromonas* species. The enzyme was purified and study for its characteristics. Further the enzyme need to be purified at HPLC grade and studied for its structure. This study might pave way for the elucidation and characterization of the molecular pathogenesis associated with the lipase gene. Hence novel antibacterial targets can be found which might help the biologists to design effective drugs against many of the antibiotic resistant nosocomial infections.

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