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ORIGINAL ARTICLE

Screening, characterization, and optimization of lipase enzyme producing bacteria isolated from dairy effluents contaminated muddy soil

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Abstract

Lipases, particularly microbial lipases, are important industrial biocatalysts. As a result, lipase enzyme screening, synthesis, and purification from microbial strains are constantly evolving to meet the needs of the pharmaceutical and food industries. Thus, the goal of this study was to identify the most potential lipase-producing bacterial strains from Aavin dairy industry effluent contaminated soil. Furthermore, growth parameters, such as pH, temperature, carbon and nitrogen sources, were optimized for lipase enzyme production from selected bacterial strains. According to the findings, 9 strains (V1–V9) of 15 bacterial isolates were found to be lipase producers. However, three strains (V1, V7, and V8) predominated and demonstrated significant lipase-producing activity. These V1, V7, and V8 bacterial strains were identified as *Bacillus pumilus* V1, *Bacillus pumilus* V7, and *Bacillus subtilis* V8 through 16S rRNA sequencing. About 16.6 to 27.8 μg mL^{-1} of lipase production was recorded under the optimal growth conditions: pH 8, temperature 37 °C, fructose and yeast extract as suitable carbon and nitrogen source. Among these 3 strains *B. pumilus* V1 showed excellent lipase productivity than others. The molecular weight of this lipase produced by bacteria was determined to be 35 kDa using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE).

Keywords Dairy effluent soil · *Bacillus* sp. · Lipase · Optimization · Production

Introduction

In several biotechnology sectors, lipolytic enzymes (EC 3.1.1) can potentially catalyze a wide range of biological reaction molecules. Their usage ranges from food applications to biochemical applications in medicine, detergents production, pesticide synthesis, leather tanning, wastewater treatment (Whangchai et al. 2021), and cosmetics production (Sarmah et al. 2018, Anusha et al. 2021). Lipolytic enzymes are also used in alcoholics, acidolysis, esterification and aminolysis as biocatalysts (Mehta et al. 2021). Below is how lipases work as a biocatalyst: one of the most important benefits of rigorous biochemical research since the 1940s has been enzymes in illness diagnosis, providing a basis for clinical chemistry (Narayanan et al. 2021a). Lipases are found in every kingdom of life, including bacteria, archea and eukaryotes, including plants, livestock and fungi (Kandasamy et al. 2021a,b). Microbial lipase is more valuable than plant and animal enzymes because it is easy to control the genetically engineered and capable of rapid growth in a wide range of catalytic activity (Ponniah et al. 2021, Narayanan et al. 2021b). In addition, no seasonal oscillations impact microorganisms to ensure they are constantly supplied and high levels of fatty lipases from microbial cells are obtained. More stable than plants and animal derivatives

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are also microbial lipases, and their synthesis is easier and safer for industrial and research purposes (Filho et al. 2019; Egbuna et al. 2021). Due to their increased activity, neutral or alkaline pH optimum, bacterial enzymes are chosen over fungal enzymes (Kandasamy et al. 2021a,b). Bacterial cells are more easily genetically and environmentally modified due to their short generation times, simple dietary requirements, and the production of readily desirable qualities to increase cell yield and enzyme activity or secrete altered enzymes (Hunter et al. 2019, Saranyadevi et al. 2021). Bacterial lipases are mainly glycoproteins, but lipoproteins are some extracellular lipases (Kumarasamy et al. 2020). Bacterial extracellular lipases are often produced by nitrogen and carbon sources, inorganic salts, lipid presence, temperature, and oxygen availability (Soman et al. 2020a, Vijayan et al. 2020). The gram-positive and gram-negative bacteria create different lipases. Main lipases derive from Gram-positive, such as *Bacillus* sp., *Staphylococcus* sp., *Streptococcus* sp., and *Enterococcus* sp., are the most important bacterial species (Nguyen et al. 2020; Narayanan et al. 2021c). Few Gram-negative species, such as *Achromobacter* sp., *Alcaligene* sp., *Burkholderi* sp., and *Chromo bacterium* sp. Strains, were reported as lipase producer (Matias et al. 2021; Soman et al. 2020b; Narayanan et al. 2021d). The lipase enzyme produced from *Bacillus* strains is the most familiar and preferably involved in various industry sectors. However, the demand for lipase enzymes is increasing in industries; hence, the researchers focus on enumerate most potential lipase enzyme producing bacterial isolates from various sources. In this connection, this research was designed to enumerate the predominant and most efficient lipase-producing bacterial isolates from dairy (Aavin) effluent contaminated soil. Furthermore, the optimal growth conditions required for evaluating the maximum lipase enzyme producing competence of bacterial test isolates were studied.

Materials and methods

Sample collection

A muddy soil sample (10 g) contaminated with dairy effluent was collected at a 15–30 cm depth from the Aavin Dairy Industry in the Dharmapuri District of Tamil Nadu. The sample was contained in a sterile ziplock bag and immediately transported to the laboratory, where it was kept in a refrigerator until further processing (Uchida et al. 2018).

Enumeration of bacterial population and preliminary screening

The cultivable bacteria populations present in the collected dairy effluent contaminated muddy soil sample were enumerated by following standard serial dilution technique (Sirisha et al. 2010). In brief, about 1 g of collected muddy soil sample was dissolved in 10 mL of sterilized distilled water and used as stock solution. The standard serial dilution process was followed from this stock solution as 10^{-1}–10^{-9}. Then from the 10^{-6}th dilution, 0.1 mL was taken and inoculated on sterilized Rhodamine olive oil agar (contains: peptone 5 g L^{-1}, yeast extract 3 g L^{-1}, NaCl 4 g L^{-1}, agar 15 g L^{-1}, rhodamine dye (1 mg mL^{-1}) 10 mL L^{-1}) Petri plates by spread plate method. The inoculated plates were incubated at 37 °C for 48 h. after 48 h of incubation, the colonies on the plates were observed, and 15 colonies were suspected as lipase producers based on colonies covered by orange fluorescent color under UV exposure. Then, as a preliminary screening step, these 15 probable colonies were inoculated individually on fresh Rhodamine olive oil agar media. The results were seen, and chosen strains were submitted to secondary screening.

Secondary screening

From the preliminary screening, 9 bacterial isolates (named as V1–V9) were subjected to secondary screening using Tween 20 agar plates (containing: peptone (667 g L^{-1}), NaCl (5 g L^{-1}), $CaCl_2.2H_2O$ (0.1 g L^{-1}), agar–agar (20 g L^{-1}), and 10 mL of (156 g L^{-1}) Tween20). A Loop full of these pure bacterial isolates was individually inoculated on sterilized Tween20 agar plates and incubated at 37 ºC for 24 h. After incubation, the colonies were observed and noted the colonies' zone of clearance (Elhussiny et al. 2020).

Quantitative analysis by lipase activity assay

The quantitative analysis was performed to determine which strain (V1–V9) produced a significant volume of lipase enzyme through lipase activity assay (Sharma et al. 2017). Then 1 mL of each selected strain (3.0×10^7 CFU mL^{-1}) was inoculated on freshly prepared production (50 mL) media containing 5 g L^{-1} (each) peptone and beef extract. After sterilization, olive oil was added as 10 mL L^{-1} (pH 7.0) in a 100 mL beaker. The culture (each) inoculated flasks were incubated at 37 ºC for 24 h. After incubation, the culture-grown medium was subjected to a centrifugation process at 5 K rpm for 15 min. Then the supernatant was subjected to lipase activity assay utilizing *p*-nitrophenyl laureate (p-NPL) as substrate and followed the standard estimation method. Then the absorbance of the sample was recorded at 380 nm using a nanodrop spectrophotometer (NanoDrop™ 2000, Thermo Scientific™, USA) and calculated the unit value using standard calculating formula.

Molecular characterization of bacterial test isolates

The lipase activity (quantitative) assay revealed that only three bacterial isolates (V1, V7, and V8) showed a significant volume of lipase production than others (Ilesanmi et al. 2020). Hence, these three strains were subjected to molecular characterization study by 16S rRNA sequencing for genus and species level identifications. The standard DNA extraction and 16S rRNA sequencing method were followed. Then obtained sequences of these test isolates were subjected to phylogenetic tree analysis to detect the percentage of genetic similarity using the NCBI-BLAST website.

Growth parameters optimization for lipase enzyme production

The microbes will express their maximum metabolic and growth activity under optimal conditions only. Hence, the most suitable (optimal) growth conditions for the lipase enzyme production efficiency test bacterial isolates, such as *Bacillus pumilus* V1, *Bacillus pumilus* V7, and *Bacillus subtilis* V8, were studied. The growth parameters, such as pH (6.0, 7.0, 8.0 and 9.0), temperature (25, 30, 35, 37 and 40 ºC), 300 mg L^{-1} (each) of carbon sources (glucose, sucrose, fructose, and maltose), and 300 mg L^{-1} (each) of nitrogen sources (beef extract, yeast extract, soybean, and peptone), on production medium are as per the requirement. Triplicates were performed for each growth parameter, and lipase yield was estimated by the nanodrop spectrophotometer method and a standard formula calculated yield (Sahoo et al. 2020).

Production of lipase

The extended lipase-producing activities of *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8, were studied under optimized conditions, such as pH 8, temperature 37 ºC, fructose (carbon source), and yeast extract (nitrogen source) in production medium and incubated for 48 h, in a shaking incubator. After incubation, the quantification (yield) of lipase enzyme was determined by spectrophotometer analysis and yield was calculated by a standard formula (Sharma et al. 2017).

Molecular weight determination of lipase by SDS-PAGE

The ammonium sulphate fractionation method was used to extract the lipase (protein) from bacterial test isolates (Sharma et al. 2017). In brief, 24 h-old bacterial culture was centrifuged at 10 K rpm for 10 min. The equal volume of ammonium sulphate (39 g 100 mL^{-1}) solution was added to the supernatant and incubated at 4 ºC overnight. Then this reaction mix was centrifuged at 10 K rpm for 15 min in a cooling centrifuge. Then obtained pellet was dissolved in 0.1 M phosphate buffer (pH 7.0) and subjected to standard SDS-PAGE analysis.

Statistical analysis

The majority of the tests were done in triplicates to ensure accuracy and reproducibility, and the values mentioned in the results are the mean and standard error (SE) of triplicates.

Results and discussion

Isolation of lipase-producing bacteria

Lipases, particularly microbial lipases, are important industrial enzymes. As a result, lipase enzyme screening, synthesis, and purification from microbial strains are constantly evolving to meet the needs of the pharmaceutical and food industries. Interestingly, the results obtained in this study are most likely matched with the lipases found in bacteria that have been reported earlier. About 15 numbers bacterial isolates were enumerated from the Aavin dairy industry effluent contaminated muddy soil sample. In the preliminary screening, out of 15 bacterial isolates, 9 cultures were identified as lipase producers, and these 9 cultures were initially named V1–V9. The preliminary screening was confirmed by developing orange fluorescent color colonies on Rhodamine olive oil agar under UV exposure. It declared that the V1–V9 bacterial isolates possess lipase-producing potential. The secondary screening on Tween 20 agar plate analysis also confirmed that these V1–V9 bacterial isolates have lipase-producing competence and it was confirmed by zone of clearance around the colonies.

Similarly, around 9 bacterial strains from forty bacterial isolates enumerated from the oil contaminated soil have been reported as excellent lipase producers confirmed by fluorescent colonies on Rhodamine olive oil agar media (Lomthaisong et al. 2012). According to another report, the 8 bacterial strains screened from oil contaminated soil can produce lipase, which was confirmed by observing the opaque and pink colour fluorescent (halos around) colonies on olive oil and rhodamine B containing agar media (Alhamdani and Alkabbi 2016). Creating a unique interaction between uranyl fatty acid ion and cationic rhodamine B causes the orange colour fluorescence around the lipase-producing bacterial colonies when observed under UV light (Mohammed 2013). The generation of excited dimers of rhodamine B, which fluoresce at longer wavelengths than the exited monomer, may be related to the mechanism of this color formation (Arbeloa et al. 2007).

Lipase production by quantitative method

The extended lipase generating activity of these V1 to V9 bacterial isolates was examined by quantitative technique in olive oil as a substrate. The results showed that out of 9 isolates 3 strains, V1, V7, and V8 produced a significant volume of lipase as 31.2 µg mL^{-1}, 22.9 µg mL^{-1}, and 18.7 µg mL^{-1}, respectively, confirmed by titration method. Furthermore, these strains were classified as a "very active strain" for lipase production than other strains. Several microbial species have been reported in the scientific community. For example, in bacteria, the species belongs to *Bacillus*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, and *Pseudomonos*, in fungi, *Penicillium*, *Fusarium*, *Aspergillus*, and so on have been recognized as a potential agent for lipase enzyme production (Chandra et al. 2020). The bacterial strain isolated from muddy soil from dairy effluents was suspected as belonging to the *Bacillus* genus and possessing substantial lipase generating capacity in this study. Willerding et al. (2011) identified around 75 bacterial strains out of 440 were identified as lipase producers through the qualitative method. Statistically, these strains were counted as 41% of the total bacterial diversity in Amazonia soil (Willerding et al. 2011). Another bacterial strain, namely *Streptomyces exfoliates* LP10 screened from petroleum contaminated soil, was recognized as an excellent lipase producer through the quantitative method and found that it yielded the lipase ranged from 1.5 to 6.9 IU mL^{-1} (Aly et al. 2012). Similarly, the *B. cereus* isolated from the contaminated soil produced around 225 IU mL^{-1}, and it was quantified by titration method (Hassan et al. 2018).

Molecular characterization of bacterial test isolates

The 16S rRNA sequencing analysis results (1500 bp-sized bacterial sequences) were subjected to NCBI – BLAST analysis and performed similarity search. Obtained results revealed that the bacterial test isolates were *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8. Three strains had a computed value of 16S rRNA gene similarity of ~99% considering the reference data. It was revealed in Fig. 1 that the obtained isolates were phylogenetically connected. It could be inferred that *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 were all relatives of the *Bacillus* genus. Strains V1, V7, and V8 were placed in their correct phylogenetic lineage using the neighbor-joining approach, and their comparative study was conducted. The tree with V1 = 0.11211510, V7 = 0.03374284 and V8 = 0.06522920 is presented here. While the tree branch lengths match those of the phylogenetic distances used to derive the phylogenetic tree, the tree itself is displayed to scale (Fig. 1). Similarly, the *P. gessardi* isolated from oil-contaminated soil has been reported as the considerable potential to produce industrially important lipase enzymes. This strain was characterized through a molecular approach (Yadav et al. 2021). The bacterial species reported from coconut oil mill contaminated soil also possess a significant lipase level producing bacterial diversity. Most of them were identified as Bacillus species, which was validated through a genetic characterization investigation (Bharathi and Rajalakshmi 2019). Another bacterial species, namely the *Pseudomonas xinjiangensis* strain CFS14 identified by 16S rRNA sequencing analysis and culture, was enumerated from oil contaminated soil with excellent lipase enzyme producing potential (Lomthaisong et al. 2012). Another species of *Pseudomonas* enumerated from the soil sample was also reported as an excellent lipase producer, and it was identified as *Pseudomonas aeuriginosa* through 16S rRNA sequencing (Ilesanmi et al. 2020).

Optimization of lipase production

The optimal growth conditions, such as pH, temperature, carbon, and nitrogen sources, are the essential factors determining bacteria lipase-producing competence (Abol-Fotouh et al. 2021). Because the optimal conditions could enhance and balance the metabolic activity of bacteria, facilitating the active lipase production (Hwang et al. 2014).

Effect of pH on lipase production

The pH is one of the most significant factors and that determine enzyme productivity. These *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 showed maximum lipase productivity at pH 8 as 27.8 µg mL^{-1}, 21.5 µg mL^{-1} 20.8 µg mL^{-1}, respectively (Fig. 2a). Furthermore, the lowest lipase production activity was found at pH 6 (2.08 µg mL^{-1}) and pH 9 (8.33 µg mL^{-1}). As a result of these findings, pH 8 was determined to be a suitable pH for lipase production by these bacterial strains (*B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8). The pH of the culture strongly influences cell growth and enzyme production. Some key aspects to consider for the commercial application of enzymes included an enzyme's maximum activity and stability under a mildly alkaline pH 8 (Ibrahim et al. 2021). The elevated lipase activity was observed in the pH range of 7–11, with pH 11 exhibiting the highest activity. Enzyme activity was found to be reduced in the acidic environment. According to the current report, the organism is an alkalophilic bacteria (Ilesanmi et al. 2020). Lipase-producing bacteria have been found to prefer pH levels around 8. Similarly, the suitable pH for *B. stearothermophilus* and *S. rimosus* was pH 7.0 and 8.0, respectively (Dikshit and Kim 2020). The suitable pH of lipase producers, such as *Staphylococcus* sp. (Sirisha et al. 2010) and *Pseudomonas monteilli* (Rasmey et al. 2017), was found as pH 7.0 pH 6.0, respectively, and produced around 35 IU mL^{-1}.

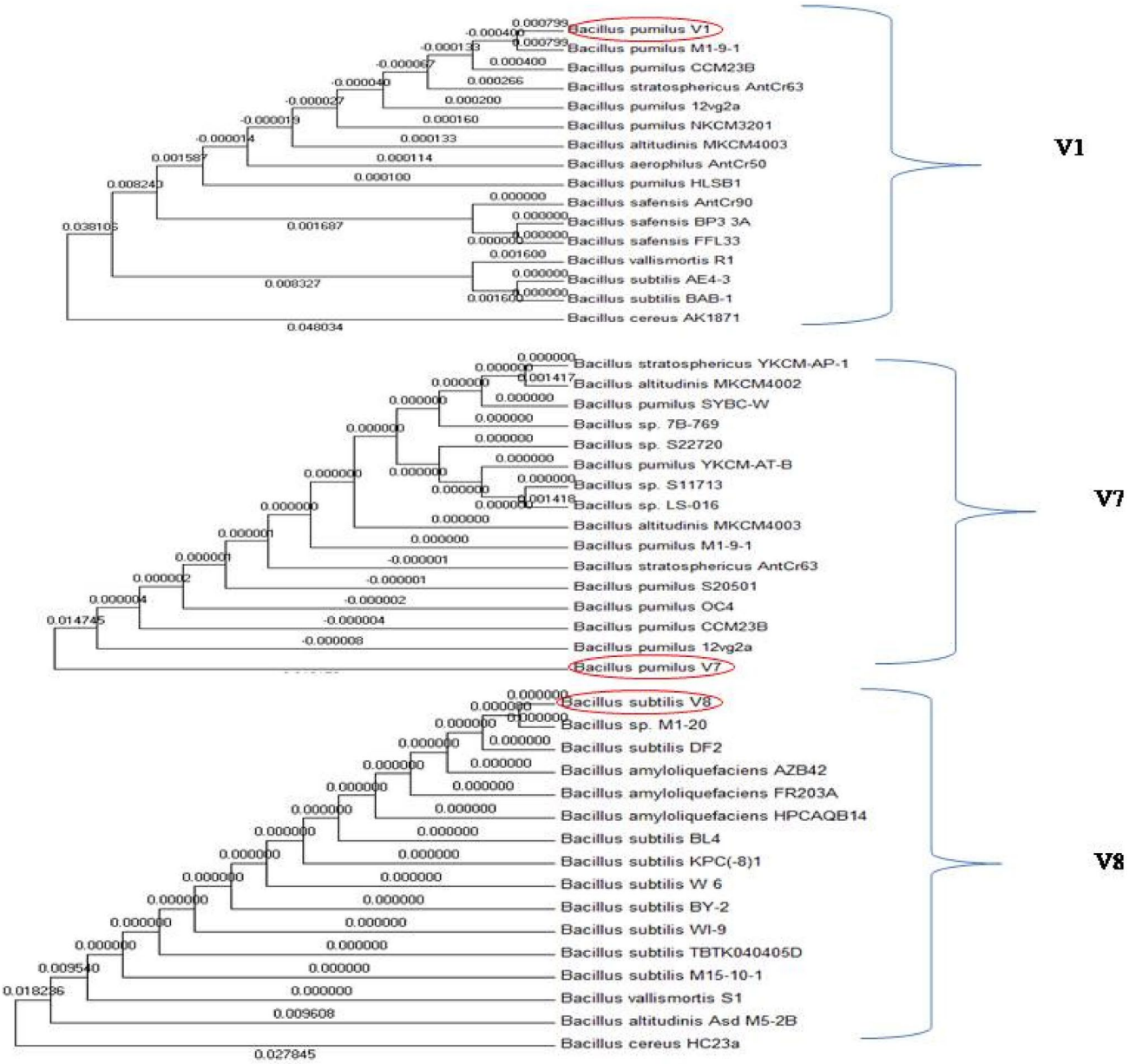

Fig. 1 Evolutionary relationship of excellent lipase enzyme-producing bacteria

Effect of temperature on lipase production

The influence of temperature on lipase synthesis by *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 was examined with various temperatures, such as 25, 30, 35, 37, and 40 °C, for 48 h. The results revealed that the optimal temperature for these strains for lipase production was found as 37 °C. At this optimal temperature (37 °C), *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 produced 27.3, 25.0, and 20.8 μg mL^{-1} lipase enzyme, respectively (Fig. 2b). Temperature influences lipase synthesis primarily through cell development, as demonstrated by a cell growth curve that increased correspondingly with increasing lipase activity (Rajendran et al. 2008). Several researchers investigated the effect of temperature on lipase fabrication over a temperature range of 30–80 °C while keeping all other conditions constant and noted that certain microbial strains are showed excellent lipase activity at increased temperature (Rodrigues et al. 2008). Temperature control enzyme synthesis at the mRNA transcription and, most likely, translation levels of proteins, increasing enzyme stability and production. Another reason for increased synthesis at higher temperatures could be because temperature affects their secretion, possibly by altering the physical features of the

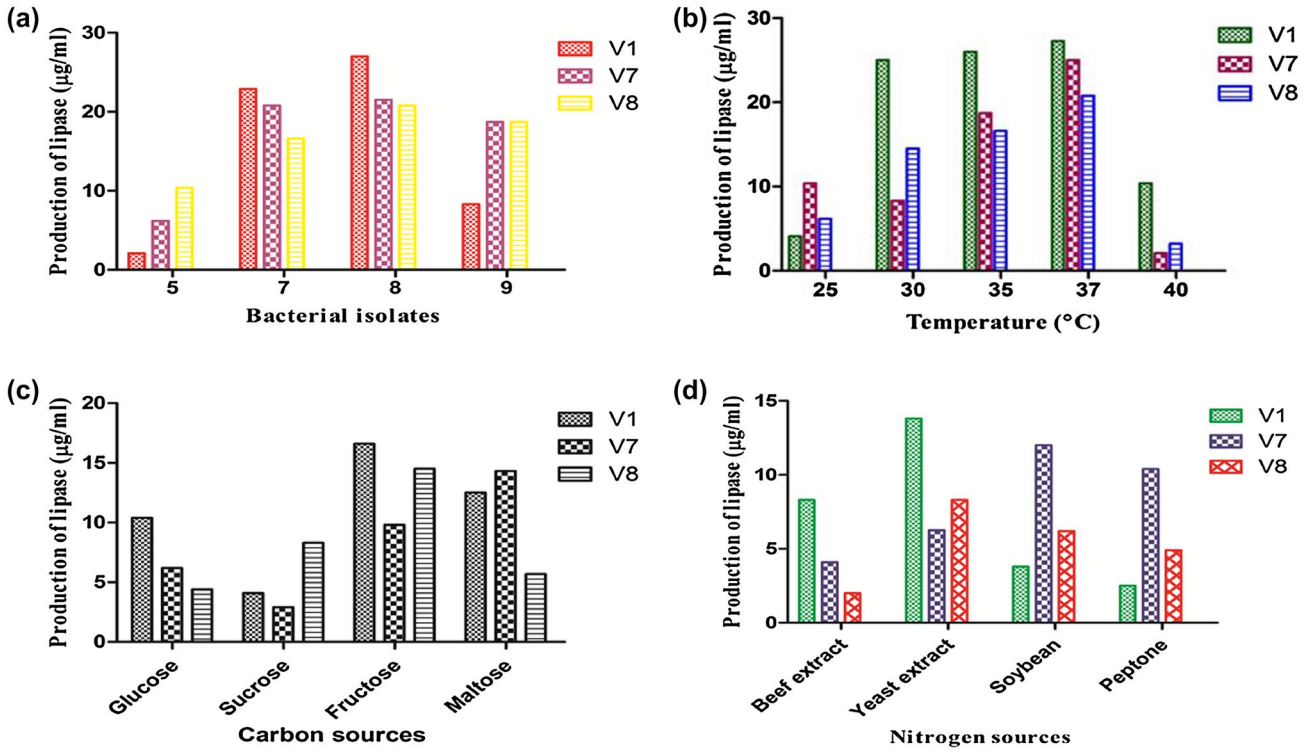

Fig. 2 Determination of Lipase production using olive oil substrate. **a**. Effect of pH in lipase activity, **b**. Effect of temperature in lipase activity. **c**. Effect of carbon sources in lipase activity, **d**. Effect of nitrogen sources in lipase activity

cell membrane. The thermostable *Anoxybacillus* sp. ARS-1 isolated from Taptapani Hotsprin showed the maximum lipase productivity of 29.4 IU g^{-1} at an optimal temperature of 57.5° (Sahoo et al. 2020).

Effect of carbon and nitrogen sources on lipase production

Carbon supply is the primary contributor to the induction of lipase productivity. Both *B. pumilus* V1 and *B. subtilis* V8 prefer fructose as a carbon source and yielded 16.6 µg mL^{-1} and 14.5 µg mL^{-1} (Fig. 2c), respectively. The *B. pumilus* V7 has effectively utilized the maltose as a suitable carbon source and yielded 14.8 µg mL^{-1} of lipase (Fig. 2c). The isolated strains have not efficiently used glucose and sucrose as a carbon source for the lipase production metabolisms. Similarly, among various nitrogen sources, the yeast extract, followed by soybean and peptone, showed a significant level of influence on lipase production in *B. pumilus* V1 (yeast extract), *B. pumilus* V7 (soybean and peptone), and *B. subtilis* V8 (yeast extract) as 14.8, 13.8, and 8.3 µg mL^{-1} (Fig. 2d). While the beef extract has had no significant impact on lipase production by all the bacterial isolates. Carbon sources have proven essential to the production of lipase activity because this enzyme is an inducible one. A higher lipase synthesis in titration is caused by nitrogen-rich nutrients (Magdouli et al. 2017). In our study, the highest level of lipase synthesis was found in yeast extract at a concentration of 12.4 mg/mL. The study reported that minimum lipase activity was calculated to be 0.15 mg/mL in glucose and yeast extract.

The maximum lipase activity in glucose plus peptone was calculated to be 0.24 mg/mL. However, compared to our results, the study results demonstrate that the maximum lipase activity was obtained by providing olive oil to yeast extract for two days. On the other hand, increased lipase levels were produced using *C. cylindracea* to grow in a complex medium containing either 10 or 33% olive oil (Ciafardini et al. 2006). According to the latest study, olive oil is an excellent carbon source for lipase production by bacteria (Ilesanmi et al. 2020). For this study, we found that yeast extract, olive oil, and fructose all had a greater impact on lipase production in *Bacillus sp*. This enzyme can act as the oil–water interface, which makes it a great choice for industrial processes. The bacterial lipases are thermo-stable while plant and fungal lipases are non-specific substrates (Chandra et al. 2020).

Production of lipase

Under optimized conditions, such as pH 8, temperature 37 ºC, fructose (carbon source), yeast extract (nitrogen

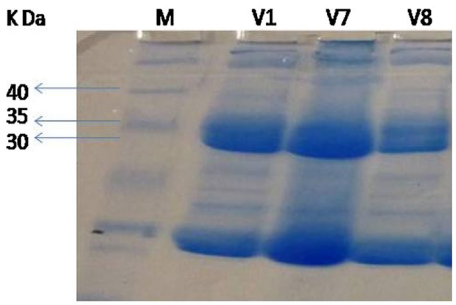

Fig. 3 SDS-PAGE image of the protein pattern of the efficient bacterial strains Lane 1: protein marker, Lane 2: *B. pumilus* (V1), Lane 3: *B. pumilus* (V7), Lane 4: *B. subtilis* (V8)

source), and bacterial test culture *B. pumilus* V1, *B. pumilus* V7 and *B. subtilis* V8 produced 27.8, 25.2, and 16.6 µg mL^{-1} of lipase, respectively. Because, under optimal conditions, cell proliferation, cell metabolism, and similar activities may be more effective, resulting in active production of primary and secondary metabolites (Murthy et al. 2014). Similarly, the growth conditions for *Bacillus* strain KS4 isolated from oil-contaminated soil samples were optimized as 1.16% olive oil, 0.12% tween 80, 5.99 mM $MgCl_2$, and 7.16% inoculum size for highest lipase production as increased from 0.612 IU mL^{-1} to 2.17 IU mL^{-1}. It was calculated as 3.54-fold higher than un-optimized conditions (Sharma et al. 2014). Another report stated that the optimized growth conditions for thermostable *Staphylococcus warneri* isolated from oil-contaminated soil were 55 °C, pH 8.0, 120 rpm, and 2% inoculums yielded 17.21 IU mL^{-1} (Yele and Desai 2015).

SDS-PAGE analysis

The molecular weight of the lipase enzyme produced from *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 were determined by SDS-PAGE analysis. The obtained SDS-PAGE image (Fig. 3) analysis revealed that the lipases extracted from *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 had a similar appearance on an SDS-PAGE gel. They were supposed to have a molecular weight of roughly 35 kDa based on the position of the protein marker (Fig. 3). This result was partially correlated with the findings of Balaji and Jayaraman (2014), who extracted and reported that the molecular weight of lipase enzyme derived from *Bacillus* sp. was found as 31.40 kDa to 50.0 (Balaji and Jayaraman 2014, Sharma et al. 2017). The *Bacillus* sp.-based lipase enzyme possesses excellent temperature stability and alkaline-intolerant detergent resistance, making them an industrial-applicable bacterium (Kroll et al. 2010). *Pseudomonas aeruginosa* HFE733, isolated from residential waste soil samples, generated alkaline lipase with a molecular weight of 51.0 kDa (Hu et al. 2018). Interestingly, another report states that the *Bacillus* sp. isolated from an oil contaminated soil, produced lipase molecular mass was found as 24 kDa (Sivaramakrishnan and Incharoensakdi 2016), and *Pseudomonas aeruginosa* LX1 yielded 56 kDa of lipase (Ji et al. 2010).

Conclusion

Extracellular lipases derived from bacteria have received far more attention than those derived from other microbial kingdoms. In this study, 9 bacterial isolates with lipase-producing activity were identified from Aavin dairy effluent-contaminated muddy soil. Nonetheless, three strains (V1, V7, and V8) out of nine demonstrated significant lipase production, which was confirmed by a quantitative lipase activity assay. These 3 strains were identified as *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 through 16S rRNA sequencing. The optimal growth conditions for these test bacterial isolates for lipase production were found as pH 8, temperature 37 ℃, fructose (carbon source), and yeast extract (nitrogen source). Under these optimized conditions, the test bacterial culture *B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8 yielded 27.8, 25.2, and 16.6 µg mL^{-1} of lipase correspondingly. Furthermore, the molecular size of this study was found as 35 kDa through SDS-PAGE analysis. These findings suggest that the bacterial strains identified from the dairy effluent-contaminated muddy soil (*B. pumilus* V1, *B. pumilus* V7, and *B. subtilis* V8) may be suitable for large-scale production after a fermentation and other growth parameter optimization study.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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