

# Screening and identification of lipolytic potential of Actinobacteria from cemetery in Bonoloyo, Surakarta, Central Java, Indonesia

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**Abstract.** Hardianto AGMP, Rahayu T, Tyastuti EM, Agustina L, Setiyadi NA, Sidiq Y. 2025. Screening and identification of lipolytic potential of Actinobacteria from cemetery in Bonoloyo, Surakarta, Central Java, Indonesia. *Biodiversitas* 26: 2786-2793. Cemetery soil is a unique reservoir of Actinobacteria with a significant lipolytic potential, in which lipids are 12.51-23.60% of the human body, a fact that has been largely overlooked in previous studies. However, studies on this subject are limited. This study, therefore, aimed to assess the lipolytic potential of 29 isolates from the Actinobacteria collection and to characterize the selected isolates further. Lipolytic screening was conducted on Tributyrin Agar (TBA) media for 72 hours, and the potential was determined by Lipolytic Index (LI). Subsequently, the colony morphology and spore type of the selected isolates were characterized through binocular microscopy and Scanning Electron Microscope (SEM). Identification was further conducted based on the 16S rRNA gene. These results showed that almost all tested isolates possess lipolytic ability, with the highest LI of 3.18 (T22). In general, the lipolytic potential of Actinobacteria was found to increase gradually after up to 72 hours of incubation. The aerial and vegetative mycelia were T5 for white and T22 for yellow, respectively, with closed spiral spores. Molecular identification showed that the T22 isolate is closely related to the *Streptomyces* sp. strain GSENDO-0580, with 99.93% similarity. In conclusion, this research underscores the urgent need for an in-depth study of *Streptomyces* sp. strain GSENDO-0580 is urgently needed, considering the extensive application of lipase enzymes in various fields.

**Keywords:** Actinobacteria, cemetery, lipase, lipolytic, lipid

## INTRODUCTION

The phylum Actinobacteria is a group of prokaryotes with a phenotype similar to that of fungi due to slow growth, spore production, and formation of aerial mycelium. The colonies have typical powdery surfaces that are strongly attached to agar media. Given that the nuclear membrane does not cover chromosomes, this group is classified as bacteria with high guanine and cytosine contents in DNA sequences. Based on Gram staining, Actinobacteria are classified as Gram-positive bacteria, with the colonies often producing various unique pigments (Anandan et al. 2016). Their primary habitat is in the soil (Zhang et al. 2019), and as most of the members are aerobic, Actinobacteria are abundant at the surface (Bhatti et al. 2017). According to the previous study, Actinobacteria are present in the cemetery ground at depths greater than 50 cm (Rahayu et al. 2024).

Various beneficial bioactive molecules are produced by Actinobacteria, including antibiotics, enzymes, herbicides, pigments, antitumor agents, growth-promoting hormones, and antiviral compounds (Amin et al. 2020; Selim et al. 2021; Meenakshi et al. 2024). Some members from the genus *Streptomyces*, such as *Streptomyces griseochromogens*, *Streptomyces galbus*, *Streptomyces actuosus*, and *Streptomyces aureofasciculus*, can produce enzymes, including lipases, proteases, cellulases, and amylases, which can be applied in various industries (Filho et al. 2019; Chandra et al. 2020;

Patel et al. 2021). Lipase enzyme is not only produced by microbes but also derived from plants, specifically grains, certain cells of animals, which function to digest fat and lipids, and microbes, including fungi, yeast, and bacteria (Filho et al. 2019). Especially for lipases, microbial ones are dominantly used due to their ability to be produced extracellularly, their stability, and the microbes' simple genetic modification when needed. Microbial lipases from lipolytic Actinobacteria have broad industrial applications, such as waste treatment and biodiesel production (Semache et al. 2021; Zulaika et al. 2021). Members of the genus *Streptomyces* produce lipases with significant activity induced by lipids as a carbon source (Alsaïdy et al. 2023). Moreover, Actinobacteria can produce bioactive compounds such as antimicrobial and antitumor agents (Bawazir et al. 2019; Allouch-Kerboua et al. 2023). These microbes are capable of growing under extreme conditions such as high salinity and high temperature, which enables the production of enzymes that are relatively resistant to harsh environments (Swarna and Gnanadoss 2020).

The place that has the potential to harbor lipolytic microbes is the cemetery, which is a burial site for corpses. In Indonesia, corpses are buried to a depth of 1.5 m. In this site, the process of human body decomposition occurs (Finley et al. 2015), and each body mostly contains lipids (12.51-23.60%), proteins (14.3-18.62%), and water (55.13-67.85%) (Ioan et al. 2017). Some studies have proven the existence of lipolytic microbes inside the cemetery soil.

Studies by Mandiri et al. (2023) and Rini et al. (2023) reported 36 and 30 isolates of lipolytic bacteria collected from a cemetery in Surakarta.

The lipolytic activity of microbes is influenced by incubation time. It has been proven by the study of Mandiri et al. (2023) that the Lipolytic Index (LI) of cemetery soil bacteria fluctuates. After 96 h of incubation, most bacterial isolates presented the highest LI, which then gradually decreased. Another study confirmed that the production of lipases reached a maximum at 48 h of incubation for *Bacillus coagulans* and *Pseudomonas putida* (Semache et al. 2021; Zulaika et al. 2021), at 72 h of incubation for *Pseudomonas* sp. 37 and *Bacillus thuringiensis* (TS11BP) (Bhatti et al. 2017; Alsaïdy et al. 2023), and at 96 h of incubation for *Achromobacter xylosoxidans* J2 (TS2MCN) (Bae et al. 2018).

In our previous study, a total of 23 isolates were collected from cemetery soil at depths of 30 cm and 70 cm. Among the many isolates, 7 were Actinobacteria, which have not been screened for lipolytic potential. Since the literature references on the lipolytic potential of Actinobacteria from cemetery soil are very limited, we aimed to assess the lipolytic potential of Actinobacteria isolates and identify the selected isolate based on the 16S rRNA gene. The results are expected to lead to the discovery of isolates of lipolytic potential Actinobacteria. Lipase is producible and usable in various industries, including bioremediation.

## MATERIALS AND METHODS

### Culture media

Actinomycetes Isolation Agar (AIA) media (HiMedia) was used to grow Actinobacteria isolates (Bawazir et al. 2019), which were prepared by weighing 21.7 g/L AIA and adding 25 µg/mL cycloheximide antibiotic (Sigma Aldrich) to reduce fungal contamination (Bae et al. 2018). The screening of lipolytic potential was performed through the use of Tributyrin Agar (TBA) (Faradila et al. 2020). 10 g of tributyrin, 20 g of bacteriological agar, 5 g of peptone, and 3 g of yeast extract were weighed and then mixed into 1 L of distilled water inside an Erlenmeyer flask to homogenize by heating. Subsequently, Nutrient Broth (NB) media (Oxoid) was used to grow Actinobacteria isolates for DNA genome extraction by weighing in 8 g of the media and dissolving it in 1 L of distilled water. All media were sterilized inside an autoclave at 121°C for 15 minutes (Febrianti and Harun 2022).

### Screening for lipolytic bacteria

The collected Actinobacteria were refreshed with new AIA media through the streaking technique and incubated at room temperature for 7-10 days. Subsequently, lipolytic tests were conducted through the agar plug diffusion method (Ortellado et al. 2021). The incubated isolates were plugged out using a 6 mm diameter sterile cork borer, placed on the surface of selective media of tributyrin agar, and incubated for 72 hours at room temperature. Isolates were considered positive for lipolytic activity when a clear zone around the agar plug formed. The observation was

conducted every 24 h (Rajanikanth and Damodharam 2017). Isolates with a high Lipolytic Index (LI) were assumed to have high lipolytic activity (Chairunnisa et al. 2019). According to Admassie et al. (2022) and Soleha and Retnaningrum (2020), LI can be measured using the formula:

$$\text{Lipolytic Index} = \frac{\text{Clear zone diameter}}{\text{Colony diameter}}$$

### Characterization of the selected Actinobacteria isolates

The selected isolates of Actinobacteria with strong lipolytic ability were characterized for colony morphology and spore type. The colony morphology included the color of the vegetative mycelium, aerial mycelium, elevation, margin, surface, and pigmentation. The spore type was observed under a binocular microscope and Scanning Electron Microscope (SEM). Morphological characteristics of the bacterial isolates were identified using a Scanning Electron Microscope (SEM) after incubation at room temperature for 14 days on Actinomycetes Isolation Agar (AIA) media (HiMedia) without any chemical fixation (Damayanti et al. 2021). After incubation, one loop of bacterial biomass was added evenly on the surface of the carbon-tape-covered stub. The sample was air dried and coated with Au (using Au ion sputter-Hitachi MC1000) and was set at 20 mA for 60 seconds, and observed using SEM Hitachi SU3500 (Japan). The SEM was set in high vacuum mode, accelerating voltage of 3 kV, spot intensity of 30%, and magnification of 5,000x and 10,000x.

### Identification of lipolytic potential isolates based on the 16S rRNA gene

The identification of potentially lipolytic isolates was started with DNA genome extraction using the PrestoTM Mini gDNA Bacteria Kit (Geneaid) procedure with slight modifications to the cell lysis process. About 0.25 g of glass beads were added to 200 µL of Gram+Buffer, and 60 µL of lysozyme stock (20 mg/mL) was added (Ambarwati et al. 2023; Sawitri et al. 2024). DNA genome amplification was performed through 2 universal primers, namely 27F-5'-AGA GTT TGA TCM TGG CTG AG-3' and 1492R-5'-CGG TTA CCT TGT TAC GAC TT-3' (Ambarwati et al. 2023). About 25 µL of mic PCR (PowerPol), 22 µL of ddH<sub>2</sub>O, and 1 µL of each primer were mixed, and 1 µL of template DNA was added. DNA genome was amplified using miniPCR under conditions of initial denaturation at 96°C for 180 s, denaturation at 95°C for 30 s, annealing at 55°C for 30 s, elongation at 72°C for 60 s, and a final extension at 72°C for 300 s (Syakuri et al. 2024). PCR products were visualized through electrophoresis on a 0.8% agarose gel (Deswiniyanti and Astarini 2018). The amplicons were sequenced at the 1<sup>st</sup> base, and the sequencing data were analyzed in comparison to the NCBI database using BLAST and further constructed into a phylogenetic tree through MEGA 11 (Keklik 2023). Interspecies relationships were examined on the basis of GC composition in the 16S rRNA sequences through the composition computational model of MEGA 11 (Keklik 2023).

## RESULTS AND DISCUSSION

### Screening for lipolytic Actinobacteria

In total, 29 isolates of Actinobacteria showed lipolytic activity on tributyrin agar media. LI of each isolate varied at 24, 48, and 72 h of incubation, ranging from 0.00 (S6) to  $2.14 \pm 0.80$  (T8),  $1.22 \pm 0.03$  (S1) to  $2.56 \pm 0.30$  (T1), and  $0.70 \pm 0.00$  (T23) to  $3.18 \pm 0.74$  (T22). In general, LI increased with higher incubation time for isolates T1, T3, T5, T8, T10, T22, T23, T25, T27, T31, T33, T34, T42, T43, S1, S6, S17, and S19. Some isolates presented decreasing LI, including T9, T15, T28, T35, T36, and T41. The rest of the isolates were relatively stable for up to 72 h (Table 1).

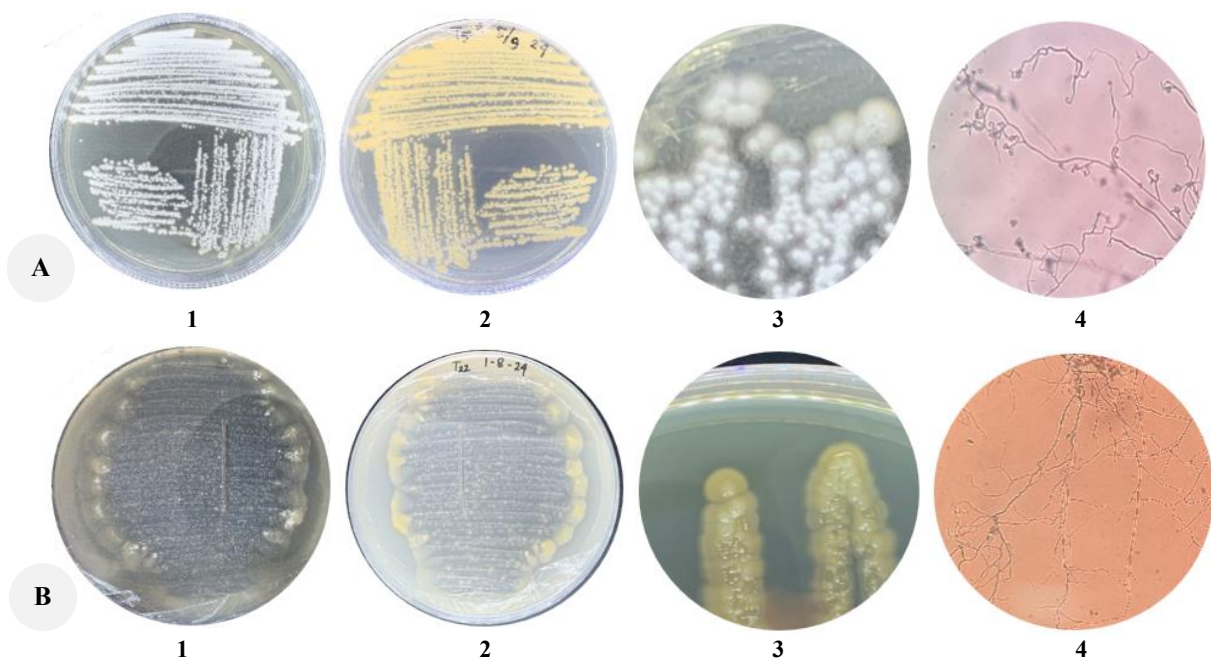
### Characterization of selected Actinobacteria isolates

Lipolytic screening results showed 2 selected isolates, T5 and T22, with a high level of LI of  $2.5 \pm 0.32$  and  $3.18 \pm 0.74$ , respectively (Table 1) with special morphological characters that are close to the Actinobacteria group. We did not select T1 because of its morphology did not show a powdery-like texture, which is typical form Actinobacteria character. The two isolates presented similar morphological characteristics, including yellow vegetative mycelium, powdery surfaces, umbonate elevation, and rounded margins. In comparison, the color of the aerial mycelium was white for T5 and yellow for T22. Both colonies produced diffuse pigments and had closed spiral spores (Figures 1 and 2). The results of Scanning Electron Microscope (SEM) observations of the spore morphology of Actinobacteria isolates at  $10,000\times$  magnification showed morphology, hyphae, and spore chain. These morphological characteristics indicate that the isolates observed have typical morphological characteristics of the genus *Streptomyces*, namely the formation of spores in the form of chains at the ends of

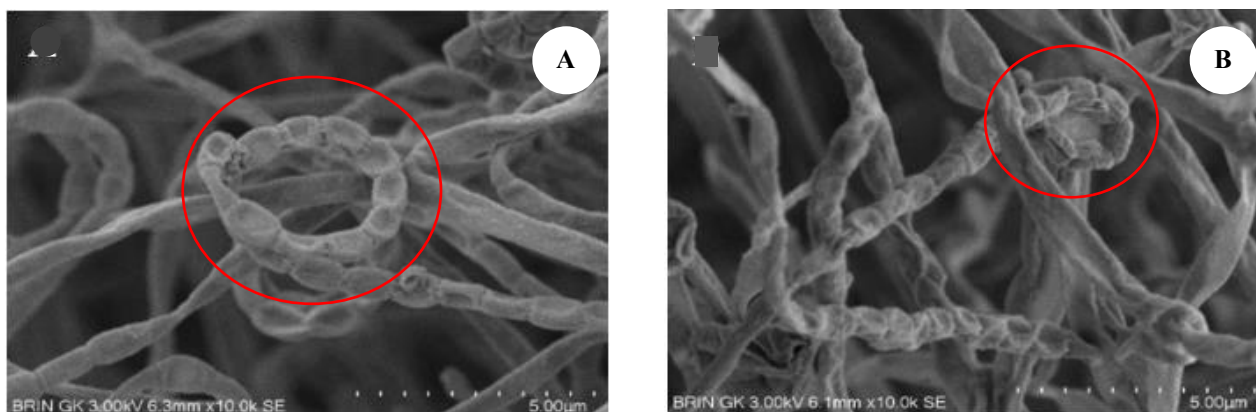
hyphae. These results support the identification of the isolates as a group of Actinobacteria.

**Table 1.** Screening results of the lipolytic potential of the Actinobacteria isolate collection

Isolate code	Lipolytic index hour -		
	24	48	72
T1	$2.01 \pm 0.21$	$2.56 \pm 0.30$	$2.60 \pm 0.20$
T3	$1.50 \pm 0.07$	$1.60 \pm 0.13$	$1.67 \pm 0.0$
T5	$1.65 \pm 0.63$	$2.10 \pm 0.30$	$2.50 \pm 0.32$
T8	$2.14 \pm 0.80$	$2.32 \pm 0.17$	$2.47 \pm 0.02$
T9	$2.06 \pm 0.21$	$2.26 \pm 0.44$	$2.23 \pm 0.12$
T10	$2.00 \pm 0.25$	$2.36 \pm 0.16$	$2.40 \pm 0.25$
T15	$1.04 \pm 0.70$	$1.76 \pm 0.34$	$1.70 \pm 0.06$
T22	$0.90 \pm 0.00$	$2.44 \pm 0.17$	$3.18 \pm 0.74$
T23	$0.95 \pm 0.00$	$1.62 \pm 0.04$	$1.77 \pm 0.22$
T25	$0.70 \pm 0.00$	$1.55 \pm 0.15$	$1.90 \pm 0.30$
T27	$1.43 \pm 0.13$	$1.47 \pm 0.02$	$1.48 \pm 0.06$
T28	$1.69 \pm 0.10$	$1.43 \pm 0.06$	$1.20 \pm 0.06$
T31	$1.50 \pm 0.30$	$2.25 \pm 0.02$	$2.38 \pm 0.20$
T32	$1.22 \pm 0.27$	$1.55 \pm 0.00$	$1.55 \pm 0.00$
T33	$1.15 \pm 0.05$	$1.45 \pm 0.28$	$1.48 \pm 0.29$
T34	$1.20 \pm 0.00$	$1.30 \pm 0.10$	$1.60 \pm 0.20$
T35	$1.30 \pm 0.00$	$2.45 \pm 0.05$	$2.27 \pm 0.00$
T36	$1.59 \pm 0.22$	$1.67 \pm 0.00$	$0.70 \pm 0.00$
T41	$0.95 \pm 0.00$	$1.62 \pm 0.29$	$1.47 \pm 0.14$
T42	$1.20 \pm 0.00$	$1.51 \pm 0.11$	$1.95 \pm 0.00$
T43	$0.60 \pm 0.00$	$1.45 \pm 0.05$	$1.65 \pm 0.05$
T46	$0.37 \pm 0.00$	$1.37 \pm 0.09$	$1.73 \pm 0.06$
S1	$1.10 \pm 0.04$	$1.22 \pm 0.03$	$1.47 \pm 0.11$
S6	0.00	$1.30 \pm 0.10$	$1.68 \pm 0.06$
S17	$0.80 \pm 0.00$	$1.80 \pm 0.00$	$2.45 \pm 0.84$
S19	$0.65 \pm 0.00$	$1.33 \pm 0.13$	$1.47 \pm 0.21$
S20	$1.88 \pm 0.21$	$1.69 \pm 0.14$	$1.69 \pm 0.14$
S22	$1.20 \pm 0.00$	$1.76 \pm 0.00$	$1.76 \pm 0.00$
S23	$1.57 \pm 0.22$	$2.35 \pm 0.00$	$2.35 \pm 0.00$



**Figure 1.** Colony and spore morphology of Actinobacteria isolates: A. T5, B. T22. 1 and 3: Macroscopic morphology aerial mycelium on AIA media, 2: Macroscopic morphology vegetative mycelium on AIA media, 4: Microscopic morphology spore on a binocular microscope with 1000x magnification



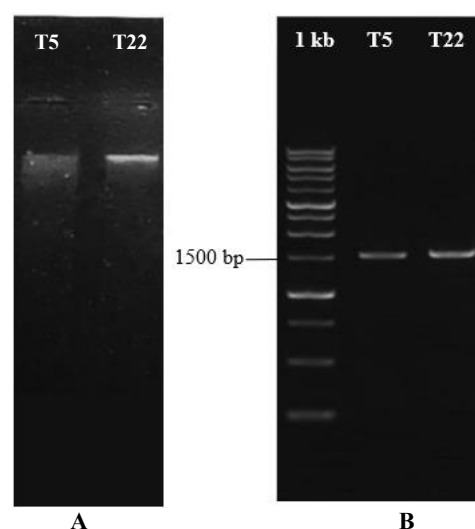
**Figure 2.** Macroscopic morphology on AIA Media and microscopic morphology on SEM 10,000× of magnification: A. Morphology of *Streptomyces* sp. VEL17 (T5); B. *Streptomyces* sp. GSEND0-0580 (T22)

### Molecular identification of potential lipolytic isolates of Actinobacteria

Two selected isolates, T5 and T22, were molecularly identified based on the 16S rRNA gene, starting with DNA genome extraction and then continuing with amplification by the universal primers 27F and 1492F, resulting in a 1500 bp amplicon (Figure 3). T5 has the closest match to *Streptomyces* sp. VEL17, with 99.86% similarity, while T22 has a very close relationship with *Streptomyces* sp. GSEND0-0580, with 99.93% similarity (Table 2). A phylogenetic tree was constructed to determine the kinship relationships among T5, T22, and other isolates deposited in GenBank (Figure 6).

**Table 2.** Nucleotide similarity analysis of T5 and T22 isolate compared with NCBI database

Closest species	Similarity (%)	Accession number
<b>T5</b>		
<i>Streptomyces</i> sp. VEL17	99.86	AB914463.2
<i>Streptomyces indiaensis</i> strain IF 5 1	99.51	FJ951435.1
<i>Streptomyces</i> sp. MAH25	99.44	AB899157.1
<i>Streptomyces</i> sp. VITTKGB	99.44	GU358071.1
<i>Streptomyces exfoliatus</i> strain MMA 1033	98.88	KY580807.1
<i>Streptomyces</i> sp. strain UeEPI15	98.60	MH553633.1
<i>Streptomyces</i> sp. strain HmuEPI22	98.12	MH553624.1
<i>Streptomyces</i> sp. TIR12	98.46	AB899158.1
<i>Streptomyces exfoliatus</i> strain A156.7	97.21	MH547398.1
<i>Streptomyces indiaensis</i> strain A164	97.02	MH547399.1
<b>T22</b>		
<i>Streptomyces</i> sp. GSEND0-0580	99.93	GQ924491.1
<i>Streptomyces</i> sp. ACT-0090	99.93	GQ924530.1
<i>Streptomyces</i> sp. strain BS-AP-5	99.50	OQ438806.1
<i>Streptomyces</i> sp. BS-2	99.43	OQ438812.1
<i>Streptomyces</i> sp. strain MJM14731	99.36	MG980052.1
<i>Streptomyces adustus</i> strain TN105	99.36	ON406141.1
<i>Streptomyces pseudovenezuelae</i> strain TU15	99.36	MH482875.1
<i>Streptomyces</i> sp. CS-2 (2012)	99.36	JQ065719.1
<i>Streptomyces</i> sp. ACTYS11	99.36	JX430841.1
<i>Streptomyces adustus</i> strain WH-9	99.29	NR 151949.1



**Figure 3.** Electrophoregram of isolates T5 and T22: A. Genomic DNA extraction results, B. PCR products

### Discussion

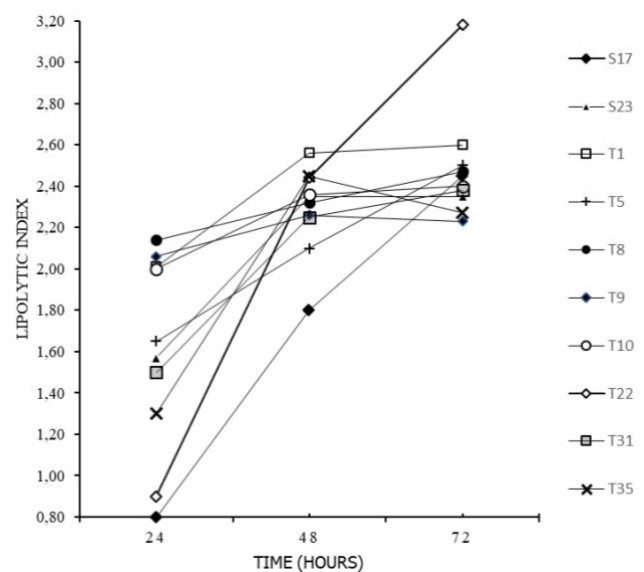
The lipolytic activity of bacteria isolates on tributyrin media was indicated by the formation of a clear zone around the colony (Oktavia and Wibowo 2017). The clear zone can be attributed to the hydrolysis of tributyrin by lipolytic bacteria, resulting in the production of a water-soluble butyric acid (Oktavia and Wibowo 2017; Mandiri et al. 2023). Tributyrin-selective media ease the observation of the result while supporting the growth of bacteria (Pratama et al. 2017). There are many other lipolytic selective media aside from tributyrin, but none yield better results than tributyrin does. Tween 80 provides a less clear visual image, resulting in bias (Hassan et al. 2018), and media with 9-(2-Carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-iminium chloride olive oil provide a qualitative result, causing a subjective reading (Jaiganesh and Jaganathan 2018). Tributyrin media is also capable of selecting lipolytic bacteria more precisely than other media, such as olive oil, soybean oil, groundnut oil, and coconut oil (Welz et al. 2021; Nimkande et al. 2023).

Almost all isolates of Actinobacteria showed lipolytic activity (Table 1). Lipolytic bacteria usually inhabit a sample containing lipids. For example, 28 bacterial isolates from hospital liquid waste were successfully purified, and 92.86% of the samples showed lipolytic activity (Amin et al. 2019). In addition, *Litopenaeus vannamei* pond sediment and soil in Antarctica store lipolytic Actinobacteria, each accounting for 50% and 76.6%, respectively, of the total purified isolates (Lamilla et al. 2017; Ayuningrum et al. 2022). The correlation between decomposing microbes and substrate availability is significantly strong (Valdés et al. 2020). Indeed, the existence of decomposing microbes depends on the availability of substrates. For example, when lipids exist, lipolytic bacteria will also be found (Lamilla et al. 2017). A study in Iran showed that nutrient-poor source samples from deserts produced only 3 isolates of Actinobacteria with lipolytic activity (Abedi-Dolatabadi et al. 2024).

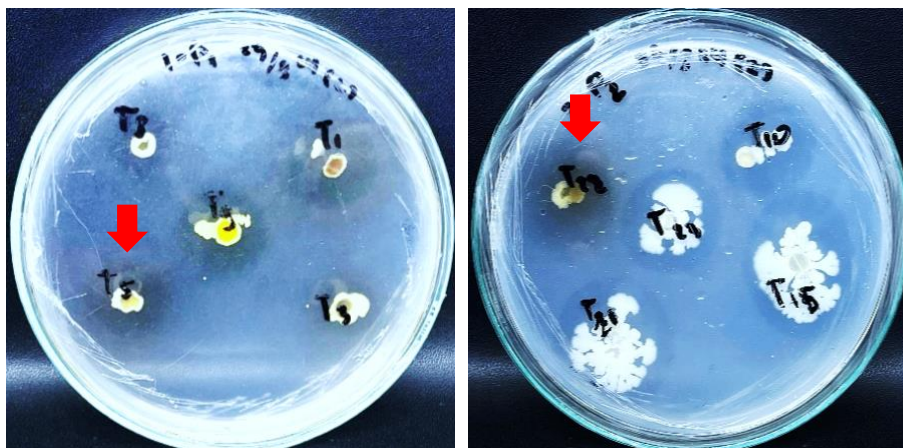
The strong lipolytic activity of Actinobacteria from cemetery soil was shown by LI of  $2.50 \pm 0.32$  and  $3.18 \pm 0.74$  for T5 and T22 isolates, respectively (Table 1 and Figure 4), which is considered high lipolytic activity. This is presumably due to cemetery soil as a corpse burial site that contains the human body 12.51-23.60% lipids (Ioan et al. 2017). The screening results for lipolytic activity from various lipid-containing sources also showed that bacteria isolates with high Lipolytic Activity (LI) were collected from oil-containing waste at 3.12 (Golani et al. 2016), shrimp farming pond sediment at 6.5 and 3.2 (Ayuningrum et al. 2022), CPO at 8.8 (Soleha and Retnaningrum 2020), Pracimaloyo cemetery soil at 2.50 (Mandiri et al. 2023), and Bonoloyo cemetery soil at 5.43 (Rini et al. 2023).

LI of isolates after 72 h of incubation increased, although some gradually decreased (Table 1 and Figure 5). This shows that the incubation time affects the production of lipase except for the carbon source, nitrogen source, pH, and agitation rate (Golani et al. 2016). In this study, tributyrin was used as the primary carbon source to induce lipase production. According to Panyachanakul et al. (2024), the presence of fats and oils in the medium is not

necessarily a key determinant for lipase induction. Abedi-Dolatabadi (2024) reported that lipase was produced at the end of the logarithmic phase. A similar result was reported by Abedi-Dolatabadi (2024), specifically for Actinobacteria. Other groups of bacteria also presented various lipase activities. A study by Mandiri (2023) reported that the LI of cemetery soil bacteria fluctuates; after 96 h of incubation, most of the bacteria presented the highest LI. Another study reported that *Pseudomonas* sp. produced lipase at a maximum of 72 h of incubation, while *Bacillus coagulans* and *Pseudomonas putida* 922 produced lipase at 48 h (Fatima et al. 2014; Bharathi et al. 2019). Lipase production is inducible, which probably also leads to different results among bacteria isolates (Mehta et al. 2017).



**Figure 5.** Lipolytic activity of Actinobacteria isolates at 24, 48, and 72 h of incubation

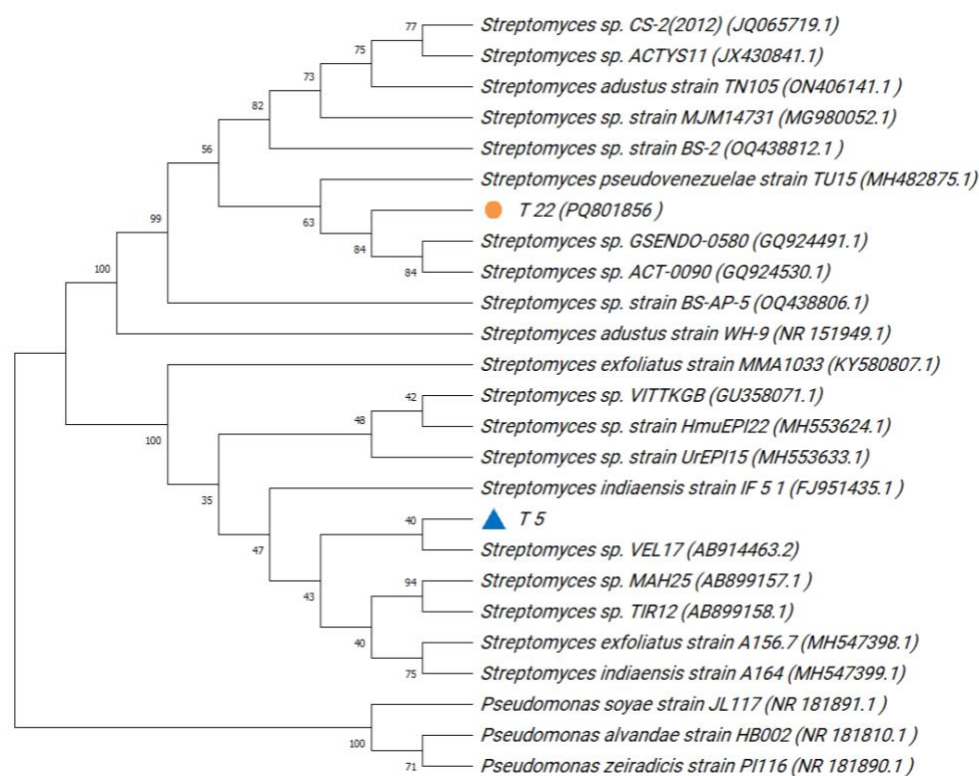


**Figure 4.** Lipolytic activity of Actinobacteria on tributyrin media. Extracellular microbial lipase release by Actinobacteria isolates formed the halo zone surrounding the colony. The halo/clear zone can be seen surrounding the isolate code T5 and surrounding isolate T22

Two isolates, namely T5 and T22, were selected for the highest LI and then subjected to molecular identification based on the 16S rRNA gene. This gene is the reference for bacteria identification since it is a universal marker that is highly conserved in all prokaryotic species. The length of the 16S rRNA gene is 1600 bp, and it contains nine hypervariable regions, V1-V9 (Fukuda et al. 2016; Antil et al. 2023), which differ among bacterial strains. Here, amplification from the bacteria's DNA genome was carried out through the universal primers 27F and 1492R, which amplify the whole 16 rRNA gene, approximately 1550 bp long (Qurban and Ameen 2020), with the electrophoregram showing a suitable result (Figure 3). There is also a large amount of accessible data on 16S rRNA gene of bacteria, easily accessible in GenBank (Qurban and Ameen 2020).

Morphologically, both T5 and T22 isolates have Actinobacteria characteristics, including a powdery colony surface, strong attachment to agar media, the production of

pigments, and the formation of spores (Anandan et al. 2016). Moreover, the analysis of the GC content of the 16S rRNA showed compositions of 54.97% and 58.20% (Table 3), which are reassuringly consistent with the GC content commonly found in Actinobacteria (Anandan et al. 2016). The two selected isolates are related to *Streptomyces* sp. VEL17 and *Streptomyces* sp. GSENDO-0580, each with 99.86% and 99.93% similarity, respectively (Table 2). As stated by Drancourt and Raoult (2002), a similarity value of more than or equal to  $\geq 99\%$  indicates that isolates are the same species, while a similarity value  $\geq$  less than 97% suggests isolates are of the same genus. The reconstruction of the phylogenetic tree also showed the proximity among species in one group (Figure 6). According to SEM analysis, the isolated showed spore chain formation, which is typical of *Streptomyces*, with straight chains or flexible (Shrivastava et al. 2015; Rani et al. 2024).



**Figure 6.** Phylogenetic tree of selected potentially lipolytic isolates

**Table 3.** The 16S rRNA nucleotide gene composition

Species	Nucleotides composition (%)						Total
	T(U)	C	A	G	GC	AT	
<i>Streptomyces</i> sp. strain BS-2	18.61	25.09	22.88	33.43	58.51	41.49	1451
<i>Streptomyces</i> sp. GSENDO-0580	18.85	24.72	22.79	33.63	58.36	41.64	1448
T22	18.97	24.84	22.83	33.36	58.20	41.80	1397
<i>Streptomyces</i> sp. ACT-0090	18.96	24.61	23.00	33.42	58.04	41.96	1487
<i>Streptomyces indiaensis</i> strain IF 5	19.89	23.64	24.94	31.50	55.15	44.84	1387
<i>Streptomyces</i> sp. MAH25	19.92	23.81	25.03	31.22	55.03	44.96	1390
<i>Streptomyces</i> sp. VEL17	19.89	23.79	25.09	31.21	55.01	44.98	1387
T5	19.95	23.84	25.07	31.12	54.97	45.02	1388

Many Actinobacteria genera are reported to have lipolytic activity (Lu et al. 2022). For example, *Streptomyces clavuligerus* produced 3000 UL-1 lipase after 36 h of incubation (dos Santos et al. 2017), *Streptomyces* sp. TEM33 produced lipase at 1.74+0.0005 U/g dry substrate (gds) (Vasconcelos 2018), and *Streptomyces* sp. A3301 produced lipase at 321 U/mL after 3 days of incubation (Panyachanakul et al. 2024). *Streptomyces* Al-Adebi-49 also yielded lipase at 172 U/mL after 6 days (Al-Dhabi et al. 2020), while *Streptomyces* sp. BT3 and *Streptomyces* sp. BT4 each produced 0.80 and 1.39 U/mg crude lipase, respectively (Welz et al. 2021). Lipase is broadly used in various fields, including detergent production (lipid stain removal), starch and fuel (synthesis of lipase-catalyzed biodiesel), baking (dough stability and conditioning), food processing (improvement of food texture), pulp and paper (pitch control, contamination control), as well as environmental application in degradation of lipid waste, removal of solid and water solutions by hydrocarbons, oils and lipids (Ramnath et al. 2017).

In conclusion, this study found that Actinobacteria isolated from cemetery soil in Surakarta have potential lipolytic activity namely the isolates T5 and T22. The two selected isolates are related to *Streptomyces* sp. VEL17 and *Streptomyces* sp. GSENDO-0580, respectively. Therefore, further research can be done on these two isolates to develop optimization strategies for increasing lipase enzyme production that can be applied in various fields.

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