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Biocatalytic Potential of a *Raoultella terrigena*-Derived Lipolytic Enzyme for High-Performance Detergents

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Abstract: Dump sites harbour microorganisms with potential for environmentally friendly industrial applications. This study assessed the lipolytic activity of municipal dumpsite-associated bacteria and evaluated the stability of the most potent isolate's lipolytic enzyme against laundry detergents. It also examined the crude lipase's ability to remove stains from cotton fabric. Among twelve bacteria isolated, five demonstrated notable halo zones on tributyrin agar plates. The diameters (mm) were MN38 (11 ± 1.4), MN1310 (8.5 ± 0.7), MN28 (6.5 ± 0.71), MN18 (7.0 ± 1.4), and MN310 (8.15 ± 0.21). Quantitative analysis revealed that MN38 exhibited the highest lipase activity (14.76 ± 0.27 U/mL), while MN1310 showed the lowest (6.40 ± 0.85 U/mL). Nucleotide sequence analysis identified the isolates as *Raoultella terrigena* veli18 (MN38), *Stenotrophomonas maltophilia* veli96 (MN1310), *Viridibacillus* sp. veli10 (MN28), *Stenotrophomonas* sp. veli19 (MN18), and *Klebsiella* sp. veli70 (MN310). The crude lipase from *R. terrigena* veli18 maintained 73.33%, 52.67%, 55.0%, and 54.0% of its original activity after 60 min of exposure to Sunlight, Surf, Maq, and Omo, respectively. Adding crude lipase to enzyme-free laundry detergents significantly enhanced their cleaning efficacy, completely removing oil stains from cotton fabric. This performance of *R. terrigena* veli18 crude lipase highlights its potential as an effective detergent bio-additive.



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1. Introduction

Hydrolases are essential enzymes contributing to sustainable development across sectors. α/β hydrolases serve as important biocatalysts in the chemical and pharmaceutical industries, facilitating chiral compound production under eco-friendly conditions [1]. In bioremediation, microbial hydrolases, especially lipases and esterases, effectively transform hazardous pollutants into less detrimental substances [2,3]. Lipases are multifunctional enzymes that facilitate long-chain acylglycerol breakdown and formation [4]. These enzymes can be extracted from animals, plants, and microorganisms, with microbial sources being the most commercially valuable [5]. Microorganisms, particularly bacteria, fungi, and yeasts, are favoured for lipase production due to their high substrate specificity, stability, and cost-effective yield [6]. Lipase applications span industries such as fat and oil processing, food processing, detergent production, environmental remediation, and biodiesel synthesis [7–9]. They also play crucial roles in the pharmaceutical, cosmetic, and waste management industries [10]. Lipases' widespread use is attributed to their exceptional ability to operate at water–lipid interfaces and reverse reactions in non-aqueous environments [5].

Bacterial lipases offer benefits over fungal equivalents, including resilience to harsh conditions, simplified mass production, and susceptibility to genetic modification. Their molecular weights span 19–96 kDa, with optimal functioning conditions between 15 and 70 °C and pH 5.0 and 10.8 [11]. The catalytic centre features a triad resembling serine proteases, shielded by a lid-like formation that shifts upon substrate interaction [12]. Recent progress in protein engineering and immobilization methods has enhanced bacterial lipases' characteristics, boosting their industrial applicability [11].

Isolating lipase-producing bacteria from dump sites offers benefits for enzyme production, as these locations contain microorganisms capable of breaking down waste through enzyme secretion [13]. Several bacterial species, including *Pseudomonas* spp., *Bacillus* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Arthrobacter* spp., *Alcaligenes* spp., and *Achromobacter* spp., have been recognized as effective lipase producers [8,9,13–15]. Gram-negative bacteria have been reported as lead producers of lipases [16].

Microbial lipases have become essential in developing environmentally friendly detergents due to their adaptability and ecological benefits. These enzymes break down lipid-based stains, reducing the need for harmful chemical components in cleaning products [17]. Bacterial lipases from *Staphylococcus arlettae* JPBW-1, *Staphylococcus aureus* ALA1, and *Pseudomonas aeruginosa* showed remarkable stability when exposed to surfactants, commercial detergents, and oxidizing agents [18–20]. Alongside nonionic detergents, these lipases can improve washing effectiveness, eliminating up to 62% of oil from stained fabrics [19]. Crude lipases produced through solid-state fermentation could enhance washing performance, increasing effectiveness from 52% to 74% when used with detergents [21]. This highlights the potential of microbial lipases in creating eco-friendly and efficient detergent formulations. The current research aimed to isolate bacterial strains from a municipal dump site and assess their lipolytic potential. The identity of the isolates producing copious extracellular lipases was confirmed using molecular techniques. The study examined the stability of the lipolytic enzyme from the most effective lipase-producing strain, identified as *Raoultella terrigena* veli18, in commercial laundry detergents. The crude lipase's effectiveness in removing stains from fabric was also assessed for industrial prospects.

2. Materials and Methods

2.1. Sample Collection, Processing, and Bacteria Isolation

The soil samples used for the isolation of lipolytic bacteria were obtained from a municipal dump yard that receives various waste materials from local enterprises and households in Raymond Mhlaba Municipality, South Africa. The samples were processed within six hours of collection. A small portion (5 g) of each soil sample was dissolved in 50 mL of sterile distilled water and mixed thoroughly using a vortex mixer (Digisystem Laboratory Instruments Inc., Xizhi Dist., New Taipei City, Taiwan). A 10-fold dilution of the samples was prepared using sterile distilled water. From each dilution, 100 µL was withdrawn and inoculated onto basal salt media (BSM) agar prepared with the following compositions (g/L): 0.5 yeast extract, 0.5 NaCl, 5 (NH₄)₂SO₄, 1.0 K₂HPO₄, 0.5 MgSO₄, KH₂PO₄, 15 bacteriological agar (Merck chemicals (Pty) Ltd., Modderfontein, Gauteng, South Africa), and 10 mL of olive oil (Faithful to Nature, Muizenberg, Cape Town, South Africa). The isolation media were amended with 50 mg/L of nystatin (Merck chemicals (Pty) Ltd., Modderfontein, Gauteng, South Africa) to stop the growth of fungal species. The plates were inspected for bacterial growth after incubation at 30 °C for 72 h. Colonies with variable morphological characteristics were selected and purified by streaking them onto newly prepared BSM agar until axenic colonies were obtained. The BSM slants were prepared, the pure bacterial isolates were transferred onto them, and they were grown for 24 h at 30 °C. The slants were kept at 4 °C for further analysis.

2.2. Fresh Inoculum Preparation and Qualitative Activity Evaluation

Axenic bacteria were inoculated onto freshly prepared BSM supplemented with olive oil, and plates were placed in an incubator (Labotec (Pty) Ltd., Midrand, Gauteng, South Africa) set at 30 °C. After 24 h, the plates were retrieved from the incubator, and grown bacterial colonies were transferred into microtubes containing sterile saline and homogenized by vortexing. The optical density of the bacterial suspension was properly adjusted to read 0.1 at 600 nm using a spectrophotometer (PerkinElmer, Singapore). The homogeneous bacterial solution served as a fresh inoculum for the subsequent experimentation.

2.2.1. Screening for Lipolytic Potential Using Tributyrin Agar

Tributyrin agar plates were prepared with 3 g/L peptone, 2 g/L yeast extract, 10 mL/L tributyrin (Merck (Pty) Ltd., Modderfontein, Gauteng, South Africa), and 15 g/L bacteriological agar (the pH of the medium was adjusted to 8.0) [22]. The media were sterilized with an autoclave (Already Enterprise Inc., Beitou District, Taipei City, Taiwan) at 121 °C and 15 psi and allowed to cool below 45 °C before pouring into Petri dishes. After adequate gelling of the media, the plates were inoculated with 20 µL of the various freshly prepared bacterial suspension and incubated at 30 °C for 72 h. After incubation, the plates were removed from the incubator and observed for a clear zone around the colonies due to the degradation of tributyrin.

2.2.2. Screening for Lipolytic Potential Using Tween-20 Agar

The isolates that showed appreciable halo zones on tributyrin agar plates were further screened in this step. Tween-20 (Sigma-Aldrich, St. Louis, MO, USA) agar plates were prepared using the following compositions: 10 g/L peptone, 5 g/L NaCl, 0.1 g/L CaCl₂, 10 mL/L Tween-20, 15 g/L bacteriological agar, and the medium pH was adjusted to 8.0 [23]. The media were sterilized by autoclaving at 121 °C and 15 psi. Holes (5 mm) were made at the centre of the Tween-20 agar plates, which were then inoculated with 50 µL of the selected bacterial suspensions and incubated at 30 °C for 72 h. The plates were assessed for calcium salt precipitate around the cork-bored holes post-incubation.

2.2.3. Screening for Lipolytic Potential Using Phenol Red Agar

Additionally, the selected isolates were evaluated for lipolytic potential using phenol red agar prepared with the following compositions: 3 g/L yeast extract, 5 g/L peptone, 1.0 g/L CaCl₂, 0.1 g/L phenol red (Merck (Pty) Ltd., Modderfontein, Gauteng, South Africa), 10 mL/L olive oil, 15 g/L bacteriological agar [22,24]. The medium was adjusted to pH 7.3, the endpoint pH for phenol with NaOH [25]. The medium was then autoclaved at 121 °C and 15 psi. Holes (5 mm) were made at the centre of the phenol red agar plates, which were then inoculated with 50 µL of the selected bacterial suspensions and incubated at 30 °C for 72 h. After incubation, the media plates were monitored for a colour change around the holes containing the bacterial cultures. The drop in pH from neutral to acidic condition due to the hydrolysis of phenol red by lipolytic bacteria causes a colour change in the media near the well.

2.3. Quantitative Evaluation of Isolates for Lipolytic Potential

The production of the extracellular lipases by the selected isolates was carried out via submerged fermentation using the following compositions: 0.5 g/L yeast extract, 0.5 g/L NaCl, 5 g/L (NH₄)₂SO₄, 1.0 g/L K₂HPO₄, 0.5 g/L MgSO₄, 0.8 g/L KH₂PO₄, and 10% (v/v) olive oil. The media flasks were sterilized at 121 °C and 15 psi and cooled. The flasks were inoculated with the standardized bacterial suspensions (2%, v/v) and then incubated in an orbital shaker for 72 h at 30 °C and 130 rpm. The cell-free extracts of the fermentation media

were recovered by centrifugation (HERMLE Labortechnik GmbH, Siemensstr, Wehingen, Germany) at $15,000 \times g$ for 10 min and were used for the lipolytic activity assay.

2.4. Lipolytic Activity Assay

A lipolytic activity assay was conducted using 4-nitrophenyl palmitate (Gentham Life Sciences, Corsham, UK), as Ramnath et al. [22] described. The substrate mixture consisted of 3 mM 4-Nitrophenyl palmitate substrate in 20% methanol, 50 mM Tris-HCl buffer (pH 8), and 0.1% triton X-100. The assay mixture containing 200 μ L of the substrate and 30 μ L of the crude supernatant was incubated at 37 °C for 1 h. After incubation, the assay mixture was monitored for the release of p-nitrophenol by measuring the absorbance at 405 nm using a SYNERGYMx 96-well microplate reader (BioTek Instrument Inc., Winooski, VT, USA). The amount of enzyme required to liberate 1nM of p-nitrophenol per minute under the specified assay protocol was considered as one unit (U) of lipolytic activity.

2.5. Identification of Lipolytic Bacteria

The genomic deoxyribonucleic acid (DNA) of bacterial isolates showing good lipolytic activity was isolated from whole bacterial cells using the salting-out method [26]. DNA templates were used to conduct a polymerase chain reaction (PCR) on the T100 Thermal Cycler (Bio-Rad, Hercules, CA, USA). Taq 2 $\times$ master mix, universal oligonucleotides [27], and other molecular reagents utilized for the PCR were purchased from Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, Gauteng, South Africa). The PCR microtubes with a reaction volume of 25 μ L consisted of nuclease-free water (5.5 μ L), master mix (12.5 μ L), forward primer (1.0 μ L), reverse primer (1.0 μ L), and DNA template (5.0 μ L). The PCR assay followed the previously reported conditions [28]. After the reaction, amplicons were visualized with gel on ethidium bromide-stained agarose using an ultraviolet transilluminator (Uvitec, Cambridge, UK). The PCR products were further purified, and cycle sequencing was performed using an Applied Biosystems 3500xL series Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The .ab1 files obtained were analysed for evolutionary relationships against GenBank. The isolates' nucleotide sequences were deposited in the National Center for Biotechnology Information database.

2.6. Evaluation of Enzyme Stability with Commercially Available Detergents

The lipolytic enzyme stability in commercially available laundry detergents was evaluated, as reported previously [29]. Laundry detergents purchased from supermarkets, including Sunlight, Omo, Surf (Unilever, uMhlanga, Durban, South Africa), and Mag (Bliss brands (Pty) Ltd., Longdale, Johannesburg, South Africa), were diluted in tap water to give a final concentration of 7 mg/mL. The endogenous enzymes in the detergents were inactivated by heating the detergent solution for 30 min at 100 °C before the crude lipase addition. The enzyme–detergent solution was thoroughly mixed and allowed to stand for 30 min at 37 °C. Aliquots were used to determine the residual enzyme activity under the standard assay conditions. The control experiment was performed by substituting the detergent solution with 100% distilled water.

2.7. Lipolytic Enzyme Evaluation for Oil Stain Removal

The capacity of the lipolytic enzyme to remove oil from cotton fabrics was assessed using the method described by Paul et al. [30]. The application of lipolytic enzyme as a detergent additive was studied using white cotton cloth pieces (5 cm $\times$ 5 cm) stained with oil. The stained fabrics were placed into separate flat-bottom flasks. One flask contained only tap water (50 mL), the second flask contained tap water (50 mL) with Sunlight (7 mg/mL) and was lipase-free, and the third flask contained tap water (10 mL) with Sunlight (7 mg/mL) and was enzyme-free with 40 mL of crude lipase solution. After

incubation at 37 °C for 15 min, the cloth pieces were removed, rinsed with distilled water, and dried. The stain removal capability of the crude lipolytic enzyme was visually assessed. The oil-stained fabric immersed in tap water without adding either the detergent solution or the crude lipolytic enzyme served as the control.

2.8. Statistical Analysis

The data accrued from triplicate experiments were analysed using Duncan's multiple range test for analysis of variance in the Statistical Package for the Social Sciences version 23. The mean difference was compared at $p < 0.05$.

3. Results

3.1. Bacterial Isolation and Qualitative Evaluation of Lipolytic Activity

Municipal dump yards receive a significant amount of solid organic waste, and the microbial community in this habitat actively decomposes these waste materials. The variety of solid waste found in landfills could, therefore, influence the plasticity and metabolic diversity of the microbiome. Consequently, we explored the municipal dumpsite for bacterial isolates with lipolytic potential. Twelve isolates were recovered from the soil samples based on their distinctive morphological characteristics. On initial evaluation using tributyrin agar (Figure 1a), 5 out of the 12 isolates demonstrated good lipolytic activity with the following diameters of halo zones (mm): MN38 (11.0 ± 1.4), MN1310 (8.5 ± 0.7), MN28 (6.5 ± 0.71), MN18 (7.0 ± 1.4), and MN310 (8.15 ± 0.21), as presented in Table 1. Further assessment of these five isolates for lipolytic activity on Tween-20 agar indicated that there were differential deposits of calcium salts around the holes (Figure 1b). While on phenol red agar, the five isolates changed the colour of the media around the bacterial culture from red to yellow (Figure 1c).

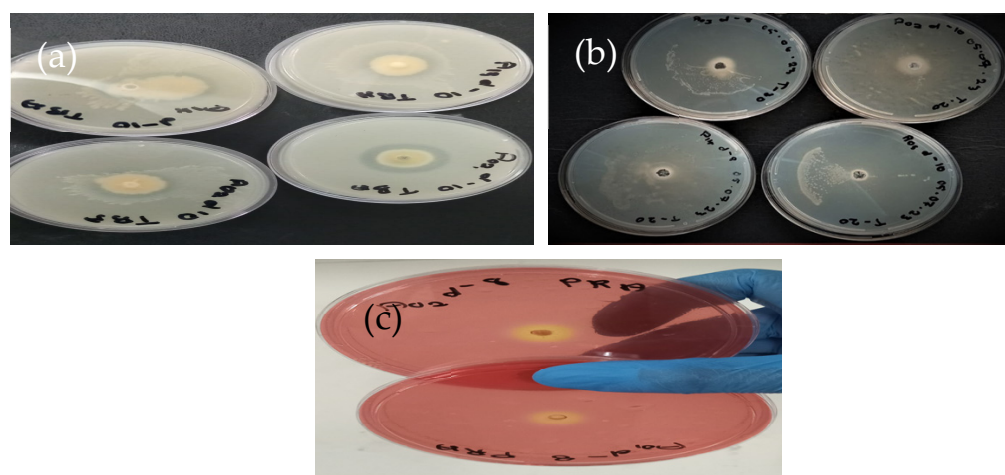


Figure 1. Evaluation of bacterial strains from the municipal dump site for lipolytic potential on (a) tributyrin agar, (b) Tween-20 agar, and (c) phenol red agar.

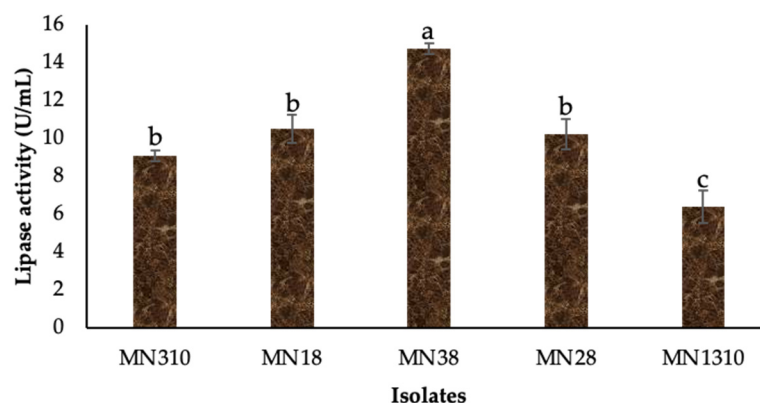
3.2. Quantitation of Lipase Production and Bacterial Identification

The quantitative evaluation of the bacterial strains for lipolytic activity showed that MN38 had the highest lipase titre of 14.76 ± 0.27 U/mL among the isolates (Figure 2). The remaining isolates—MN310, MN18, MN28, and MN1310—had extracellular lipase activities of 9.10 ± 0.28 , 10.53 ± 0.74 , 10.25 ± 0.79 , and 6.40 ± 0.85 (U/mL), respectively.

Table 1. Diameter of halo zones formed by lipolytic bacteria on tributyrin agar.

S/N	Isolate Code	Diameter (mm) *
1.	MN38	11.0 ± 1.4^a
2.	MN1310	8.5 ± 0.7^b
3.	MN28	6.5 ± 0.71^c
4.	MN18	7.0 ± 1.4^{bc}
5.	MN310	8.15 ± 0.21^{bc}
6.	MN210	2.25 ± 0.35 ^d
7.	MN118	0
8.	MN135	0
9.	MN35	0
10.	MN15	2.25 ± 0.35 ^d
11.	MN148	2.35 ± 0.49 ^d
12.	MN48	3.1 ± 0.14 ^d

* The values with similar letters (a, b, c, d) are not statistically different from one another. The isolates in bold showed significant lipolytic activities on a plate assay using tributyrin agar.

**Figure 2.** Quantitative assessment of lipolytic enzyme production by the bacterial strains isolated from the municipal dump site. The bars with similar letters (a, b, c) are not statistically different from one another.

The sequencing of the 16S rRNA gene and phylogenetic analysis showed that MN38 and MN1310 showed a high percentage of sequence homology with *Raoultella terrigena* PKG-3X-1D (accession number: KJ685494) (98.92%) and *Stenotrophomonas maltophilia* L15 (accession number: KF358258) (100%); consequently, they were identified as *Raoultella terrigena* veli18 and *Stenotrophomonas maltophilia* veli96, respectively (Table 2). In addition, MN28 had 98.21% sequence similarity with *Viridibacillus arenosi* YT114 (accession number: MW405667) and *Viridibacillus arvi* (accession number: HG798348); MN18 showed 99.40% sequence similarity with *Stenotrophomonas maltophilia* SN_33 (accession number: MT448767) and *Stenotrophomonas geniculata* NM208 (accession number: MT114514); and MN310 demonstrated 99.46% sequence similarity with *Klebsiella oxytoca* RCB646 (accession number: KT260858) and *Klebsiella pneumoniae* E3-2 (accession number: KP058371). Based on the incomplete discrimination among the species of the bacterial strains, MN28, MN18, and MN310 were identified as *Viridibacillus* sp. veli10, *Stenotrophomonas* sp. veli19, and *Klebsiella* sp. veli70, respectively. The nucleotide sequences of *R. terrigena* veli18, *S. maltophilia* veli96, *Viridibacillus* sp. veli10, *Stenotrophomonas* sp. veli19, and *Klebsiella* sp. veli70 were submitted to GenBank and were assigned the accession numbers PQ278865, PQ278868, PQ278866, PQ278867, and PQ278869, respectively (Table 2).

Table 2. Identification of lipolytic-enzyme-producing bacteria using 16 rRNA gene sequencing.

S/N	Isolate Code	Reference Organism	Percentage Similarity (%)	Sequence Identity	Accession Number
1.	MN38	<i>Raoultella terrigena</i> PKG-3X-1D (KJ685494)	98.92	<i>Raoultella terrigena</i> veli18	PQ278865
2.	MN28	<i>Viridibacillus arenosi</i> YT114 (MW405667) <i>Viridibacillus arvi</i> (HG798348)	98.21	<i>Viridibacillus</i> sp. veli10	PQ278866
3.	MN18	<i>Stenotrophomonas maltophilia</i> SN_33 (MT448767) <i>Stenotrophomonas geniculata</i> NM208 (MT114514)	99.40	<i>Stenotrophomonas</i> sp. veli19	PQ278867
4.	MN1310	<i>Stenotrophomonas maltophilia</i> L15 (KF358258)	100	<i>Stenotrophomonas maltophilia</i> veli96	PQ278868
5.	MN310	<i>Klebsiella oxytoca</i> RCB646 (KT260858) <i>Klebsiella pneumoniae</i> E3-2 (KP058371)	99.46	<i>Klebsiella</i> sp. veli70	PQ278869

3.3. Stability Profile and Washing Performance of the Crude Lipase Against Laundry Detergents

The stability study with commercially available laundry detergents showed that the crude lipolytic enzyme retained 73.33% of its original activity after 60 min of treatment with Sunlight (Figure 3). The enzyme showed residual activities of 52.67%, 55.0%, and 54.0% against Surf, Maq, and Omo.

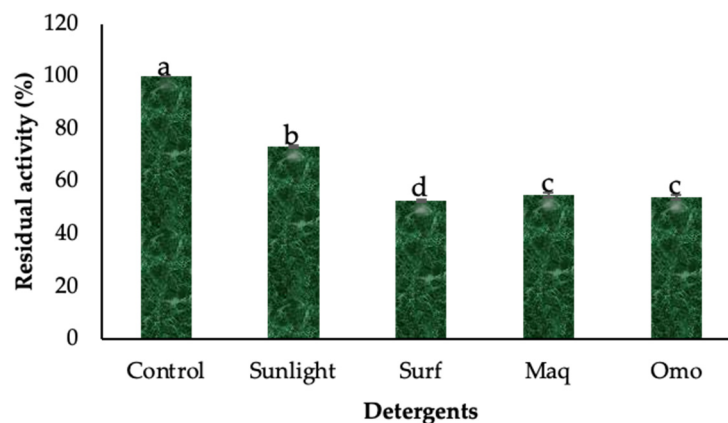


Figure 3. Effect of laundry detergent on the stability of lipolytic enzyme from *R. terrigena* veli18. The bars with similar letters (a, b, c, d) are not statistically different from one another.

Based on the stability profile of the enzyme (i.e., retaining above 50% of the original activity after 60 min of incubation with the various laundry detergents), the oil-stained fabric washing performance of the crude lipolytic enzyme was assessed (Figure 4). Sunlight detergent with deactivated endogenous enzymes significantly removed the oil stain on the cotton fabric (Figure 4b) compared to the control (Figure 4a). But, when the sunlight was supplemented with the crude lipolytic enzyme, the washing performance improved remarkably, with complete removal of the oil stain from the cotton fabric observed visually (Figure 4c).

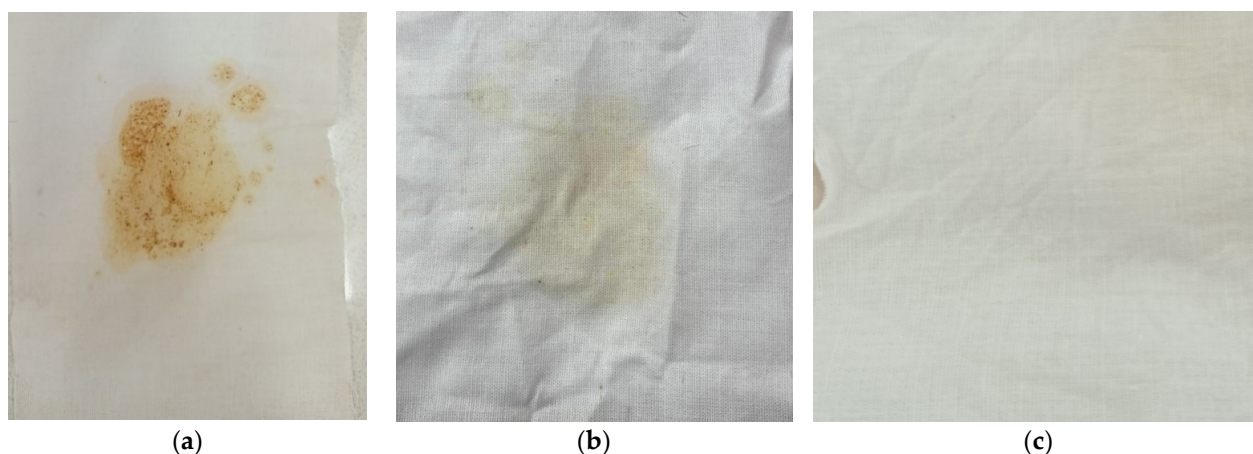


Figure 4. Washing performance evaluation of crude lipolytic enzyme from *R. terrigena* veli18: (a) cotton fabric stained with oil; (b) oil-stained cotton fabric washed with enzyme-free detergent; and (c) oil-stained cotton fabric washed with detergent supplemented with lipolytic extract.

4. Discussion

The study of lipolytic bacteria has gained immense attention from researchers developing cleaner and sustainable technological processes. Consequently, different environmental habitats have been explored for isolating microbial species with robust genetic and metabolic properties for producing versatile lipolytic enzymes [19]. Municipal dump sites are complex chemical and microbial ecosystems where the microbial community converts diverse renewable and synthetic materials into nutrients for their adaptation and survival. Therefore, this study explored the microbial diversity of a local municipal dump site for bacteria with lipolytic potential. The initial qualitative screening of the isolates for lipolytic properties was implemented using a tributyrin agar plate assay. The clear zone around the colony indicated an exo-production of lipolytic enzymes, and this method has been widely employed for the initial evaluation of wild microbial isolates for lipase production [25,31]. However, tributyrin hydrolysis indicates the presence of lipases or other esterases [32]. While esterases primarily catalyse the hydrolysis of ester bonds in short-chain fatty acids, lipases exhibit a significantly wider range of substrate specificity than esterases [33]. The lipolytic potential of the bacterial strains with appreciable halo zones on tributyrin agar was further confirmed on Tween-20 agar plates. The observed calcium salt precipitate around the cork bore hole demonstrated the presence of bacterial lipase in the extracellular medium. Lipolytic enzyme secretion by the bacterial strain has been identified in other reports through the formation of insoluble calcium salt of the fatty acid, emanated from Tween hydrolysis [34]. Both Tween-20 and Tween-80 yield similar results in agar plate assays. However, Tween-20 has been affiliated with esterase activity as it contains lower fatty acid esters, while Tween-80 has been associated with lipase activity in the presence of oleic acid esters [35]. The released fatty acids are further conjugated with the calcium salts in the medium, forming insoluble crystals around the inoculation site [36]. The last step of screening the isolates for lipase activity was on a phenol red agar plate, where the liberated free fatty acids caused a drop in medium pH around the culture, thereby causing a change in the media colour from red to yellow [37]. This step was used as a confirmatory test to validate the lipolytic activity of the bacterial isolates from the municipal dump site. This screening method has been strongly recommended for evaluating and selecting potent lipase-producing strains [22,38]. Rhodamine B and triolein agar plate assays have also been utilized to confirm the presence of lipase [32]. As identified with the qualitative screening, the bacterial strains' expression of both esterase and lipase activity is a promising indicator for developing sustainable bioprocesses. Though isolated from the same environment, the

differential extracellular lipase activity by the isolates on solid media demonstrates their genetic distinctiveness and metabolic diversity.

The identification of the isolates indicated that they are predominantly (80%) Gram-negative bacteria, except for *Viridibacillus* sp. Gram-negative bacteria have shown great potential in biotechnological processes, leading to innovative developments. *R. terrigena* veli18 showed the maximum lipase activity among the isolates characterized in this study, and a recent study by Zhao et al. [39] likewise reported lipolytic-enzyme-producing and oil-degrading *R. terrigena*. Gram-negative bacteria are manipulatable for expressing desired traits, which is crucial from industrial and biotechnological viewpoints. The sourcing of efficient lipolytic isolates from petrol-contaminated soil indicated that Gram-negative bacteria, including *Escherichia* sp., *Staphylococcus* sp., *Pseudomonas* sp., and *Klebsiella* sp., were predominantly present among the best-performing isolates [40]. Similarly, Luz and colleagues reported in their study that among 25 isolates that showed extracellular lipase activities, *Serratia marcescens* and *Pseudomonas fluorescens*, which are within the confines of Gram-negative bacteria, expressed the highest lipolytic activity [41]. Out of 196 bacteria analysed for fat, oil, and grease (FOG) degradation, 11 isolates showed remarkable activity during lipid degradation profiling. These isolates comprised ten Gram-negative bacteria ($\approx 91\%$), including *Acinetobacter* sp., *Aeromonas* sp., *Pseudomonas* sp., and *Staphylococcus* sp., and one Gram-positive bacterium—*Bacillus* sp. [42]. Among these isolates, *Aeromonas* sp. and *Staphylococcus cohnii* demonstrated an outstanding biodegradation of FOG substrates by removing 37.9% of oleic acid and 19.1% of triolein, respectively, after 7 days [42]. *Chryseobacterium gleum* and *Bacillus velezensis* were the top lipase-producing isolates among the bacteria isolated from palm oil sewage sludge [43]. The isolation of Gram-negative bacteria with lipolytic-enzyme-producing capacity from contaminated sights underscores their involvement in bioremediation and nutrient recycling. Considering this, Sutar and co-workers recently characterized *Staphylococcus petrasii* sub sp. *jettensis* VSJK R1 with the capacity to degrade lipid-rich materials from restaurant wastewater [44].

Though Gram-negative bacteria have shown to be the workhorses in various industrial and biotechnological developments, their applicability has been drastically impeded by the fact that they are associated with public health problems due to their high resistance to antibiotics [45]. Compared to Gram-positive bacteria, Gram-negative bacteria exhibit greater resistance to antimicrobial agents. These microorganisms employ various strategies to combat antimicrobials, such as utilizing efflux pumps, producing enzymes that break down the drugs, and modifying the sites where the antimicrobials bind. Gram-negative bacteria's rapid growth and genetic stability in large-scale cultures make them appealing candidates for producing important enzymes [46]. Therefore, modifying bacterial isolates through genetic engineering to remove unwanted genes could offer a solution to generating reliable strains suitable for multifaceted applications. An alternative approach in perspective would be to express the desired enzyme-secretory machinery of the Gram-negative bacteria within an industrially competent host to minimize the need for handling pathogenic strains.

The evaluation of crude lipase stability in different laundry detergents showed a decrease in enzyme activity after 1 h of incubation, and this lower residual activity observed could be attributed to the negative impact of the detergent's ingredients on the structural configuration of the lipolytic enzyme. Lipase from *Pseudomonas plecoglossicida* S7 showed variable stability in the presence of the tested commercial detergent, with 29% residual activity against Ariel, even in an immobilized state [47]. Contrariwise, crude lipases assessed for their potential compatibility with various commercial detergents (Sufi, Brite, Ariel, Surf excel, Express power, Bonus) demonstrated enzyme stability profiles $>50\%$ [48]. Additionally, a lipolytic enzyme from *S. arlettae* JPBW-1 showed remarkable structural stability with the various commercial detergents tested, retaining above 80% of the original activity against

Surf excel, Tide, Wheel, Ariel, and Nirma [19]. Detergent ingredients, including colourants, fragrances, pH modifiers, optical brighteners, surfactants, antiredeposition agents, and water softeners, among others, have been identified to affect the stability of enzymes in detergents [49]. In modern detergents, enzymes have transitioned from minor additives to crucial components, offering substantial benefits over traditional chemical processes in terms of sustainability and efficiency [50]. Ensuring enzyme–detergent compatibility is crucial for application purposes [47]. The fundamental advantage of incorporating bio-additives into detergents is their ability to effectively eliminate stains at significantly lower temperatures while decreasing water usage. This dual benefit makes them eco-friendly and cost-effective, as they reduce energy consumption without compromising cleaning efficiency. Researchers have investigated the use of encapsulated enzymes to improve the stability of enzymes in detergent formulation [51]. The incorporation of enzymatic encapsulation in detergent formulations provides multiple advantages, including (1) enhanced catalytic stability against fluctuations in temperature and pH during washing and (2) improved resilience to organic solvents and other inhibitory substances present in detergents [52]. Earlier studies also reported the use of recombinant DNA technology in creating robust microbial lipase for industrial applications [53].

The efficient oil removal performance of crude lipase from *R. terrigena* veli18 underpins its application potential as a detergent bio-additive. The study conducted by Chauhan and colleagues indicated that a buffer–detergent–lipase admixture significantly improved oil removal from cotton fabric compared to buffer and detergent only [19]. Similarly, the supplementation of heat-treated Surf excel with crude lipase extracts improved its stain removal capacity on cotton fabric stained with egg yolk, ketchup, olive oil, and butter [48]. As industries and consumers search for methods to efficiently and effectively remove various stains without harming fabrics or the environment, enzymes are becoming an increasingly crucial ingredient in detergents. Several enzymes, including lipases, proteases, mannanases, cellulases, pectinases, and amylases, are already commonly incorporated into cleaning products to tackle a wide array of typical stains. Lipases stand out as one of the most significant biocatalysts in biotechnology due to their versatility and commercial viability [48]. These enzymes are environmentally friendly, non-toxic, easily mass-produced, and adaptable for various industrial processes. Lipase functions as a green additive in detergents by degrading fatty/oily stains, allowing the detergent’s surfactants to attach to and remove the remaining stain particles from materials. Lipase creates a fabric–enzyme complex that forms a protective barrier when applied to material surfaces. This barrier prevents the enzyme from washing away and stops oily substances from adhering to the material [54]. Detergents’ endogenous enzymes break down stains, whereas non-biological detergents employ alternative stain removal methods. This may include more potent chemicals, higher wash temperatures, or extended soaking periods, which might be eco-unfriendly and involve high energy consumption. Bio-based detergents are efficient at lower temperatures and in shorter wash cycles than their non-biological counterparts. This clean technological development results in cost savings for consumers and low environmental impact for manufacturers [55]. The ability of *R. terrigena* veli18 crude lipase to efficiently destain oily cotton fabric in static washing conditions is noteworthy and warrants further studies.

5. Conclusions

In conclusion, this study explored the bacterial diversity of a local municipal dumpsite for lipase-producing bacteria. Isolate MN38, identified as *R. terrigena* veli18, demonstrated remarkable lipolytic potential with a high extracellular lipase titre. This shows that municipal dumpsites are a good source of microbial species with industrial and biotechnological application potential. The crude extract was moderately stable in the presence of selected

laundry detergents. Incorporating enzymes into cleaning products has been deemed necessary in modern-day formulations, as these bio-additives enhance their effectiveness in stain removal while minimizing environmental harm. Enzymes enable detergents to remove various stains with minimal energy use. Hence, the excellent washing performance of *R. terrigena* veli18 crude lipase against oil-stained cotton fabric underscores the enzyme's potential as a detergent additive. Therefore, future research will focus on cloning and the heterologous expression of the lipase-encoding genes from *R. terrigena* veli18 in industrially competent *E. coli*, as well as the molecular modification, optimization, and upscale production of lipase. Purification, as well as biochemical and structural characterization of the enzyme, are also being considered for their suitability in industrial applications.

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Abbreviations

The following abbreviations are used in this manuscript:

BSM	Basal salt media
FOG	Fat, oil, and grease
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
U	Unit

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