Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Identification of Lipase Producing *Staphylococcus saprophyticus* Li-B5 with Potential Bioremediation Applicability

Asmita Shrestha[1,2], Gyanu Raj Pandey[1,2], Sudip Silwal[1], Raj Kumar Shrestha[1], and Dinesh Tiwari[1]
[1]Shubham Biotech Nepal Pvt. Ltd., Bharatpur-29, Chitwan, 44200, Nepal
[2]Biotechnological Research and Development Center Pvt. Ltd., Bharatpur-29, Chitwan, 44200, Nepal
Received: 02 Feb 2025; Revised: 03 Jul 2025; Accepted: 17 Jul 2025; Published online: 31 Jul 2025

Abstract
Bioremediation is a biological treatment process that uses microorganisms to biodegrade environmental pollutants. In this study, lipase production, emulsification of hydrocarbons, growth in the presence of inhibitors, and decolorization of triphenylmethane dyes by the selected isolate were tested. Lipase production was confirmed by the development of a halo region around the colonies on tributyrin agar by the isolate. The isolate was identified as *Staphylococcus saprophyticus* by 16S rRNA gene sequencing and biochemical features. Among different hydrocarbons used, the maximum emulsification index was found on xylene (83.33 ± 3.33) % and minimum on diesel (26.67 ±3.33) %. The isolate was able to decolorize malachite green, phenol red, fuchsin, and crystal violet with maximum decolorization was observed for malachite green (96.53 ± 0.69) % and minimum with crystal violet (27.04 ± 1.13) %. The isolate could grow in the presence of growth inhibitors like phenol and lead acetate but was unable to grow in the presence of mercuric chloride. This study suggests that the identified isolate, *Staphylococcus saprophyticus* Li-B5, is suitable for bioremediation because it can emulsify different hydrocarbons, decolorize various dyes, and produce lipase enzymes.

Keywords: Bioremediation, *Staphylococcus saprophyticus*, Bioemulsifiers, Lipase, Triphenylmethane dyes.

Corresponding author, email: asmitashrstha@gmail.com

Introduction
The growth of urbanization and industrialization has exposed the environment to a variety of toxins that are potentially harmful to living beings. Pollutants emitted by many industrial operations are significant contributors to soil and aquatic pollution. Many pollutants, such as antimony, chromium, and mercury are present in the wastewater from dye-producing companies (1). The use of herbicides, pesticides, and fertilizers in agriculture introduces pollutants like aluminum, copper, zinc, nickel, lead, and arsenic into the environment (2,3). Crude oil is also a major environmental pollutant due to pipeline vandalism, transportation leak, and/or accidental spillage (4). The majority of compounds in crude oil are hydrocarbons, including hazardous substances such as benzenes, poly-aromatic hydrocarbons, and oxygenated polycyclic aromatic hydrocarbons, all of which pose serious threats to humans, animals, and plants in the environment (5).

The leakage of crude oil and other harmful dyes such as cotton blue, crystal violet, malachite green, and methyl violet from the textile, leather, cosmetic, pharmaceutical, and paper industries represents a direct threat to the environment due to their toxicity and carcinogenicity (6,7). As a result, these pollutants must be addressed by physical, chemical, or biological approaches (8). Physical and chemical approaches have been utilized for years, but they have difficulties, such as the requirement for an expert and specific equipment for the chemical remediation operation, while the physical remediation procedure is costly (9). The best alternative for physical and chemical remediation processes is biological remediation, also known as bioremediation.

Bioremediation is a biological treatment process that uses bacteria and fungi for the biodegradation of environmental pollutants (10). Following the 1989 leak of 41 million liters of petroleum from the Exxon Valdez in Alaska, bioremediation techniques were significantly improved. From 1993 to 1997, Exxon invested more than ten million dollars in bioremediation investigations, resulting in several patents (11). Bioremediation is used to lower the concentration and/or toxicity of many compounds, including petroleum derivatives (aliphatic and aromatic hydrocarbons), industrial solvents, insecticides, and metals using microorganisms (12). Microorganisms offer numerous advantages when used as pollutant-removing agents in soil, water, and sediment environments. They help restore the natural conditions by preventing further pollution (13).

Recent studies have identified more than 79 bacterial genera capable of degrading petroleum hydrocarbons (14). Some of the bacteria include *Achromobacter*, *Acinetobacter*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*,

Burkholderia, *Staphylococcus*, *Streptobacillus*, *Streptococcus*, and *Rhodococcus* (15–17). Fungal genera including *Amorphoteca*, *Neosar toryagraphium*, *Aspergillus*, *Penicillium*, *Cephalosporium*, *Talaromyce,* and yeast genera *Yarrowia*, *Candida*, and *Pichia* are capable of degrading hydrocarbons pollutants in the environment (18). Various enzymes produced by these organisms, such as cytochrome P450, laccase, dehalogenase, dehydrogenase, hydrolase, protease, and lipase play vital roles in bioremediation (19).

Among the enzymes produced by microorganisms, lipases are particularly useful in the bioremediation of greasy effluents comprising oils, lipids, and proteins released from a variety of sources (20). A recent study found that *Pseudomonas sp.* from petroleum oil-contaminated areas tested positive for lipase production, supporting its potential use in breaking down petroleum oil-polluted soils. This highlights the importance of considering lipase-producing microorganisms as a crucial component in formulating bioremediation strategies for petroleum oil spills (21). In addition to lipase production, the emulsification of hydrocarbons and decolorization of triphenylmethane dyes are also important factors for bioremediation (7,22). However, to date no recent studies have investigated the role of *Staphylococcus saprophyticus* in the bioremediation of heavy metals and oil.

In this study, we isolated a bacterial strain *Staphylococcus saprophyticus*, as a contaminant on tributyrin agar. The strain was tested for its ability to produce lipase, emulsify hydrocarbon, and decolorization of triphenylmethane dyes. The present paper reports on the identification of the isolate and its potential for use in the bioremediation of petroleum hydrocarbons and triphenylmethane dyes.

Materials and methods

Isolation, screening, and culture maintenance

The organisms isolated were contaminants on tributyrin agar (5 g/L peptone; 3 g/L yeast extract; 10 mL/L tributyrin; 15 g/L agar; pH 7.5) (23). Lipase-producing isolates were selected based on the presence of halo zones surrounding colonies on tributyrin agar. These isolates were subsequently subcultured onto nutrient agar plates for further purification and analysis. All the organisms were preserved by lyophilization and stored at -80℃ for long-term storage and bacterial slants were prepared and stored at 4℃ for research activities. The isolate with the highest index on tributyrin agar was selected for further research. The index was calculated by the formula: (colony diameter + halo diameter)/ colony diameter.

Identification of selected isolate

Molecular characterization

DNA extraction and sequencing

The isolate was cultured on nutrient broth and incubated at 30 ℃ for 24 hours. DNA extraction was performed according to the procedure described by Sambrook and Russel (24). The 16S rRNA gene of the isolate was amplified using universal primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) and then sequenced at Macrogen Inc., South Korea.

The raw sequence was assembled and clipped using Codon Code Aligner version 11.0.3 and deposited at the NCBI GenBank, with accession number MT256296.1. Subsequently, the sequence was compared with the National Center Biotechnology Information database using BLASTN. Twelve highly similar sequences were obtained in FASTA format for phylogenetic analysis.

Maximum likelihood analysis of taxa

The evolutionary history was inferred by using the maximum likelihood method and the Tamura-Nei model (24). The bootstrap consensus tree inferred from 2000 replicates, was taken to represent the evolutionary history of the taxa analyzed (25). Evolutionary analyses were conducted in MEGA11 (26).

Morphological and biochemical characterization

The selected isolate was identified using morphological and biochemical characteristics according to Bergey's Manual of Systemic Bacteriology (27). For macroscopic characterization, colony form, elevation, margin, texture, opacity, surface appearance, chromogenesis, and diameter of the colony were observed. Gram's test was performed, and the organism was viewed under the microscope for microscopic characterization. For the sugar utilization test, the isolate was cultured on phenol red broth base (10 g/L peptone; 5 g/L Nacl; 0.018 g/L phenol red; pH 7.4) supplemented with 10 g/L carbohydrates. For salt tolerance, the organism was cultured on nutrient agar supplemented with 10% and 15% NaCl; for temperature tolerance in nutrient broth at 45 ℃ and 60 ℃ and for pH tolerance test, in nutrient broth at different pH (4.5, 6.0, 7.2, 8.0, 9.5). Growth was observed after 24 hours of incubation.

Screening for hemolysis activity

The selected isolate was cultured on the blood agar plate (3 g/L beef extract; 5 g/L peptone; 5% sheep blood; 5 g/L NaCl; 15 g/L agar; pH 7) and incubated at 37 ℃ for 24 hours. The appearance of clear zones around the colony

indicates complete hemolysis while translucent greenish color indicates partial hemolysis (28).

Screening for hydrolytic enzyme production

The isolate was cultured on starch agar (Nutrient agar with 1% starch) and incubated at 30 ℃ for 48 hours. After incubation, the agar medium was flooded with iodine solution for a few minutes and drained off. Starch hydrolysis is indicated by a clear halo region around the colony (29).

Gelatinase production was observed by stabbing gelatin agar (7.5 g/L agar) in a test tube. After 48 hours incubation at 30 ℃, cultured test tubes were placed at 4 ℃ in a refrigerator until the bottom resolidified. Gelatin hydrolysis can be confirmed if the medium remains liquid after refrigeration (30).

For the protease test, the isolate was cultured on skim milk powder agar (5% skim milk powder) and incubated at 30 ℃ for 48 hours. The clear halo region indicates the production of protease by the isolate (31).

Emulsification of various hydrocarbons

The selected isolate was grown on liquid medium described by Abu-Ruwaida et al. (1991) and incubated at 30 ℃ for seven days without shaking (32). The culture was centrifuged at 10,000 rpm for 10 minutes, filtered and the filtrate was used for determination of emulsification activity. Emulsifying activity was determined using the method described by G.A. Plaza et al. (2006) (33). 7 mL of supernatant were poured into test tubes which were overlaid with 3 mL of diesel, petrol, xylene, and toluene. The mixture was then vortexed for 1 min. After 24 hours, the emulsion stability was assessed, and the emulsification index (EI-24) was calculated using Equation 1. Each hydrocarbon was tested in triplicate for emulsification test. If the EI-24 was 50% or higher, the emulsion was considered stable (34).

$$EI-24 = \frac{height of\ emulsion\ layer}{height\ of\ hydrocarbons\ phase} * 100 \qquad 1$$

Decolorization of triphenylmethane dyes

(Crystal violet, Fuchsin, Malachite green, and Phenol red) were the dyes used in this study to observe the ability of decolorization by the isolate, following the method described by L. Ayed et al. (2010) (35). 1 mL of culture grown on nutrient broth was transferred to Mineral salts medium (MSM) containing 0.1 g/L $MgSO_4$; 0.6 g/L $(NH_4)_2SO_4$; 0.5 g/L NaCl; 1.36 g/L K_2HPO_4; 0.02 g/L $CaCl_2$; 1.1 mg/L $MnSO_4$; 0.2 mg/L $ZnSO_4$; 0.2 mg/L $CuSO_4$; 0.14 mg/L $FeSO_4$; 7 mM glucose; 0.1% yeast extract; 50 mg/L dye; pH 7.0 (36). The decolorization of dyes was determined spectrophotometrically using different wavelengths for each dye (Crystal violet – 592 nm, Fuchsin – 530 nm, Malachite green – 618 nm, and Phenol red – 431 nm). Each dye was tested in triplicate for decolorization by the isolate. The decolorization percentage was calculated using Equation 2.

$$\text{Decolorization (\%)} = \frac{AI-At}{Ai} * 100 \qquad 2$$

Where Ai is the initial absorbance (uninoculated MSM medium with dye) and At is the absorbance at incubation time t.

Growth in the presence of inhibitors

The isolate was cultured in nutrient broth and incubated at 30 ℃ for 24 hours. The confluently grown liquid culture was streaked over a nutrient agar plate in a zigzag pattern. Three bores were made in the culture media using a sterile borer. 200 µl of different concentrations of phenol (0.1%, 0.5%, 1%); mercuric chloride (100 ppm, 500 ppm, 1000 ppm); lead acetate (1 mM, 5 mM, 10 mM) was pipetted into the bores, and the zone of inhibition was observed. Three replicate bores were used for each inhibitor to ensure consistent and reliable results.

Statistical analysis

Data analysis was performed using Origin Pro and Microsoft Excel. ANOVA was conducted for quantitative data where the Tukey test was used to compare the means at a 95% confidence interval.

Result and Discussion

Isolation and screening

Among ten isolates, isolate B5 (Figure 1a) had a significantly greater hydrolysis index (3.08 ± 0.083) compared to other isolates at $p < 0.05$ confidence interval as shown in Figure 1b. The organism with the highest index on tributyrin agar was selected for further study. Lipase-producing bacteria can be used for bioremediation because they aid in the degradation of xenobiotic compounds such as petroleum, insecticides, fertilizers, pesticides, plastics, and other hydrocarbon-containing substances, which are constantly degrading our natural environment (37,38).

Identification of selected isolate

Molecular characterization of the isolate

Based on 16s rRNA gene sequencing, the selected isolate showed high similarity to *Staphylococcus saprophyticus* (**Figure 2**). BLAST result using "*16S ribosomal RNA* sequences (Bacteria and Archaea)" database, indicated that B5 showed 100% similarity with *Staphylococcus edaphicus* strain CCM 8730, *Staphylococcus saprophyticus subsp. saprophyticus* ATCC *15305*, *Staphylococcus*

saprophyticus strain JCM 2427, and *Staphylococcus saprophyticus* strain 102446.

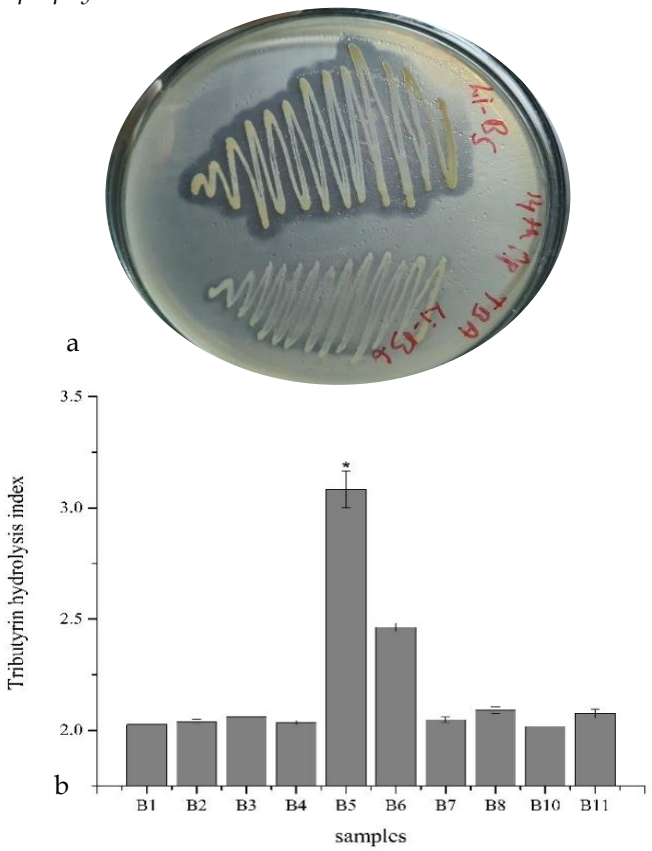

Figure 1. a – halo region demonstrated by B5 in tributyrin agar compared with another isolate. **b –** Graph showing the Tributyrin hydrolysis index of various organisms. *The hydrolysis index is significantly greater at a $p < 0.05$ confidence interval. Data represent mean ± SE (n = 3).

Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (2000 replicates) was shown next to branches. Initial trees for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model and then selecting the topology with superior log likelihood values.

Morphological and biochemical characterization

Table 1. Phenotypic features of selected isolate and comparison with closely related species based on BLAST result.

Test	Li-B5	*S. saprophyticus* **subsp.** *Saprophyticus* (27)	*Staphylococcus edaphicus strain CCM8730* (39)
Colony morphology			
Colony form	circular	ND	circular
Elevation	slightly raised	Raised	slightly convex
Margin	entire	entire	entire
Texture	butyrous	glistening	glistening
Opacity	opaque	opaque	ND
Surface appearance	smooth	smooth	smooth
Chromogenesis	Creamy White	slightly yellow	whitish
Diameter (mm)	1.0 to 2.0	>5	2
Pigmentation	-	-	-
Gram staining	Gram-positive	Gram-positive	Gram-positive
Microscopy	Violet cocci clustered in group	Singly and form pairs.	spherical or irregular in cocci
Cell Size (μm)	<1	0.6-1.2	0.8
Growth on NaCl agar			
10% Nacl	+	+	+
15% Nacl	+	d	-
Alkaline phosphatase	-	-	+
pH 8.0	-	ND	ND
pH 10.0	-	ND	-
Temperature tolerance test			
45 °C for 24 h	+	d	-
45 °C for 72 h	+	ND	ND
60 °C for 24 h	-	ND	ND
Growth at pH			
4.5	-	ND	-
6	+	ND	ND
7.2	+	ND	ND
8	+	ND	ND
9.5	+	ND	ND
Citrate Utilization	-	ND	+
Bile Esculin Hydrolysis	-	-	-
Reduction of nitrate		ND	ND
VP Test	+	+	+
Indole Production	-	ND	+
Motility	-	-	-
Hydrogen Sulphide Production	-	ND	+
Coagulase Test	-	ND	-
Hemolysis	-	ND	+ (weak)
Arginine dehydrolase	-	-	-
Lysine decarboxylase	-	ND	-
Ornithine decarboxylase	-	-	-
Pyruvate Utilization	-	ND	+
Production of			
Lipase	+	ND	+
Amylase	-	ND	ND
Gelatinase	+	ND	-

Protease	-	ND	ND
Urease	+	+	+
Catalase	+	ND	+
Oxidase	-	-	-
Sugar utilization			
Glucose	+	+	+
Sucrose	+	+	+
Fructose	+	+	+
Maltose	+	+	+
Xylose	-	-	-
Galactose	-	-	+
Lactose	+	-	-
Mannitol	+	d	+
Arabinose	-	-	-
Ribose	-	-	+
Glycerin	+	ND	+
Sorbose	-	ND	-
Melezitose	+	ND	-
Sorbitol	-	ND	+
Resistant to antibiotics	AMP, P, CB, CIP, GEN, TE, CTX, AM, CXM, AZM, E	ND	ND
Sensitive to antibiotics	MO, LE, VA, RIF	ND	ND

d – not defined, ND – not determined, AMP – ampicillin (10 mcg); P – penicillin (1 unit); CB – carbenicillin (100 mcg); CIP – ciprofloxacin (5 mcg); GEN – gentamycin (10 mcg); TE – tetracycline (30 mcg); CTX – cefotaxime (30 mcg); AMC – amoxicillin (10 mcg); CXM – cefuroxime (30 mcg); MO – moxifloxacin (5 mcg); LE – levofloxacin (5 mcg); AZM – azithromycin (15 mcg); E – erythromycin (15 mcg); VA – vancomycin (5 mcg); RIF – rifampicin (5 mcg).

Morphological and biochemical tests, as shown in Table 1 suggest that the selected isolate was a gram-positive coccus of size <1µm and non-motile. After 24 hours of incubation on nutrient agar medium, colonies were creamy white, smooth, butyrous, slightly elevated, circular, and 1-2 mm in diameter at 30 °C. The isolate tested positive for urease and catalase but negative for oxidase. It also tested positive for VP but negative for citrate utilization, bile esculin hydrolysis, nitrate reduction, indole formation, hydrogen sulfide production, arginine dehydrolase, lysine decarbolase, ornithine decarbolase, and pyruvate utilization. It was able to utilize glucose, sucrose, fructose, maltose, lactose, mannitol, glycerin, and melezitose. The isolate was susceptible to moxifloxacin (5 mcg), levofloxacin (5 mcg), vancomycin (5 mcg), and rifampicin (5 mcg). It tolerated salt well, growing up to 15% NaCl. The isolate grew well at 45 °C but not at 60 °C. In the pH tolerance test, the isolate grew confluently at pH 6, 7.2, 8, and 9.5, but not at pH 4.5.

The phenotypic features of the selected isolate were compared with *Staphylococcus saprophyticus* and *Staphylococcus edaphicus* (27,39). Table 1 suggests that the selected isolate belongs to *S. saprophyticus* because most of the morphological and biochemical features were similar. The only difference between the selected isolate and *S. saprophyticus* was the fermentation of lactose. Phenotypic features of B5 that differ from *S. edaphicus* were growth in 15% NaCl, alkaline phosphate production, citrate utilization, indole production, H_2S production, hemolysis, pyruvate utilization, production

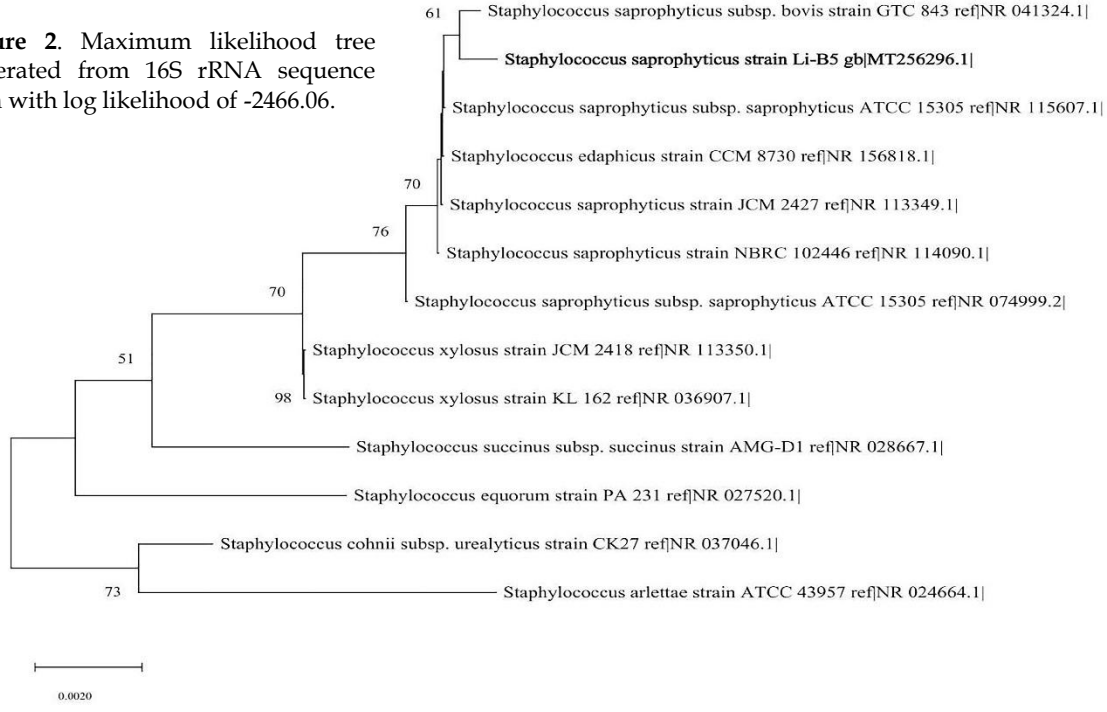

Figure 2. Maximum likelihood tree generated from 16S rRNA sequence data with log likelihood of -2466.06.

of gelatinase, fermentation of galactose, lactose, ribose, melezitose, and sorbose.

Screening for hemolytic activity and production of hydrolytic enzymes

The selected isolate was screened for hydrolysis of blood and production of other hydrolytic enzymes. The isolate was nonhemolytic, coagulase negative, and H_2S negative; such nonpathogenic nature ensures safety for industrial and environmental applications (40). The organism produced gelatinase but was unable to produce amylase and protease. Production of more than one enzyme by the same organism is beneficial for industrial applications because more than one enzyme can be produced simultaneously under the same condition (41). Microbial enzymes degrade crude oil spills in oceans, pesticides, and other industrial wastes aiding in bioremediation and controlling environmental pollution (42).

Emulsification of various hydrocarbons

The emulsification index varied from 26.67% to 83.33% as shown in Figure 3. The emulsification of diesel (26.67 ± 3.33) % and kerosene (53.33 ± 3.33) % was significantly lower compared to xylene (83.33 ± 3.33) % at a $p < 0.05$ confidence interval. The emulsification of petrol (70 ± 5.77) % and toluene (76.67 ± 6.67) % was comparable to xylene (83.33 ± 3.33) % at $p < 0.05$ confidence interval. Similar studies have reported that emulsification by bacterial species on xylene was found to be maximum, with an emulsification index of 87% on xylene, 71.23% on kerosene, 67% on diesel, and 74% on petrol (43–46). Bioemulsifiers are often found to improve hydrocarbon biodegradation in liquid media, soil slurries, water, and soil microcosms (22). Bioemulsifiers produced by different bacteria have different mechanisms that aid in bioremediation such as; stabilizing oil-in-water emulsions, decomposing and neutralizing polychlorinated biphenyls, microbial-enhanced oil recovery, and in situ biodegradation of oil sludge (47,48). A similar study reported that *Pseudomonas* spp. isolated from oil-contaminated soil showed high potential for oil degradation and biosurfactant production, and the biosurfactant showed emulsification activity in kerosene, mannitol, glycerol, and glucose which confirmed its applicability against different hydrocarbon pollution (49). Bioemulsifiers are non-toxic and biodegradable, making them very useful for bioremediation (48).

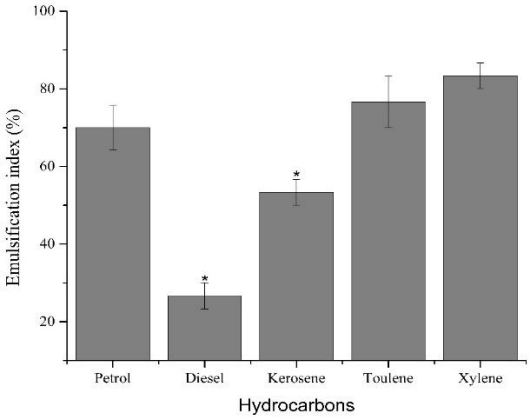

Figure 3. Graph showing the amount of emulsification of various hydrocarbons by selected isolate. *The mean emulsification index is significantly lower in comparison to xylene at a $p < 0.05$ confidence interval. Data represent mean ± SE (n = 3).

Decolorization of triphenylmethane dyes

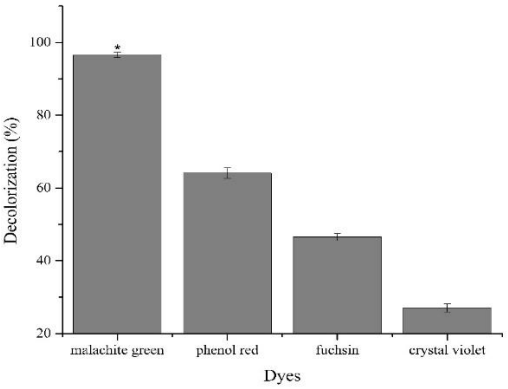

Figure 4. Graph showing decolorization of various dyes by the selected isolate. * The mean decolorization is significantly higher in comparison to other dyes at a $p < 0.05$ confidence interval. Data represent mean ± SE (n = 3).

The percentage decolorization of the triphenylmethane dyes ranges from 27.04% to 96.53% as shown in Figure 4. The decolorization of malachite green (96.53 ± 0.69) % was significantly greater than phenol red (64.09 ± 1.41) %, fuchsin (46.52 ± 0.96) %, and crystal violet (27.04 ± 1.13) % at $p < 0.05$ confidence interval. A study reported that the average percentage decolorization of malachite green, phenol red, fuchsin, and crystal violet was 33%, 80%, 37%, and 62% respectively (35). Another study showed that the decolorization of Malachite Green, Methyl Violet, Crystal Violet, and Cotton Blue was (94.7%), (91.8%), (86.6%), (68.4%) respectively (50). The results demonstrate the ability of the isolate to remove triphenylmethane dyes through biosorption or biodegradation. The decolorizing ability of the isolate would be favorable for bioprocessing dye-containing wastewater, degradation of a wide range of pollutants, and for industrial applications (51). Another study showed that *Staphylococcus saprophyticus* was very efficient in degrading the dye Navy N5RL1 commonly

used in carpet industry (52). Thus, isolate could be useful in bioremediation because of its ability to decolorize the harmful dyes in the environment.

Growth in the presence of inhibitors

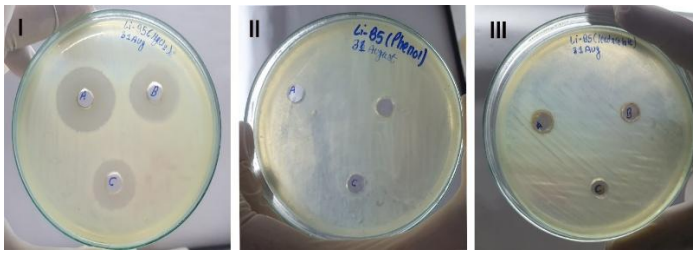

Figure 5. Growth of selected isolate on different concentrations of various inhibitors. I: mercuric chloride (A – 100 ppm, B – 500 ppm, C – 100 ppm). II: phenol (A – 1%, B – 0.5%, C – 0.1%). III: lead acetate (A – 10 mM, B – 5 mM, C – 1 mM).

The growth of selected isolate on nutrient agar containing various concentrations of inhibitors is shown in Figure 5. Isolate B5 was able to grow in the presence of different concentrations of phenol (0.1%, 0.5%,1%) and lead acetate (1 mM, 5 mM, 10 mM). Mercuric chloride inhibited the growth of the isolate, forming a zone of inhibition with diameters of 27.5 mm, 25 mm, and 19 mm at concentration of 1000 ppm, 500 ppm, and 100 ppm respectively. The result indicates that the isolate was able to grow in the presence of phenol and lead acetate but unable to grow in the presence of mercuric chloride. The isolate also degrades different pollutants like hydrocarbons and decolorizes different harmful dyes. Hence, it stands poised for impactful bioremediation applications, adept at not only degrading environmental pollutants but also resilient against inhibitors like phenol and lead acetate, which fail to impede its growth.

As an opportunistic pathogen requiring BSL-2 containment, environmental applications of *S. saprophyticus* demand risk assessment focusing on exposure vectors (aerosols, fomites, water) for immunocompromised hosts; microbial resilience (biofilm persistence, antimicrobial resistance); and containment efficacy. To assess the risks associated with the environmental use of *Staphylococcus saprophyticus*, a thorough risk assessment should be conducted, focusing on exposure pathways, pathogenicity, and environmental persistence. Mitigation strategies, such as engineering controls (e.g., closed processing systems), personal protective equipment (PPE), and strict decontamination protocols (e.g., chemical disinfectants, UV treatment), can be implemented to minimize exposure. Regular sampling and fail-safe sterilization (e.g., autoclaving effluent) to prevent unintended release.

Conclusion

The selected isolate, *Staphylococcus saprophyticus* Li-B5, demonstrated remarkable capabilities, showcasing its proficiency in lipase production, hydrocarbon emulsification, and triphenylmethane dye decolorization. Its industrial potential and suitability for enzyme production is underscored by its elevated tributyrin agar index and nonpathogenic nature. Moreover, this isolate emerges as a potent candidate for bioremediation, excelling in the degradation of petroleum hydrocarbons and the detoxification of hazardous dyes in the environment.

Data availability

Data are available from Doi: 10.17632/nv2ktzyv6s.1. Nucleotide database is available at NCBI Genebank with accession number MT256296.1 for 16S rRNA sequence.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgements

The authors gratefully acknowledge Shubham Biotech Nepal Pvt. Ltd. for providing research space and facilities.

Funding source

This research did not receive any specific grant from external funding agencies in the public, commercial, or non-profit sectors.

Author contribution

Asmita Shrestha: Conceptualization, Methodology, Visualization, Writing, Validation, Investigation, Review and editing. **Gyanu Raj Pandey**: Methodology, Visualization, Writing, Validation, Investigation, Software, Data curation, Review and editing. **Sudip Silwal**: Writing, Validation, Investigation, Software, Data curation, Review and editing. **Raj Kumar Shrestha**: Investigation. **Dinesh Tiwari**: Investigation.

References

1. Methneni N, Morales-González JA, Jaziri A, Mansour H Ben, Fernandez-Serrano M. Persistent organic and inorganic pollutants in the effluents from the textile dyeing industries: Ecotoxicology appraisal via a battery of biotests. Environ Res [Internet]. 2021 May;196(October 2020):110956. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0013935121002504
2. Prabagar S, Dharmadasa RM, Lintha A, Thuraisingam S, Prabagar J. Accumulation of heavy metals in grape fruit, leaves, soil and water: A study of influential factors and evaluating ecological risks in Jaffna, Sri Lanka. Environ Sustain Indic [Internet]. 2021 Dec;12(August):100147. Available from: https://doi.org/10.1016/j.indic.2021.100147
3. Dhankhar N, Kumar J. Impact of increasing pesticides and fertilizers on human health: A review. Mater Today Proc [Internet]. 2023 Apr;

Available from: https://www.sciencedirect.com/science/article/pii/S2214785323018382
4. Ogunlaja A, Ogunlaja OO, Okewole DM, Morenikeji OA. Risk assessment and source identification of heavy metal contamination by multivariate and hazard index analyses of a pipeline vandalised area in Lagos State, Nigeria. Sci Total Environ [Internet]. 2019 Feb 15;651(Pt 2):2943–52. Available from: https://doi.org/10.1016/j.scitotenv.2018.09.386
5. Al-Rubaye AH, Jasim DJ, Ameen HFM, Al-Robai HA, Al-Assal JR. The impacts of petroleum on environment. IOP Conf Ser Earth Environ Sci [Internet]. 2023 Apr 1;1158(3):032014. Available from: https://iopscience.iop.org/article/10.1088/1755-1315/1158/3/032014
6. Asif Z, Chen Z, An C, Dong J. Environmental impacts and challenges associated with oil spills on shorelines. J Mar Sci Eng [Internet]. 2022 May 31;10(6):762. Available from: https://www.mdpi.com/2077-1312/10/6/762
7. Gao T, Qin D, Zuo S, Peng Y, Xu J, Yu B, et al. Decolorization and detoxification of triphenylmethane dyes by isolated endophytic fungus, *Bjerkandera adusta* SWUSI4 under non-nutritive conditions. Bioresour Bioprocess [Internet]. 2020 Dec 6;7(1):53. Available from: https://doi.org/10.1186/s40643-020-00340-8
8. Ayilara MS, Babalola OO. Bioremediation of environmental wastes: the role of microorganisms. Front Agron [Internet]. 2023 May 30;5(May):1–15. Available from: https://www.frontiersin.org/articles/10.3389/fagro.2023.1183691/full
9. Mahmood A, Bilal B, Naeem Z, Iram S. Physical, chemical, and biological remediation techniques for textile effluents in context with developed and developing countries. In: Kumar V, Prasad R, Kumar M, editors. Rhizobiont in Bioremediation of Hazardous Waste [Internet]. Singapore: Springer Singapore; 2021. p. 409–41. Available from: https://doi.org/10.1007/978-981-16-0602-1_18
10. Sayed K, Baloo L, Sharma NK. Bioremediation of total petroleum hydrocarbons (tph) by bioaugmentation and biostimulation in water with floating oil spill containment booms as bioreactor basin. Int J Environ Res Public Health [Internet]. 2021 Feb 24;18(5):2226. Available from: https://www.mdpi.com/1660-4601/18/5/2226
11. Seabra PCS, Bomtempo J V., Costa NO. Biotechnology in the petroleum industry: 1988–1997. Tech Bull 42, PETROBRAS, Rio Janeiro, Brazil [Internet]. 1999; Available from: https://onepetro.org/WPCONGRESS/proceedings-abstract/WPC16/All-WPC16/201996
12. Alin I, Prisecaru M, Stoica I, Calin M, Cristea T. Biological remediation of soil polluted with oil products : an overview of available technologies. Stud SI Cercet Stiint Univ BACAU Ser Biol [Internet]. 2016 May 1;101. Available from: https://www.researchgate.net/publication/309901995_BIOLOGICAL_REMEDIATION_OF_SOIL_POLLUTED_WITH_OIL_PRODUCTS_AN_OVERVIEW_OF_AVAILABLE_TECHNOLOGIES
13. Kaur S, Sarao L. Microbial Bioremediation. In: New Vistas In Microbial Sciences-1 [Internet]. Integrated Publications; 2022. p. 47–66. Available from: https://www.researchgate.net/publication/357839226_Microbial_Bioremediation
14. Tremblay J, Yergeau E, Fortin N, Cobanli S, Elias M, King TL, et al. Chemical dispersants enhance the activity of oil- and gas condensate-degrading marine bacteria. ISME J [Internet]. 2017 Dec 11;11(12):2793–808. Available from: http://dx.doi.org/10.1038/ismej.2017.129
15. Sarkar P, Roy A, Pal S, Mohapatra B, Kazy SK, Maiti MK, et al. Enrichment and characterization of hydrocarbon-degrading bacteria from petroleum refinery waste as potent bioaugmentation agent for in situ bioremediation. Bioresour Technol [Internet]. 2017 Oct;242:15–27. Available from: http://dx.doi.org/10.1016/j.biortech.2017.05.010
16. Varjani SJ, Upasani VN. A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. Int Biodeterior Biodegradation [Internet]. 2017 May;120:71–83. Available from: http://dx.doi.org/10.1016/j.ibiod.2017.02.006
17. Hossain MF, Akter MA, Sohan MSR, Sultana DN, Reza MA, Hoque KMF. Bioremediation potential of hydrocarbon degrading bacteria: isolation, characterization, and assessment. Saudi J Biol Sci [Internet]. 2022 Jan;29(1):211–6. Available from: https://doi.org/10.1016/j.sjbs.2021.08.069
18. Sharma N. Microbes and their Secondary Metabolites: Agents in bioremediation of hydrocarbon contaminated site. arch pet environ biotechnol [Internet]. 2019 Oct 24;4(1). Available from: https://www.gavinpublishers.com/articles/review-article/Archives-of-Petroleum-Environmental-Biotechnology/microbes-and-their-secondary-metabolites-agents-in-bioremediation-of-hydrocarbon-contaminated-site
19. Bhandari S, Poudel DK, Marahatha R, Dawadi S, Khadayat K, Phuyal S, et al. Microbial enzymes used in bioremediation. Silva CG, editor. J Chem [Internet]. 2021 Feb 8;2021:1–17. Available from: https://www.hindawi.com/journals/jchem/2021/8849512/
20. Basheer SM, Chellappan S, Beena PS, Sukumaran RK, Elyas KK, Chandrasekaran M. Lipase from marine *Aspergillus awamori* BTMFW032: production, partial purification and application in oil effluent treatment. N Biotechnol [Internet]. 2011 Oct;28(6):627–38. Available from: http://dx.doi.org/10.1016/j.nbt.2011.04.007
21. Jacob EL, Mohan AP, Joseph AV. Bioremediation of petroleum-polluted soil using biosurfactant producing bacteria, *pseudomonas* sp. J Sci Res [Internet]. 2022;66(01):224–31. Available from: https://www.bhu.ac.in/research_pub/jsr/Volumes/JSR_66_01_2022/24.pdf
22. Calvo C, Manzanera M, Silva-Castro GA, Uad I, González-López J. Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. Sci Total Environ [Internet]. 2009 Jun;407(12):3634–40. Available from: http://dx.doi.org/10.1016/j.scitotenv.2008.07.008
23. Ilesanmi OI, Adekunle AE, Omolaiye JA, Olorode EM, Ogunkanmi AL. Isolation, optimization and molecular characterization of lipase producing bacteria from contaminated soil. Sci African [Internet]. 2020 Jul;8:e00279. Available from: https://doi.org/10.1016/j.sciaf.2020.e00279
24. Sambrook J, Russell DW. Molecular cloning : a laboratory manual [Internet]. 3rd ed. cold spring harbor laboratory press. Cold Spring Harbor, N.Y. SE -: Cold Spring Harbor Laboratory Press; 2001. Available from: https://www.cshlpress.com/pdf/sample/2013/MC4/MC4FM.pdf
25. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution (N Y) [Internet]. 1985 Jul;39(4):783. Available from: https://www.jstor.org/stable/2408678?origin=crossref
26. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary genetics analysis version 11. Battistuzzi FU, editor. Mol Biol Evol [Internet]. 2021 Jun 25;38(7):3022–7. Available from: https://academic.oup.com/mbe/article/38/7/3022/6248099
27. Brenner DJ, Krieg NR, Garrity GM, Staley JTTA-TT-. Bergey's manual® of systematic bacteriology [Internet]. 2nd ed NV. Brenner DJ, Krieg NR, Staley JT, Garrity GM, editors. Boston, MA: Springer US; 2005. Available from: http://link.springer.com/10.1007/0-387-28021-9
28. Lee J, Kim S, Kang C-H. Screening and probiotic properties of lactic acid bacteria with potential immunostimulatory activity isolated from kimchi. Fermentation [Internet]. 2022 Dec 21;9(1):4. Available from: https://www.mdpi.com/2311-5637/9/1/4
29. Timilsina PM, Pandey GR, Shrestha A, Ojha M, Karki TB. Purification and characterization of a noble thermostable algal starch liquefying alpha-amylase from *Aeribacillus pallidus* BTPS-2 isolated from geothermal spring of Nepal. Biotechnol Reports [Internet]. 2020;28:e00551. Available from: https://doi.org/10.1016/j.btre.2020.e00551
30. Balan SS, Nethaji R, Sankar S, Jayalakshmi S. Production of gelatinase enzyme from *Bacillus* spp isolated from the sediment sample of Porto Novo Coastal sites. Asian Pac J Trop Biomed [Internet]. 2012 Jan;2(3):S1811–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S2221169112605000
31. Pandey GR, Shrestha A, Karki TB, Neupane S, Ojha S, Koirala P, et al. Screening and identification of thermotolerant and osmotolerant *Bacillus amyloliquefaciens* BKHE isolated from kinema of eastern nepal for alkaline protease production. Int J Microbiol. 2022;2022. Available from: https://www.hindawi.com/journals/ijmicro/2022/6831092/
32. Abu-Ruwaida AS, Banat IM, Haditirto S, Salem A, Kadri M. Isolation of biosurfactant-producing bacteria, product characterization, and evaluation. Acta Biotechnol [Internet]. 1991 Jan 2;11(4):315–24. Available from: https://onlinelibrary.wiley.com/doi/10.1002/abio.370110405

33. Płaza GA, Zjawiony I, Banat IM. Use of different methods for detection of thermophilic biosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. J Pet Sci Eng [Internet]. 2006 Jan;50(1):71–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0920410505001646
34. Bosch P, Robert M, Mercade M, Espuny M, Parra J, Guinea J. Surface active compounds on microbial cultures. Tenside Surfactants Deterg [Internet]. 1988 Jul 1;25(4):208–11. Available from: https://doi.org/10.1515/tsd-1988-250402
35. Ayed L, Chaieb K, Cheref A, Bakhrouf A. Biodegradation and decolorization of triphenylmethane dyes by *Staphylococcus epidermidis*. Desalination [Internet]. 2010 Sep;260(1–3):137–46. Available from: http://dx.doi.org/10.1016/j.desal.2010.04.052
36. Chaieb K, Hagar M, Radwan NRE. Biodegradation and decolorization of azo dyes by adherent *Staphylococcus lentus* strain. Appl Biol Chem [Internet]. 2016 Jun 15;59(3):405–13. Available from: http://link.springer.com/10.1007/s13765-016-0169-4
37. Blandón LM, Marín MA, Quintero M, Jutinico-Shubach LM, Montoya-Giraldo M, Santos-Acevedo M, et al. Diversity of cultivable bacteria from deep-sea sediments of the Colombian Caribbean and their potential in bioremediation. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol [Internet]. 2022 Mar 23;115(3):421–31. Available from: https://doi.org/10.1007/s10482-021-01706-4
38. Ali S, Khan SA, Hamayun M, Lee I-J. The recent advances in the utility of microbial lipases: A review. Microorganisms [Internet]. 2023 Feb 17;11(2):510. Available from: https://www.mdpi.com/2076-2607/11/2/510
39. Pantůček R, Sedláček I, Indráková A, Vrbovská V, Mašlaňová I, Kovařovic V, et al. *Staphylococcus edaphicus* sp. nov., isolated in Antarctica, harbors the *mecC* gene and genomic islands with a suspected role in adaptation to extreme environments. Drake HL, editor. Appl Environ Microbiol [Internet]. 2018 Jan 15;84(2). Available from: https://journals.asm.org/doi/10.1128/AEM.01746-17
40. Hapsari Rt, Susilowati A, Pangastuti A. Isolation and identification of lipolytic bacteria from the digestive tract of eel (*Anguilla bicolor bicolor*). Int J Bonorowo Wetl [Internet]. 2020 Apr 16;10(1):27–36. Available from: https://smujo.id/bw/article/view/10506
41. Shrestha S, Chio C, Khatiwada JR, Kognou ALM, Qin W. Optimization of multiple enzymes production by fermentation using lipid-producing *Bacillus* sp. Front Microbiol [Internet]. 2022 Nov 1;13(November):1–13. Available from: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1049692/full
42. Paladhi AG, Joshi JT, George A, Manohar MV, Vallinayagam S, Malik JA. Lipase and lactic acid bacteria for biodegradation and bioremediation. In: Microbes and microbial biotechnology for green remediation [Internet]. Elsevier; 2022. p. 265–86. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780323904520000049
43. Rahman KSM, Rahman TJ, Lakshmanaperumalsamy P, Marchant R, Banat IM. The potential of bacterial isolates for emulsification with a range of hydrocarbons. Acta Biotechnol [Internet]. 2003 Dec;23(4):335–45. Available from: https://onlinelibrary.wiley.com/doi/10.1002/abio.200390043
44. de Sousa T, Bhosle S. Isolation and characterization of a lipopeptide bioemulsifier produced by *Pseudomonas nitroreducens* TSB.MJ10 isolated from a mangrove ecosystem. Bioresour Technol [Internet]. 2012;123:256–62. Available from: http://dx.doi.org/10.1016/j.biortech.2012.07.056
45. Ali F, Das S, Hossain TJ, Chowdhury SI, Zedny SA, Das T, et al. Production optimization, stability and oil emulsifying potential of biosurfactants from selected bacteria isolated from oil-contaminated sites. R Soc Open Sci [Internet]. 2021 Oct 13;8(10). Available from: https://royalsocietypublishing.org/doi/10.1098/rsos.211003
46. Sahana TG, Fathimath Sadiya MK, Rekha PD. Emulsifying and cell proliferative abilities of the exopolysaccharide produced by leguminous plant nodule associated bacterium *Cronobacter* sp. J Polym Environ [Internet]. 2018;26(8):3382–8. Available from: http://dx.doi.org/10.1007/s10924-018-1223-6
47. Alizadeh-Sani M, Hamishehkar H, Khezerlou A, Azizi-Lalabadi M, Azadi Y, Nattagh-Eshtivani E, et al. Bioemulsifiers derived from microorganisms: Applications in the drug and food industry. Adv Pharm Bull [Internet]. 2018 Jun 19;8(2):191–9. Available from: http://apb.tbzmed.ac.ir/Abstract/apb-19533
48. Mnif I, Ghribi D. High molecular weight bioemulsifiers, main properties and potential environmental and biomedical applications. World J Microbiol Biotechnol [Internet]. 2015 May 5;31(5):691–706. Available from: http://link.springer.com/10.1007/s11274-015-1830-5
49. N. N. O, E. I. C, A. E. M. Bioremediation potentials and emulsification activity of indigenous bacteria isolated from crude oil polluted soil in Ohaji-Egbema, Nigeria. Univers J Microbiol Res [Internet]. 2020 Mar;8(1):1–6. Available from: http://www.hrpub.org/journals/article_info.php?aid=9075
50. Adenan NH, Lim YY, Ting ASY. Removal of triphenylmethane dyes by *Streptomyces bacillaris*: A study on decolorization, enzymatic reactions and toxicity of treated dye solutions. J Environ Manage [Internet]. 2022 Sep;318:115520. Available from: https://www.sciencedirect.com/science/article/pii/S0301479722010933
51. Li G, Peng L, Ding Z, Liu Y, Gu Z, Zhang L, et al. Decolorization and biodegradation of triphenylmethane dyes by a novel *Rhodococcus qingshengii* JB301 isolated from sawdust. Ann Microbiol [Internet]. 2014 Dec 25;64(4):1575–86. Available from: http://link.springer.com/10.1007/s13213-014-0801-7
52. Kumari L, Verma AK, Tiwary D, Giri DD, Nath G, Mishra PK. Biodegradation of Navy N5RL1 carpet dye by *Staphylococcus saprophyticus* strain BHUSS X3. 3 Biotech [Internet]. 2015 Oct 31;5(5):775–82. Available from: http://dx.doi.org/10.1007/s13205-015-0276-7