

Effect of Rhamnolipids on Pathogenicity Characteristics of Microorganisms in Organic Compost



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ABSTRACT

Background: Rhamnolipids are hydrophilic glycolipids, often classified as biosurfactants. They are produced by different bacterial species. Rhamnolipids are extensively studied in biological research because of their interesting features like antimicrobial, antifungal, and antiviral activities.

Materials and Methods: To study the antimicrobial effect of rhamnolipid, we conducted a cross-sectional study on the eight different pathogenic bacterial strains from November 2019 to June 2020. These bacterial strains were isolated from the organic compost. Both disk diffusion and broth microdilution methods were used to evaluate the inhibitory effects of rhamnolipids on these pathogenic bacteria. Also, protease and amylase enzyme activities were evaluated in these eight bacterial isolates.

Results: For Bacillus stearothermophilus, within the area of 31.5 mm, no growth was observed, hence proving the inhibitory effect of rhamnolipid. After calculating the Minimum Inhibitory (MIC) and Minimum Lethal Concentrations (MLC) for each bacterial strain, it has been found that the studied bacteria were more susceptible to rhamnolipids than most of the antibiotics. Strains were also quantified for their enzymatic activity of proteases and amylases. The bacterial strains of Bacillus stearothermophilus, Brevibacillus brevis, Bacillus licheniformis, and Bordetella petrii showed maximum protease activity. Whereas Escherichia coli, Enterococcus faecalis, Enterobacter aerogenes, and Pseudomonas aeruginosa showed high amylase activity.

Conclusion: Rhamnolipids can be used as a potential antimicrobial agent for treatment of infections.

1. Introduction

Rhamnolipids are extensively studied glycolipids. They are originally produced by Pseudomonas aeruginosa and various bacterial species. They are often known as “biosurfactants.” They

consist of a hydrophilic group, having one or two L-rhamnose molecules bonded via glycosidic linkage to one or two β -hydroxy fatty acids, which is the hydrophobic part of the molecule. Mono-rhamnolipids consist of a single rhamnose molecule, whereas di-rhamnolipids have two rhamnose molecules [1, 2].

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Rhamnolipids are also produced at an industrial scale by microbial fermentation of *P. aeruginosa* in large titers. They are extensively studied because of their excellent physicochemical properties and ability to reach elevated levels of fermentation titers. Their properties change according to the change in length of fatty acids, which makes them a diverse group of molecules [2]. Rhamnolipids are amphipathic surfactants. So they reduce the surface tension, which helps the bacteria to adhere to different surfaces. Some microbes produce rhamnolipids only when hydrocarbons are used as a carbon source. Some rhamnolipids producing strains cause nosocomial infections, especially in people with a weakened immune system [2].

One of the most interesting features of biosurfactants such as rhamnolipids is their wide applications in industrial and biological arenas. In industries, they are used in petroleum products, food processing, cosmetics, bioremediation, and pharmaceutical products. They are extensively used in biological research because of their antimicrobial, antifungal, and antiviral activities. Rhamnolipids at a concentration of about 0.4-10.0 mg/L inhibit the growth of harmful algae *Heterosigma akashiwo* and *Prorocentrum dentatum*. Rhamnolipid AT10 isolated from *P. aeruginosa* showed antimicrobial activity against *E. coli* (32 mg/mL), *Mycobacterium phlei* (16 mg/mL), and *Staphylococcus epidermis* (8 mg/mL). It has also demonstrated its antifungal property against *Aspergillus niger* (16 mg/mL) [4, 5]. They are also used in cosmetics, treatments of skin diseases, wound healing [6-9].

Rhamnolipids are used as cleaners and detergents. They are active agents for surfaces and behave as natural emulsifiers, which lead to widespread use in soaps, shampoos, compositions of detergent, and laundry products [10, 11]. They are also used to improve soil quality in soil bioremediation and as biopesticides [12]. The rhamnolipids production cost is high because of costly raw materials used for bacterial fermentation. However, processes used to obtain purified products limit the rhamnolipids' application on an industrial scale [13]. Therefore, much research has been done for reducing costs and increasing yield by using cheap substrates [14], and optimizing production conditions [15]. The different process of production and screening natural strains and production strategies of genetic engineering needs more efficient methods for separating purifying rhamnolipids homologue. Numerous research studies have evaluated substrates at low cost, developed active fermentation processes to screen new strains, and increases rhamnolipids yields [16]. Rhamnolipids have great potential to replace synthetic medicines because of their lower toxic-

ity and higher antimicrobial activity. Much research has been carried out where rhamnolipids are mass-produced from *P. aeruginosa* and tested for their anti-bacterial potential on different strains [17, 18].

During the composting process of organic materials/wastes, humus-rich and biostable products are produced, which can improve the land and help to fertilize the plants [19]. Many studies have reported the presence of different mesophilic, thermophilic, and thermotolerant pathogenic microorganisms in organic compost. They can survive in the compost for a longer period. During the thermophilic phase, thermophilic bacteria grow and colonize in compost. After completing the thermophilic phase of the composting, mesophilic microorganisms grow fast in the compost [20, 21]. Even antibiotic-resistant microorganisms have been found in organic compost, where animal wastes have been used to prepare organic compost [20]. Many studies have proven that using such organic fertilizers causes the contamination of such pathogenic microorganisms in the soil and the vegetables/plants grown in such soil [20, 22-24]. This process could risk the transfer of pathogenic microorganisms to humans through such organic vegetable consumption.

Rhamnolipids are biosurfactants and can improve compost quality. They can directly or indirectly affect the growth of microorganisms and their enzyme production and helping in the microbial bioremediation processes, too [25]. Haba et al. have explained the antimicrobial effect of rhamnolipids [26, 27]. Hence, this study will evaluate the potential antimicrobial effect of rhamnolipids on the pathogenic microorganisms in the organic compost, which could help reduce the risk of the transfer of pathogenic microbes through the consumption of organically grown vegetables/fruits. In the current research, some bacterial strains are isolated from organic compost, and rhamnolipids are studied on them. The results are then compared to bacterial inhibition by synthetic antibiotics to get a better notion about the inhibitory capability of the rhamnolipids.

2. Materials and Methods

Isolation of pathogenic bacteria from compost

To isolate bacterial strains from composted organic matter, sampling of the compost production process was carried out at Ardebil Composting Plant. The obtained compost was first sifted to remove larger elements like stones, wood, small branches, etc. Then 100 g of the compost mixture was added to 500 mL of saline solution and stirred on the shaker for three h. The purpose

of such vigorous shaking is to clean up the sample by removing interference before analysis. After solid-phase saturation, the supernatant liquid phase was separated, which is to be used for media inoculation. The next step is to enrich microbes in the compost sample to obtain the isolated bacterial colonies on solid or liquid cultures. For this purpose, 10 mL of the above extract was added to 100 mL of four different culture media (Quelab, Canada), which use cellulose, xylene, starch, and protein as a carbon source, respectively. All these media after inoculation were placed in the rotary shaker incubator at 140 rpm with a temperature range of 37°C-62°C for one week. It was done to isolate the mesophilic bacteria and the thermophilic bacteria. After one week, 5 mL of each medium was added to 100 mL of the new medium of the same material. It means that 5 mL from the cellulose medium was transferred to 100 mL of the new cellulose medium. The same process was carried out for the other three media. These newly inoculated media were then incubated under the same conditions (140 rpm and a temperature range of 37°C- 62°C for one week). After one week of incubation, the same process was repeated, but this time only 1 mL from each medium was transferred to the new medium [28].

This process of microbial concentration was repeated several times to eliminate the opacity created by adding the former to the new environment caused by soil particles. Different dilutions were prepared from each of the last media of the enrichment process, and appropriate dilution of each medium was purified on a solid agar medium of the same material (cellulose, xylene, starch, and protein). Different strains were isolated based on the differences in bacterial colonies, and individual colonies were purified on a complete agar medium. Finally, by re-culturing, each strain was purified in the liquid medium, the ability of each bacterium to grow was demonstrated. Morphological analysis like colony morphology, gram staining, bacterial motility; and biochemical tests like oxidase, catalase, and sugar tests such as fermentation tests of glucose, sucrose, and lactose were carried out to identify the isolated bacterial strains, as per Bergey's Manual of Systematic Bacteriology [29].

Preparation of bacterial strains

Bacterial isolates were cultured in Mueller-Hinton agar medium (Merk, Germany) and incubated at 37°C for 24 h. Mueller-Hinton is a non-selective, non-differential media that are commonly used for antibiotic susceptibility testing. Subsequently, a few drops of the bacterial suspension were transferred to the Mueller-Hinton broth medium to achieve

a turbidity equivalent of 0.5 McFarland standard (1.5 x 10⁸ bacterial concentration per mL/CFU/mL) [30].

Rhamnolipid preparation

Rhamnolipid powder (Canada, Toronto Research Chemicals Inc.) was purchased and sequential dilutions of 1000, 500, 250, 125 and 50 mg/mL were prepared in <0.1% of dimethyl sulfoxide (DMSO) solution [4, 31]. Preparations of rhamnolipid dilutions were used for antibacterial studies.

Evaluation of the antimicrobial effect of rhamnolipid on isolated bacteria

Bacterial suspensions in Mueller-Hinton broth were used to determine qualitative and quantitative antimicrobial activity. In the qualitative method, the Kirby-Bauer agar diffusion method (more commonly referred to as the disk-diffusion method) was used. The first step is the aseptic inoculation of isolated bacterial strains suspensions on Mueller-Hinton agar medium plates. Then, for examining the antibacterial properties, blank paper disks (made of antibody medicine) were placed at a fixed distance from each other on the plates. Then, about 20 µL of prepared rhamnolipid concentrations containing 1000, 500, 250, 125, and 50 mg/mL diluted in dimethyl sulfoxide solution was added to the disks. Gentamicin antibiotic disk (10 µg/mL) was used as a positive control in each plate. The prepared plates were then incubated overnight at 37°C. After overnight incubation, the area of no growth (inhibition zones) was measured in mm [32].

Antibiotic susceptibility test

The antibiotic susceptibility test by disk diffusion method was also performed with standard synthetic antibiotics, and the result of the two tests was compared with the tables provided by the Standard Clinical and Laboratory Institute, which provide standards and guidelines for susceptibility testing (CLSI) [30]. To ensure the results of each of the different concentrations of rhamnolipid and antibiotics, these tests were repeated three times for each bacterial strain. Also, quantitative tests were performed to determine the minimum inhibitory (MIC) and minimum lethal concentrations (MLC) of rhamnolipid. Thus, the minimum inhibitory concentration was tested in a sterile microtiter plate using a broth microdilution method. To evaluate the performance of rhamnolipids as a potential antimicrobial agent, MIC and MLC values were calculated by using the broth microdilution method for the turbidity observed at an optical density of 650 nm as an observed bacterial growth in the broth. Then

the results were compared with the standard antibiotic Gentamicin (10 µg/mL). The assessment was done to evaluate bacteria's susceptibility against the rhamnolipids compared to the standard antibiotics. After culturing bacteria on the Mueller-Hinton agar medium, the observed colony was transferred to the broth with the help of a sterile swab. The broth is incubated at 37°C until the growth reaches turbidity equal to that of 0.5 McFarland standard. As per CLSI recommendations, the culture was adjusted with sterile broth to give turbidity equivalent to the McFarland 0.5 standard (1.5×10^8 CFU/mL) [30]. First, 100 µL of inoculated Mueller-Hinton broth was added into the six wells of the microtiter plate to get the desired microplate dilutions. Then, 100 µL of rhamnolipid was added to the first well (1000 mg/mL; the highest concentration of the rhamnolipid), and diluted concentrations of rhamnolipid (500, 250, 125, and 50 mg/mL) were serially added from the second, third, and so on to the sixth well. Cells in the wells was adjusted for 0.1 optical density. After incubation at 37°C, the turbidity was measured by spectrophotometry at 650 nm after every 12 h interval for the next three days to assess the bacterial growth. In another well, 100 µL of diluted suspension (Nutrient broth) equivalent to half McFarland tube was added as a blank [4, 33]. After overnight incubation at 37°C, the tubes were evaluated for macroscopically visible evidence of bacterial growth in the form of turbidity. Basic controls like culture medium and microbes were also separately noted for the presence or absence of bacterial growth in the specialplate.

By definition, the first well without turbidity (lowest) is placed as the minimum inhibitory concentration. Also, the minimum lethal concentration (MLC) test was determined according to the results of the minimum inhibitory concentration (MIC). The wells in which bacterial growth was entirely stopped by sterile swab sampling were cultured on nutrient agar medium and incubated at 37°C. After 24 h, the lowest concentration of rhamnolipid in which the bacteria did not grow was reported as the MLC [34].

Enzyme activity of the isolated strains

The next step was the determination of the enzyme activity of these isolated strains. There are different enzymes synthesized and secreted by microorganisms required for the degradation of organic waste during composting. Different enzymes like protease, amylase, lipase, and cellulase are involved in the degradation process. Hence, isolated bacterial strains were screened to produce the two enzymes: protease and amylase [35, 36]. The enzymatic activity of amylase and protease

was quantified using spectrometry. The amylase activity was determined using the DNS solution. For this purpose, 1 mL bacterial suspension was added in a test tube, to which 1 mL of prepared starch solution was added. Phosphate buffer solution in bacterial suspension acts as a control. Both test tubes were incubated at 37°C for 30 minutes. After incubation of 2 mL of DNS solution was added to each test tube, they were placed in a boiling water bath for 15 minutes. After cooling, the absorption was measured at 540 nm. This process was carried out for each bacterial strain [37].

For the determination of protease activity, 1 mL of casein (Padtanteb Co. Iran), which acts as the enzyme-substrate, is added in a test tube with 1 mL bacterial suspension. It is incubated at 37°C for 10 minutes. After 10 minutes of incubation, Trichloroacetic acid TCA was added to stop the reaction. The test samples are then passed through a 0.45-µm polyethersulfone syringe filter. After this, 1 mL of sodium carbonate and 1 mL of Folin's reagent are added. Absorbance was taken at 660 nm. This procedure was carried out for each bacterial strain [38].

3. Results

Isolation and identification of pathogenic bacteria from compost

From the morphological and biochemical tests, eight isolated bacterial strains from the organic compost were identified as per Bergey's Manual of Systematic Bacteriology. The colony morphology was studied on the bacterial culture plate, and morphological characteristics of the colony grown on the plate, the gram staining, and motility characteristic are observed and mentioned in Table 1.

The biochemical tests like evaluating the different types of sugars (glucose, lactose, and sucrose), catalase and oxidase creation, and The Voges-Proskauer (VP) test determine whether microorganisms can produce acetyl methyl carbinol from glucose fermentation. The biochemical characteristics of the bacterial colonies are mentioned in Table 2.

Evaluation of the antimicrobial effect of rhamnolipid on pathogenic bacteria

As different concentrations of rhamnolipid were used to determine the inhibitory zone on the bacterial isolates, it was found that 1000 mg/mL of rhamnolipid has the maximum antibacterial activity against different pathogenic strains. Gentamycin antibiotic disk (Padtanteb, Co.) with a concentration of 10 µg/mL was used as a positive con-

Table 1. Morphological characteristics of bacterial strains

Bacterial Strains	Colony Characteristics	Cell Characteristics of Bacteria		Motility
		Gram Nature	Shape	
<i>Enterococcus faecalis</i>	White, circular, smooth, and entire	Gram + ve	Cocci	Non-motile
<i>Enterobacter aerogenes</i>	White, circular, smooth, and entire	Gram – ve	Bacilli	Motile
<i>Bordetella petrii</i>	White, circular, shiny	Gram – ve	Coccobacilli	Non-motile
<i>Pseudomonas aeruginosa</i>	Large and smooth colonies	Gram – ve	Bacilli	Motile
<i>E. coli</i>	Circular, Moist, Smooth, and entire margin.	Gram – ve	Bacilli	Motile
<i>Bacillus stearothermophilus</i>	Creamy, circular, smooth, entire	Gram + ve	Bacilli	Motile
<i>Bacillus licheniformis</i>	Creamy, rough, irregular	Gram + ve	Bacilli	Motile
<i>Brevibacillus brevis</i>	Circular, Translucent, Smooth and Coalesce	Gram + ve	Bacilli	Motile



trol. The average area of inhibitory zones in mm for 1000 mg/mL of rhamnolipid is given in the [Table 3](#).

Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC)

MIC of rhamnolipid showed the lowest concentration, which can inhibit the visible growth of bacteria, whereas MLC showed the lowest concentration of rhamnolipid that can be lethal for bacteria. Determination of MIC and MLC are important in antimicrobial susceptibility testing. [Table 4](#) demonstrates the inhibitory and lethal effects of rhamnolipid on all eight isolated bacterial strains.

The result was compared to the standard provided by the US Standard Clinical and Laboratory Institute

(CLSI, 2015). The standard antibiotic susceptibility test result in percentage is also given in [Table 5](#). Analysis of the data was carried out by using descriptive statistics and frequency distribution tables. The resulting percentages of different antibiotics were calculated for the eight isolated bacterial strains. The obtained results were further categorized into resistant (R), intermediate (I), and susceptible (S) categories for the eight isolated bacterial strains from the organic compost.

The MIC of rhamnolipid on different isolated bacterial strains was compared with the standard value given by Standard Clinical and Laboratory Institute. Accordingly, it was found that *E. coli* was better inhibited by rhamnolipid (1000 mg/mL) compared to all other antibiotics. Only amikacin showed better inhibition than rhamnolipid.

Table 2. Biochemical characteristics of bacterial strains

Bacterial Strains	Catalase	Oxidase	Voges-Proskauer test	Fermentation Tests		
				Glucose	Lactose	Sucrose
<i>Enterococcus faecalis</i>	~ve	~ve	+ve	~ve	~ve	~ve
<i>Enterobacter aerogenes</i>	+ve	~ve	+ve	~ve	~ve	~ve
<i>Bordetella petrii</i>	+ve	+ve	+ve	~ve	~ve	~ve
<i>Pseudomonas aeruginosa</i>	+ve	+ve	–ve	+ve	~ve	~ve
<i>E. coli</i>	+ve	~ve	–ve	+ve	+ve	+ve
<i>Bacillus stearothermophilus</i>	+ve	+ve	–ve	+ve	~ve	+ve
<i>Bacillus licheniformis</i>	+ve	+ve	+ve	+ve	+ve	+ve
<i>Brevibacillus brevis</i>	+ve	+ve	–ve	~ve	~ve	~ve



Table 3. Inhibitory effect of rhamnolipid on isolated strains

Isolated Bacterial Strains	Gentamycin (10 µg/mL) (Positive Control)	Area of no Growth (mm) (Rhamnolipids – 1000 mg/mL)
<i>Brevibacillus brevis</i>	19.43 (S)	25
<i>Bordetella petrii</i>	21.90 (S)	18
<i>Bacillus stearothermophilus</i>	20.34 (S)	31.5
<i>E. coli</i>	18.24 (S)	28.4
<i>Enterococcus faecalis</i>	19.15 (S)	21.5
<i>Enterobacter aerogenes</i>	18.54 (S)	20
<i>Pseudomonas aeruginosa</i>	20.72 (S)	24.8
<i>Bacillus licheniformis</i>	19.25 (S)	28

(S): Susceptible.



Regarding *Bordetella petrii*, the inhibitory effect of 1000 mg/mL of rhamnolipid was higher than all antibiotics except ciprofloxacin, doxycycline, and imipenem which showed better susceptibility for *B. petrii*. Also, 1000 mg/mL of rhamnolipid has a better inhibitory effect on *Enterococcus faecalis* when compared to other antibiotics. However, ciprofloxacin, piperacillin/tazobactam, and co-trimoxazole were more effective in inhibiting the growth of *E. faecalis*. Regarding *Pseudomonas aeruginosa*, the inhibitory effect of 1000 mg/mL of rhamnolipid was higher than other antibiotics effective on this bacterium, but rhamnolipid was found to be less effective than doxycycline and ciprofloxacin antibiotics. The *Bacillus stearothermophilus* was effectively inhibited by 1000 mg/mL of rhamnolipid than other antibiotics except doxycycline antibiotic. In *Bacillus licheniformis*, the inhibitory effect of 1000 mg/mL of rhamnolipid was higher than other antibiotics effective on this bacterium.

However, rhamnolipid was less effective than the inhibitory effect of ceftazidime on this bacterium.

Enterobacter aerogenes was better inhibited by 1000 mg/mL of rhamnolipid than other antibiotics effective on this bacterium. Imipenem, chloramphenicol, and amikacin were more effective than rhamnolipid. Regarding *Brevibacillus brevis*, the inhibitory effect of 1000 mg/mL of rhamnolipid was higher than all the antibiotics except doxycycline on this bacterium.

Investigation of enzymatic activity in strains isolated from compost

Analysis of the enzymatic activities of the isolates indicated that these strains differ regarding the enzymatic activity of amylase and protease. Most of the protease activity belongs to the strains of *Bacillus stearother-*

Table 4. MIC and MBC of rhamnolipid (mg/mL)

Strain	MIC (mg/mL)	MLC (mg/mL)
<i>E. coli</i>	125	250
<i>Enterococcus faecalis</i>	125	250
<i>Enterobacter aerogenes</i>	250	500
<i>Bacillus licheniformis</i>	50	250
<i>Bacillus stearothermophilus</i>	125	250
<i>Pseudomonas aeruginosa</i>	50	125
<i>Bordetella petrii</i>	50	125
<i>Brevibacillus brevis</i>	50	250



Table 5. Antibiotic susceptibility test results

Antibiotic	Resistant (%)	Intermediate (%)	Susceptible (%)
Co-trimoxazole (25 µg)	75	25	-
Amikacin (30 µg)	62.5	25	12.5
Imipenem (10 µg)	50	-	50
Ceftazidime (30 µg)	50	-	50
Cefotaxime (30 µg)	62.5	-	37.5
Piperacillin/Tazobactam (100/10 µg)	37.5	12.5	50
Tetracycline (30 µg)	62.5	-	37.5
Chloramphenicol (30 µg)	75	12.5	12.5
Ciprofloxacin (5 µg)	37.5	12.5	50
Erythromycin (15 µg)	50	37.5	12.5
Doxycycline (30 µg)	50	12.5	37.5
Nitrofurantoin (300 µg)	37.5	12.5	50



mophilus, *Brevibacillus brevis*, *Bacillus licheniformis*, and *Bordetella petrii*. The highest amylase activity belonged to *Escherichia coli* strains, *Enterococcus faecalis*, and *Enterobacter aerogenes*. Also, *Brevibacillus brevis* produced the least amount of enzyme. *Enterococcus faecalis* produced the least amount of protease, and other strains had moderate enzyme production. The result of the quantification of enzyme activity is given below.

4. Discussion

Compost is a decomposed organic matter which is rich in nutrients and microorganisms. It is a rich source of aerobic, anaerobic, mesophilic, and thermophilic bacteria. For the current study, compost was selected because of its rich source of pathogenic and non-pathogenic bacteria. After isolation and enrichment, eight different strains were saved on agar and broth. Among these or-

Table 6. Quantitative evaluation of enzyme activities

Protease Enzyme (U/mL)	Strains
4.1	<i>Bacillus stearothermophilus</i>
5.4	<i>Brevibacillus brevis</i>
3.2	<i>Bacillus licheniformis</i>
5.7	<i>Bordetella petrii</i>
Amylase enzyme (U/mL)	Strains
0.09	<i>E. coli</i>
0.04	<i>Enterococcus faecalis</i>
0.07	<i>Enterobacter aerogenesis</i>
0.05	<i>Pseudomonas aeruginosa</i>



ganisms, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, *Escherichia coli* (ordinarily present in the human intestine but can cause diseases), and *Bordetella pertussis* are Gram-negative pathogens. But, *Enterococcus faecalis*, *Bacillus licheniformis*, *Brevibacillus brevis*, *Bacillus stearothermophilus* are gram-positive bacteria. Among these, *Bacillus licheniformis*, *Brevibacillus brevis* are rarely pathogenic.

To determine the effect of rhamnolipid on isolated bacterial strains, the inhibitory zone was calculated using the disk diffusion method. The same method for determining the rhamnolipid effect on microbial strains was used in other studies [39, 40]. The purpose of this method was to determine the susceptibility of bacterial strains to rhamnolipid. The disk diffusion method is used as a pre-evaluation method for novel antimicrobial agents [32]. About 1000 mg/mL of rhamnolipids showed the maximum inhibitory zone against different isolated strains, as shown in Table 3. *Bacillus stearothermophilus* has the maximum inhibitory zone of 31.5 mm among all other bacterial strains. The inhibitory zones showed the susceptibility of isolated bacterial strains for the rhamnolipid.

MIC and MBC were then determined to confirm further the susceptibility test for rhamnolipid. The result of the MIC is categorized as resistant, intermediate, and susceptible. If the concentration of rhamnolipid falls in the category of susceptible, it means that it is likely to inhibit the growth of that bacterium [41]. The rhamnolipid MIC results (Table 4) are compared with the antibiotic's standard MIC values (Table 5) provided by the US Clinical and Laboratory Standard Institute. When the data were analyzed with the help of descriptive statistics and frequency distribution tables, it was found that rhamnolipid is more effective than most of the synthetic antibiotics. It can also be proved by another study that the effectiveness of rhamnolipid as an antibiotic is much better than other antibiotics [42]. Rhamnolipid was more effective than most other antibiotics on bacterial strains of *Bacillus stearothermophilus*, *Bacillus licheniformis*, and *Brevibacillus brevis*. Also, rhamnolipids can be used as an excellent alternative against antibiotic-resistant pathogenic strains due to its antibacterial properties [25].

Similar results were seen in another study which proved that Gram-positive bacteria are more rhamnolipid sensitive with no effect on the pH [43]. However, doxycycline was more effective than rhamnolipid for *Bacillus stearothermophilus* and *Brevibacillus brevis*. In contrast, *Bacillus licheniformis* was more susceptible to ceftazidime than rhamnolipid.

Evaluation of amylase and protease activity showed that the strains differ in their enzymatic activity (Table 6). The bacterial strains of *Bacillus stearothermophilus*, *Brevibacillus brevis*, *Bacillus licheniformis*, and *Bordetella pertussis* showed maximum protease activity. These bacteria are isolated from compost, so high proteolytic activity indicates their involvement in the Nitrogen cycle.

A similar study deduced that Alkalophilic bacillus isolated from natural host produced protease with high activity [44]. Whereas other strains, like *Escherichia coli*, *Enterococcus faecalis*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*, showed high amylase activity, which indicates that they use sugar as the source of energy. It can be proved with another study that explained that hemoglobin gene presence indicates the increased production of amylase in *Escherichia coli* strains [45]. One other study also explained the increased production and activity of enzymes from compost microorganisms [46, 47]. Another study revealed that the rhamnolipids might improve the stability of an enzyme and its denaturation during heterogeneous hydrolysis of cellulose through enzymes. Additionally, rhamnolipids play a crucial role in removing pathogenic bacteria in composting because of their antibacterial function. It also promotes the quality of the composting products [1].

Bacterial strain for producing rhamnolipids is critically cost-effective for rhamnolipids production. Various efforts are needed for obtaining non-pathogenic strains with high production of rhamnolipid, including strains from the environment [48].

5. Conclusion

Microbiology trends are continuously changing because of new research, novel microbes, antibiotic resistance in microbes, and the side effects of synthetic antimicrobial agents. It is the need of the hour to find new alternatives to synthetic antimicrobials that are less toxic and have better inhibitory or bactericidal activity. During composting, different mesophilic and thermophilic pathogenic microbes grow in the organic compost, which increases the risk of a potential transfer of these pathogens through the vegetables and fruits grown in such pathogens (especially antibiotic-resistant strains) contaminated soil. Rhamnolipids are one such surfactant that is naturally produced by bacteria, which are less toxic and have antibacterial activity. Extensive research on these biosurfactants can improve the quality of the organic compost that could minimize the risk of contamination of pathogens through organic vegetables. Hence, the use of rhamnolipids can ensure the safety of

organic farming products. Extensive research on these biosurfactants could help improve the chances of using them for successful microbial treatment due to their antibacterial properties.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this article.

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Conflict of interest

The authors declared no conflict of interest.

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