World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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An investigation for exocellulase and endocellulase activities of thermotolerant Gram-positive bacteria from Al-Baha region, Saudi Arabia

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World Journal of Advanced Research and Reviews, 2025, 28(02), 1155-1168

Publication history: Received on 04 October 2025; revised on 10 November 2025; accepted on 13 November 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.28.2.3828

Abstract

This study was designed to explore some bacterial genera with the ability to decompose cellulosic materials from different places in Al-Baha region. All the collected and prepared compost samples in Nawan, Almakhwah and Bani Zabyan, Al-Baha were used for bacteria isolation on cellulose powder and CMC substrates as sole sources of carbon. Six isolates were obtained in both types of media. All the twelve isolates were described as thermotolerant as the incubation temperature was 45 °C. Basic morphological and physiological description for all isolates showed a high degree of similarity with bacteria of the genera *Bacillus*, *Amphibacillus*, *Paenibacillus* in case of all the spore forming isolates, and *Lactobacillus* in case of the non-spore forming isolate A2. The accuracy of the description of the isolates was confirmed by PCR detection of the cellulase genes ß-glucosidase and 6-phospho-ß-glucosidase in bacteria like the isolates under study. This assay was performed *in-silico* through an online program using primers designed specifically for Bacilli and Lactobacilli. Growth curves for all isolates showed maximum growth activity at 24 to 30 hours from the start of incubation except for isolate B1, which reached the maximum growth at 24 hours. Application of the isolates for plant waste composting under laboratory conditions in consortia was conducted. Finally, a simple experiment for planting fenugreek seeds was conducted to test quality of the compost and to ensure that it is free of any toxic substances for plants. The results showed the quality of the compost produced in both natural and laboratory conditions.

Keywords: Exocellulase; Endocellulase; Gram-positive; Bacteria; Compost

1. Introduction

Microorganisms exist in nature in many environments. Soil is one of the most natural environments rich in microorganisms. There are many different types of microorganisms in the soil. Bacteria are the most dominant microbes in different types of soil [1]. Soil varies with its types in its content of bacteria in terms of abundance and species present, depending on the environmental conditions. One of the most famous natural environments associated with soil and its content of organic matter, especially plants, is the compost environment. Compost is a form of soil that is very rich in its content of microorganisms, especially bacteria.

There are many taxonomically different bacterial groups in the soil of different types. Each bacteria group has a specific natural role according to the soil in which it lives and is abundant. Bacterial presence flourishes in the soil whenever the appropriate environmental conditions are available, as well as the availability of other organisms and multiple organic materials, especially plant ones. Based on this, we find that, for example, an environment such as compost, which is basically the product of the decomposition of plant organic matter, is one of the richest natural environments in terms of different bacterial species, both Gram positive and Gram negative.

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One of the most important and most famous bacterial groups in soil, especially in compost, is the group of Gram-positive bacteria with its different divisions. This difference comes from different environmental conditions and accordingly the roles played by one bacteria group differ from the other. Here, the importance of this diversity and the enzymatic activities of bacteria associated with it, according to the natural environment, its content of other living organisms, and the availability of specific organic materials.

One of the most prevalent organic materials in the soil is the remains of plant origin rich in its content of cellulose. Bacteria, especially Gram-positive bacteria, have multiple roles in the decomposition of these plant residues. Here, the enzymatic activities of Gram-positive bacteria vary according to the various natural conditions such as temperature, humidity, and air availability. Each genus of Gram-positive bacteria has specific enzymatic activities in the decomposition of natural substances, especially cellulose. We find here that the difference of groups and genera is of great importance, as the different genera of Gram-positive bacteria play a part in the complete decomposition in nature of polymers such as cellulose. Gram-positive bacteria have important roles in the process of decomposing cellulose in nature through their production of various enzymes responsible for this decomposition. The most famous of which are Bacilli and Lactobacilli groups. The genus *Bacillus* is distinguished from other bacteria mainly by its ability to form resting endospores [2].

Cellulose polymer consists of crystalline and non-crystalline parts [3]. Cellulase enzymes are distinguished from other glycoside hydrolases by their ability to hydrolyze the beta 1,4 linkages to liberate glucose units [4]. ß-glucosidase and 6-phospho ß-glucosidase are important cellulase enzymes required to complete the process of hydrolysis to glucose [5].

Completed hydrolysis of cellulose is facilitated by the action of three major types of enzymes namely endocellulases, exocellulases, and β-glucosidases according to the location action in cellulose. Endocellulases act internally on cellulose chain, exocellulases act on chain ends to produce glucose or cellobiose, and ultimately β-glucosidases act on cellobiose units to produce glucose [6].

Several unique cellulase enzymes with hydrolytic activity of crystalline and amorphous cellulose moieties have been discovered in species of the genus *Bacillus* and *Paenibacillus*. The endocellulase enzyme works on the amorphous parts, and therefore it can be detected by the growth of bacteria on a medium such as CMC agar, while a medium such as cellulose powder agar is used to detect the exocellulase enzyme, which works on the crystalline parts [7].

Many of the basics of the process of consumption of cellulose by microbes and the production of cellulase and its various applications are detailed in many references such as [4, 3, 8, 6].

This study was designed to explore some bacterial genera with the ability to decompose cellulosic materials from different places in Al-Baha region. The study aimed to isolate these bacteria from soil and compost samples on media containing cellulose powder or carboxymethylcellulose (CMC) as sole carbon and energy sources. A qualitative screening to these isolates for detection of exocellulase and endocellulase activities on agar media was conducted. The obtained isolates were subjected to morphological and physiological characterization. The detection of specific cellulase genes in the closest bacteria to our isolates by *in silico* PCR also was considered. The study ended with an investigation for potential of these cellulolytic bacterial isolates in degradation of some natural cellulose substrates and subsequent detecting the characteristics of the resulting compost and its suitability for plant cultivation.

2. Materials and methods

2.1. Sample collection locations

The areas were determined according to altitude, and compost samples were collected from a designated place in Nawan, Almakhwah (location coordinates: 19°33'27.5"N 41°07'29.8"E) at 24 meters above sea level. Compost samples were also collected from Bani Zabyan between Baljurashi and Al-Baha (location coordinates: 19°57'55.5"N 41°28'56.1"E) from a designated place in a home garden at 2,200 meters above sea level. In this last place, an experiment was designed to manage some plant organic waste and follow it up for a month (June-July 2022) and then use it for isolation as well. The experiment was designed on four treatments as follows: 1- Mixing the residues with the addition of agricultural soil 2- Mixing the residues without adding any soil 3- Mixing the residues with the addition of previously prepared compost in Bani Zabyan 4- Mixing the residues with the addition of previously prepared compost in Nawan, Almakhwah . All these samples were used for bacteria isolation in the laboratory as follows.

2.2. Culture medium and method of isolation

The following medium (PH 6.5-7.5) was used [9], consisting of (g/L): Cellulose substrate (Cellulose powder, SDFCL Company) or carboxymethylcellulose, CMC, LOBA CHEMIE Company), 20; KCl, 0.5; $MgSO_4.7H_2O$, 0.5; K_2HPO_4 (Anhydrous), 1.0; $NaNO_3$, 2.0; Potable Water, 1000 ml, in liquid or solid by adding 2% agar. The samples were first stimulated (1 g in 25 ml) by culturing them in a cellulose liquid medium and incubated aerobically within a rotary incubator (120 rpm) for 1 day at 40 ° C, then cultured on cellulose solid medium and continued incubation for 3-5 days at temperature of 45° C. The purity of the isolates was confirmed on the same agar medium and preserved on agar slopes.

2.3. Cellulase enzyme detection

Qualitative detection of the ability of the isolates to produce the enzyme and hydrolyzing of the cellulose in the medium was carried out by the method of [10] by adding the iodine reagent to the plates. The appearance of the decomposition areas around the bacterial growth were recorded as clear halos.

2.4. Description of the isolates

The culture characterization tests were carried out on the nutrient agar medium and incubated aerobically. Gram stain was conducted for the isolates. The motility and growth of the isolates at different temperatures were tested, according to the instructions mentioned in [11].

2.5. PCR detection of some components of the cellulase enzyme genes in bacteria like the isolates under study

This inspect was performed using an online program (www.in-silico.com) for the complete genomes of many different bacteria [12] and was determined using primers specially designed for Bacilli and Lactobacilli bacteria according to [5]. These primers are pre-designed to amplify the cellulase genes ß-glucosidase and 6-phospho-ß-glucosidase, two of the cellulase enzymes important to complete the hydrolysis process. The primers were (5'-TTTGCTGAAATGGG-3'), the forward, and (5'-GGATCAATTTGCCANCCCC-3'), the reverse. PCR was performed for all available species of *Bacillus* (91 species) and *Lactobacillus* (57 species) in the program. Only the representative species of all genera that gave positive results were selected, and they were checked for their registration in the GenBank, and all their data were recorded. To ensure the accuracy of the program's work, it was also used to detect the well-known 16s rRNA gene using general primers F27 (5'-AGAGTTTGATCCTGGCTCAG-3') the forward, and R1492 (5'- GGTTACCTTGTTACGACTT-3') the reverse, that yield around 1500 bp PCR amplicons.

2.6. Temperature tolerance of the isolates

A culture of isolates was carried out to test their ability to consume either type of cellulose media in each case, namely CMC or cellulose powder. This was done with solid media and incubation at increasing temperatures included 30, 45, 50, and 55 then followed up for three days and the results were recorded.

2.7. Creation of the growth curve for the isolates

The isolates were cultured separately with the same liquid medium on which they were first isolated (CMC and cellulose powder). Incubation was carried out at 45°C and growth was measured at intervals for each isolate (6 hours or multiples thereof) for two days to construct a growth curve for each isolate. Optical density measurements were taken with a spectrophotometer Model 6715 JENWAY at a wavelength of 600 (OD 600). Inoculations were made first with a volume of 2 ml of each isolate in each medium with an initial optical density of 0.002.

2.8. Application of the isolates in plant waste composting under laboratory conditions

The ability of the isolates was tested under laboratory conditions to hydrolyze mixture of plant organic residues, cotton linters and filter papers. The mixture was prepared homogeneously, distributed in 1L flasks (100 grams each) and sterilized. The water used was also sterilized to ensure that the mixtures were saturated and did not dry out during the incubation period. The isolates were cultured collectively, where all isolates growing on CMC were injected into one treatment, and all isolates growing on cellulose powder were injected in a second treatment. The organisms were first cultured for activation in liquid medium for 24 hours and then injected with a volume of 2 ml of each isolate in each case. The incubation was carried out at a temperature of 45 °C for 6 days and followed up to ensure the stirring and mixing well. On the sixth day, 10 grams of the soil mixture and the compost used first in the isolation process were added to each treatment and the incubation continued for 24 hours, to help ripen the resulting compost by other thermophilic microbes other than the isolates under study. The resulting compost characteristics were recorded and saved for later use.

2.9. Use of the prepared compost in seed planting

A planting test was conducted for fenugreek (*Trigonella foenum-graecum*) seeds (20 seed per treatment) to test the quality of the compost and to ensure that it is free of any toxic substances for plants. This procedure was done by using the compost mixture produced first in treatments 3 and 4 to which compost was added under natural conditions, and then the produced compost under laboratory conditions was added to it. The first treatment used in isolation, to which soil was added, was used here as control without any additions.

3. Results

3.1. Sample collection locations

Compost samples were collected from Nawan, Almakhwah at 24 m above sea level. Compost samples were also collected from Bani Zabyan between Baljurashi and Al-Baha at 2,200 m above sea level. In this last place, an experiment was designed to manage some plant organic waste and followed up for a month (June-July 2022) and then used also for isolation. The experiment was designed on four treatments as follows: 1- Mixing the residues with the addition of agricultural soil 2- Mixing the residues without adding any soil 3- Mixing the residues with the addition of previously prepared compost in Bani Zabyan 4- Mixing the residues with the addition of previously prepared compost in Nawan, Almakhwah.

3.2. Culturing and isolation

All the samples mentioned above were used for bacteria isolation on cellulose powder and CMC substrates as sole sources of carbon in the liquid or agar media at temperature of 45° C. During the isolation process, one bacterial colony was chosen among the very similar in appearance. Six isolates were obtained on the cellulose powder agar medium, and the same number was obtained on the CMC agar medium. All the sources, locations, and isolation media for the isolates are listed in (Table 1).

3.3. Cellulase enzyme detection

Qualitative detection of the ability of the isolates to produce exocellulase on cellulose powder agar medium was carried out by adding the iodine reagent to the plates. The appearance of the decomposition areas around the growth of the isolates were recorded as clear halos. The same test was performed concerning the ability of the isolates to produce endocellulase on CMC agar medium by adding the iodine reagent to the plates and recording the results (Figures 1 and 2).

Table 1 The sources, locations, and isolation media for the isolates

Isolate No.	Isolate Source	Location	Isolation medium	Isolation temperature
M1	Plant residues mixture with agricultural soil	Bani Zabyan Al-Baha	Cellulose powder	45° C
M2	Plant residues mixture without soil	Bani Zabyan Al-Baha	Cellulose powder	45° C
M3	Plant residues mixture with compost from Bani Zabyan	Bani Zabyan Al-Baha	Cellulose powder	45° C
M4	Plant residues mixture with compost from Nawan	Bani Zabyan Al-Baha	Cellulose powder	45° C
A1	Compost	Nawan, Almakhwah	Cellulose powder	45° C
A2	Compost	Nawan, Almakhwah	Cellulose powder	45° C
N1	Plant residues mixture with agricultural soil	Bani Zabyan Al-Baha	CMC	45° C
N2	Plant residues mixture without soil	Bani Zabyan Al-Baha	CMC	45° C

N3	Plant residues mixture with compost from Bani Zabyan	Bani Zabyan Al-Baha	CMC	45° C
N4	Plant residues mixture with compost from Nawan	Bani Zabyan Al-Baha	CMC	45° C
B1	Compost	Nawan, Almakhwah	CMC	45° C
B2	Compost	Nawan, Almakhwah	CMC	45° C

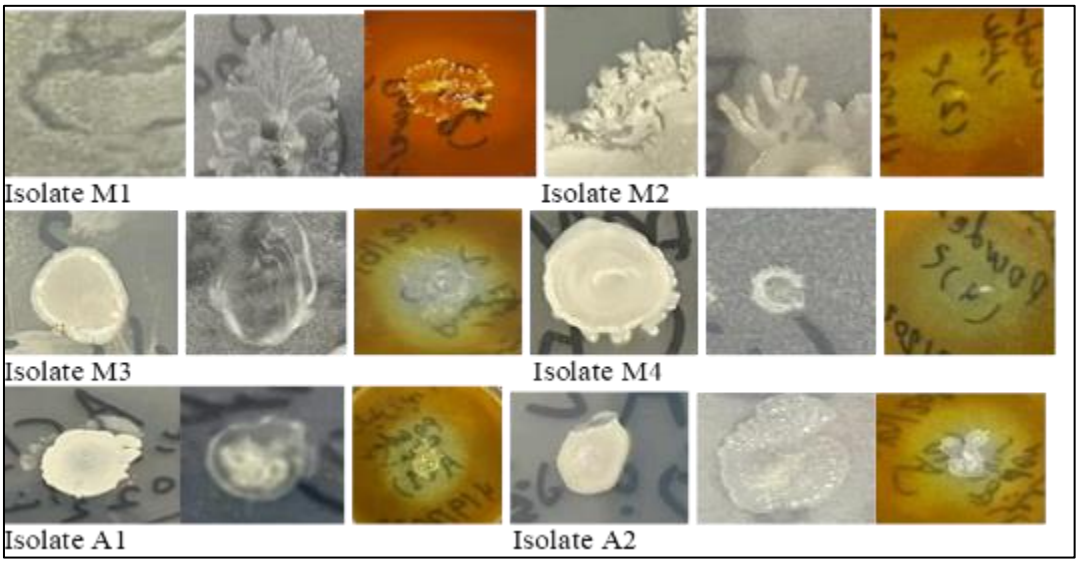

Figure 1 Growth of the Isolates which utilize cellulose powder on nutrient agar (left pic for each isolate), and their exo-cellulolytic activity (middle and right pic for each isolate), shown on cellulose powder agar before and after adding iodine solution

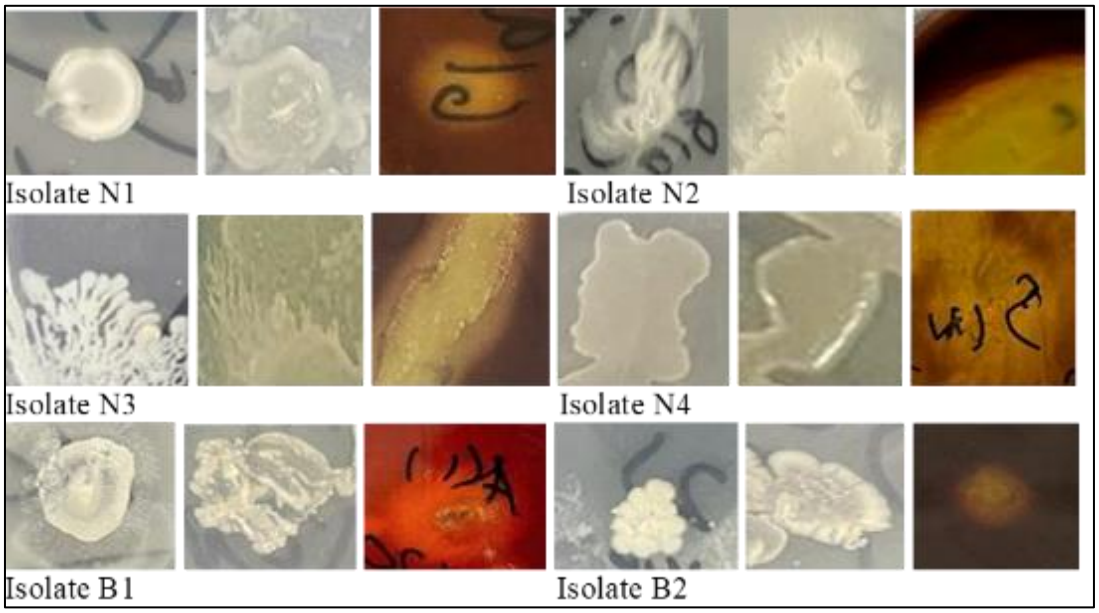

Figure 2 Growth of the isolates that utilized CMC, on nutrient agar (left pic for each isolate), and their endo-cellulolytic activity (middle and right pic for each isolate), shown on CMC agar before and after addition of the iodine solution

3.4. Description of the isolates

The culture characteristics were recorded on the nutrient agar medium (Figures 1 and 2). Gram's stain was conducted for the isolates (Figure 3), and all were Gram positive, rod shaped, and spore former bacteria except one only, the isolate A2. The motility was tested and recorded positive results for all the isolates except A2 also. Growth of the isolates at different temperatures was also carried out (Table 2). According to these characteristics collectively for all isolates and by matching them with the characteristics of Gram-positive bacteria in [11], it appeared that these isolates may be largely like the following bacterial genera: *Bacillus*, *Amphibacillus*, *Paenibacillus* in case of all the spore forming isolates, and *Lactobacillus* in case of the non-spore forming isolate A2.

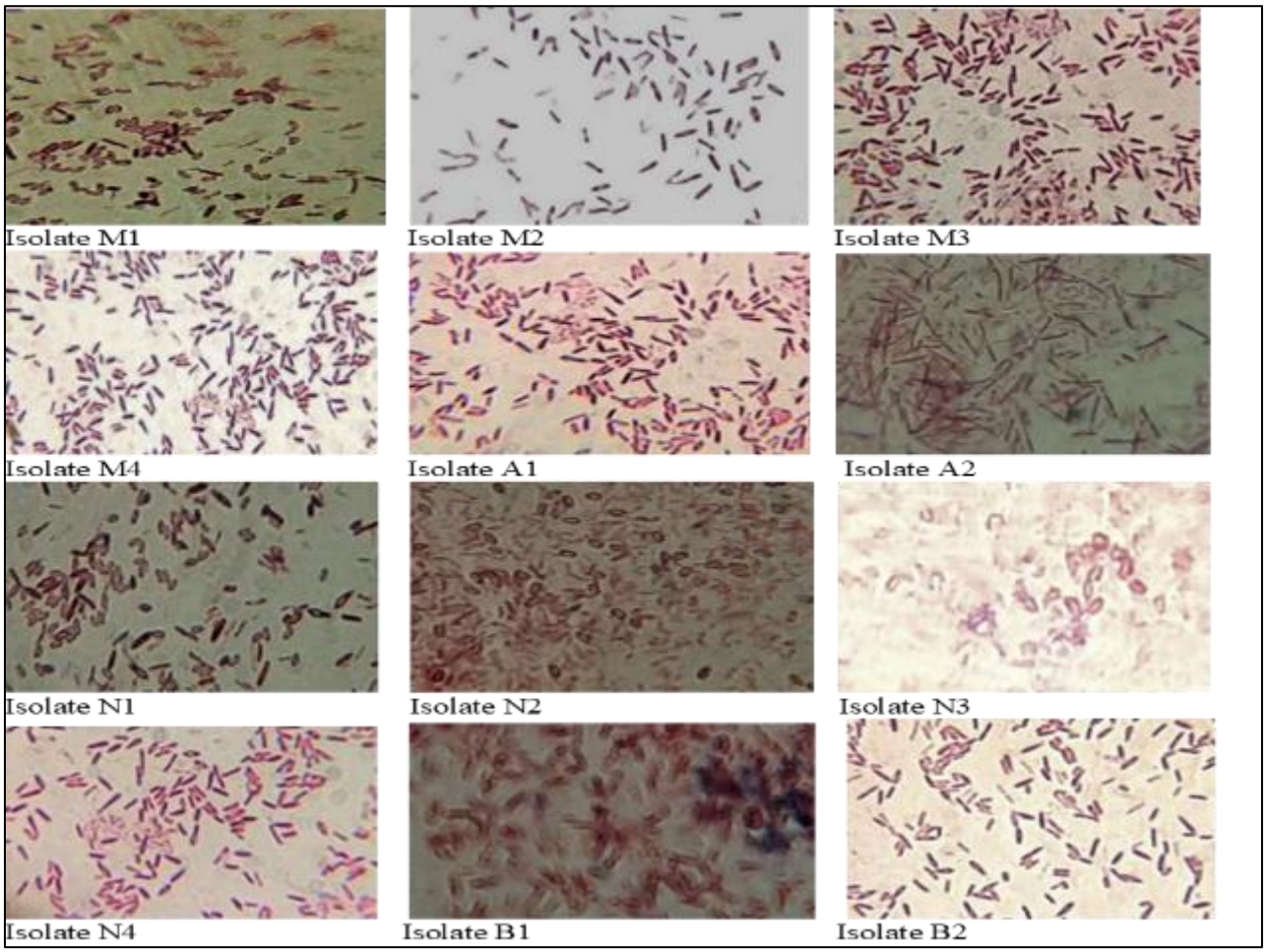

Figure 3 Photomicrographs of the isolates under oil immersion lens 1000x

Table 2 The morphological and physiological characteristics of the isolates

Test	**M1**	**M2**	**M3**	**M4**	**A1**	**A2**	**N1**	**N2**	**N3**	**N4**	**B1**	**B2**
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Endospores production	+	+	+	+	+	-	+	+	+	+	+	+
Motility	+	+	+	+	+	-	+	+	+	+	+	+
Gram's stain reaction	+	+	+	+	+	+	+	+	+	+	+	+
Aerobic or Facultative anaerobic	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 5 ^{o}C	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 55 ^{o}C	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 30 ^{o}C	+	+	+	+	+	+	+	+	+	+	+	+

Growth at 40 °C	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 50 °C	-	-	-	-	-	-	-	-	-	-	-	-

(+) Positive result (-) Negative result

3.5. PCR detection of some components of the cellulase enzyme genes in bacteria like the isolates under study

This assay was performed using an online program (www.in-silico.com) and was determined using primers specially designed for Bacilli and Lactobacilli bacteria. These primers are pre-designed to amplify the cellulase genes ß-glucosidase and 6-phospho-ß-glucosidase, two of the cellulase enzymes important to complete the hydrolysis process. PCR was performed for all available species of *Bacillus*, *Amphibacillus*, and *Paenibacillus* (91 species) and *Lactobacillus* (57 species) in the program. Only the representative species of all genera that gave positive results were selected and listed in (Table 3) with all their detailed information in the GenBank. To ensure the accuracy of the program's work, it was also used to detect the well-known 16s rRNA gene using general primers F27 and R1492 that recorded positive results in (Table 4) and confirmed the results of PCR detection of the cellulase genes.

3.6. Temperature tolerance of the isolates

The isolates in each case, CMC or cellulose powder, were tested for their ability to grow at increasing temperatures included 30, 45, 50, and 55 °C. The results were recorded, and all isolates were able to grow in each case in temperatures 30 and 45 °C. No growth was recorded on both types of cellulose media at the rest of the tested temperatures.

3.7. Growth curves for the isolates

The isolates were cultured separately with the same liquid medium on which they were first isolated, CMC and cellulose powder. Incubation was carried out at 45°C and growth was measured at intervals for each isolate as mentioned in methods for two days to construct a growth curve for each isolate. Optical density measurements were recorded, and the curves were constructed (Figures 4 and 5). The curves showed that all isolates reach their maximum growth activity at 24 to 30 hours from the start of incubation except for isolate B1, which recorded the maximum growth at 24 hours from the start of incubation.

Table 3 The representative Gram-positive bacterial genera and species which gave positive bands (812-839 bp) upon amplification using the cellulase primers

No.	Gram-positive bacteria	Fragment size In base pairs (bp)	Product enzyme	GenBank Protein accession	GenBank Sequence accession	Sequence Start position	Sequence End position
1	*Bacillus subtilis* BSn5	815	aryl-phospho-beta-d-glucosidase	ADV94750	CP002468	2039584	2040398
2	*Bacillus pumilus* SAFR-032	818	glycoside hydrolase family 1 protein (beta-glucosidase - 6-phospho-beta-glucosidase)	ABV64287	CP000813	3608869	3609686
3	*Bacillus licheniformis* ATCC 14580	818	Glycoside Hydrolase Family 1 protein	QCY01555	CP034569	776108	776925
4	*Bacillus coagulans* 2-6 (*Weizmannia coagulans* 2-6)	830	6-phospho-beta-glucosidase	AEH52551	CP002472	534021	534850
5	*Bacillus amyloliquefaciens* CC178	815	6-phospho-beta-glucosidase	AGZ58492	CP006845	3736578	3737392

6	*Amphibacillus xylanus* NBRC 15112	821	6-phospho-beta-glucosidase	BAM48390	AP012050	2393887	2394707
7	*Paenibacillus polymyxa* CR1	812	6-phospho-beta-glucosidase	AIW41298	CP006033	4577300	4578111
8	*Lactobacillus plantarum* 16	830	6-phospho-beta-glucosidase	AGO08864	CP006033	2325006	2325835
9	*Lactobacillus johnsonii* DPC 6026	839	6-phospho-beta-glucosidase	AEB92527	CP002464	223101	223939
10	*Lactobacillus rhamnosus* ATCC 8530	818	family 1 glycosyl hydrolase (aryl-phospho-beta-D-glucosidase BglH)	AER63304	CP003094	450553	451370

Table 4 Testing the accuracy of the *in-silico* program by amplifying a known 16S rRNA gene for the bacteria representing the isolates, and the selected here were *Bacillus subtilis* BSn5 and *Lactobacillus plantarum* 16

Gram-positive bacteria	**PCR Fragment size In base pairs**	**GenBank Sequence accession**	**Sequence Start position**	**Sequence End position**
Bacillus subtilis BSn5	1511 bp	CP002468	2311298	2312808
Lactobacillus plantarum 16	1529 bp	CP006033	1884668	1886196

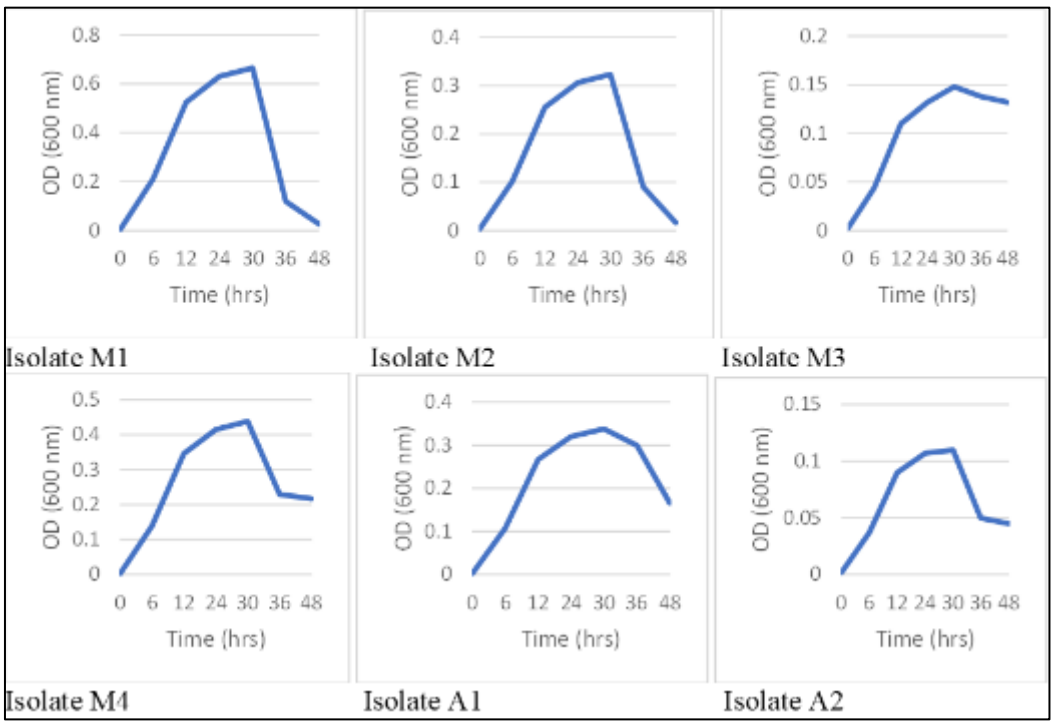

Figure 4 Growth curves for the isolates growing on cellulose powder liquid medium

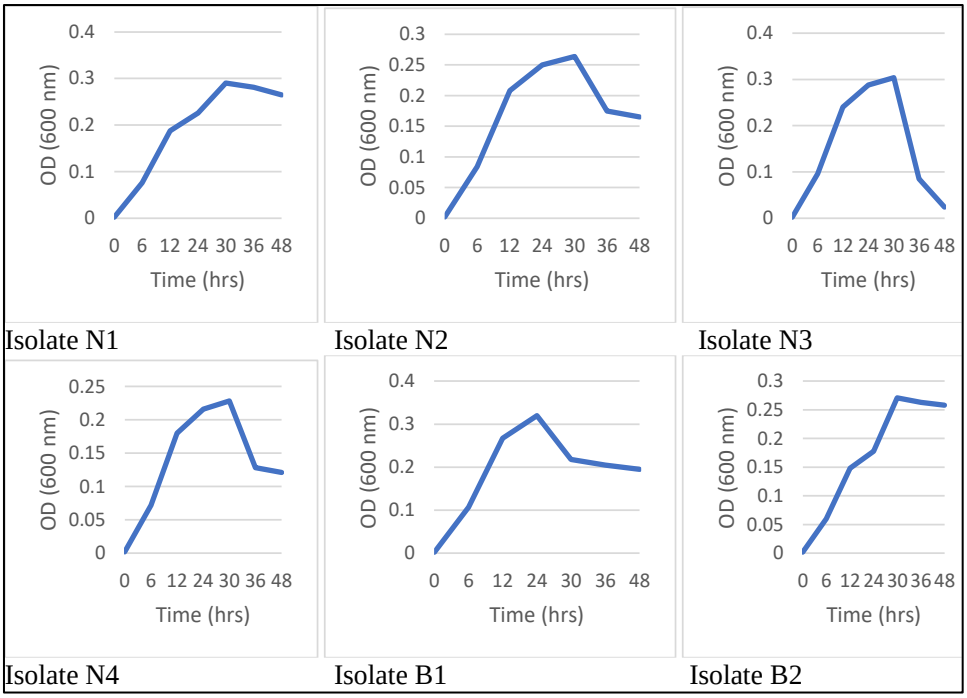

Figure 5 Growth curves for the isolates growing on CMC liquid medium

3.8. Application of the isolates in plant waste composting under laboratory conditions

Figure 6 Application of bacterial isolates for growth on a mixture of plant residues (The far left of the picture for each case, followed by the compost form after the end of the incubation). The treatments were (1) Treatment inoculated with all 6 isolates initially isolated on Cellulose powder (2) Treatment inoculated with all 6 isolates initially isolated on CMC

The ability of the isolates was tested under laboratory conditions to hydrolyze mixture of plant organic residues, cotton linters and filter papers. The mixture was prepared homogeneously, distributed in 1L flasks (100 grams each) and sterilized. The isolates were cultured collectively, where all isolates growing on cellulose powder were injected into one treatment, and all isolates growing on CMC were injected in a second treatment. The incubation was carried out at a temperature of 45 °C for 6 days and followed up to ensure the stirring and mixing well. On the sixth day, 10 grams of the soil mixture and the compost used first in the isolation process were added to each treatment and the incubation was extended for additional 24 hours, to help ripen the resulting compost by thermophilic microbes other than the thermotolerant isolates under study. The results showed the efficiency of the isolates in the two treatments in consuming the mixture of waste and decomposing it, as it shifted significantly to the form closest to maturity with a distinctive dark color and smell closest to the earthy smell (Figure 6).

3.9. Use of the prepared compost in seed planting

A planting test was conducted for fenugreek seeds to test the quality of the compost and to ensure that it is free of any toxic substances for plants. This procedure was done by using the compost mixture produced in treatments 3 and 4 for isolation, to which compost was added under natural conditions, and then mixed with the finally produced compost under laboratory conditions. The first treatment used in isolation, to which soil was added, was used here as control without any additions. The results showed the quality of the compost produced in both natural and laboratory conditions, where the seeds germinated, and the plant continued to grow naturally compared to the control grown in soil with germination percentage of about 65 % for both (Figure 7).

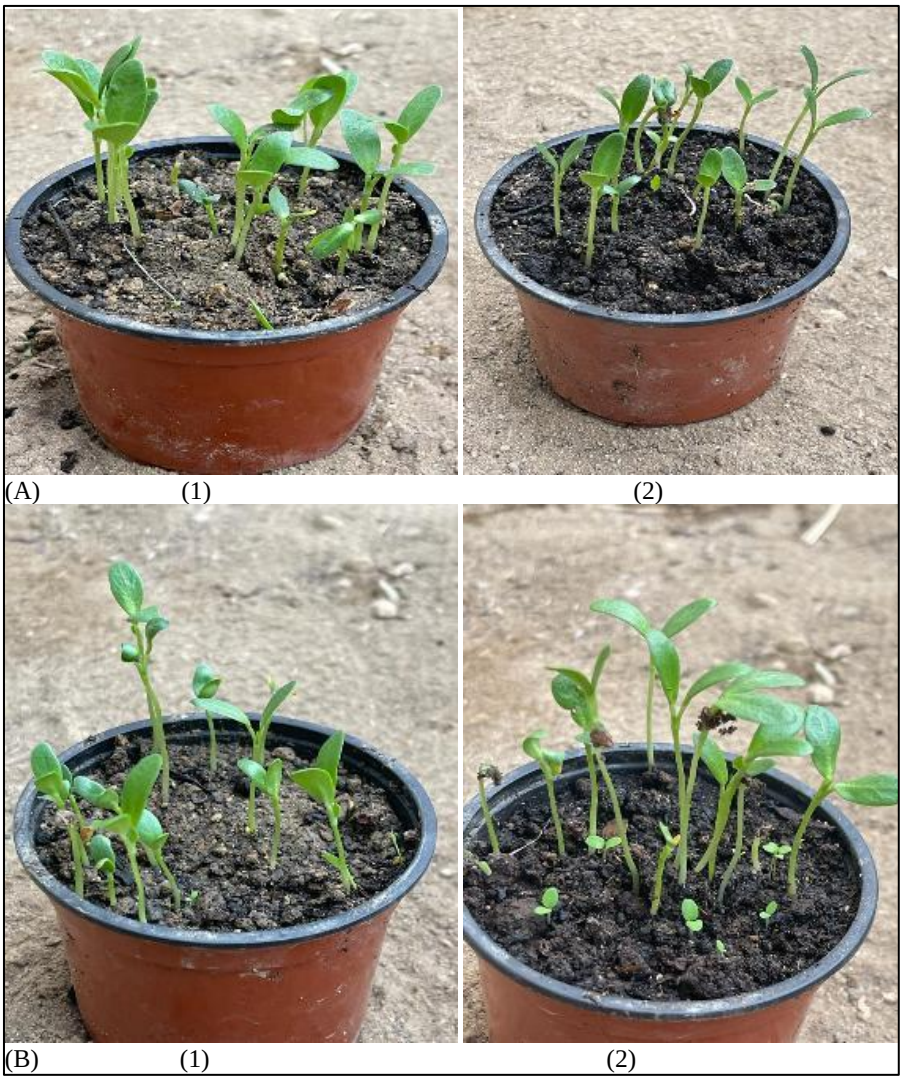

Figure 7 Germination of fenugreek seeds in (A) The case of cellulose powder grown isolates and (B) The case of CMC grown isolates; (1) Control soil (2) Compost mixture

4. Discussion

This study was designed to explore some bacterial genera with the ability to decompose cellulosic materials from different areas in Al-Baha region. Similar studies on compost and cellulase-producing Gram positive bacteria in Al-Baha, KSA include the work of [13] and [14] who reported cellulase-producing Gram positive bacteria from compost.

It was considered here that some areas are very high above sea level, and accordingly, work was chosen in two areas, one low and the other high. Compost samples were collected from Nawan, Almakhwah at 24 m above sea level. Compost samples were also collected from Bani Zabyan, Al-Baha at 2,200 m above sea level. In this last place, an experiment was designed to manage some plant organic waste and followed up for a month and then used also for isolation. The experiment was designed on four treatments as follows: 1- Mixing the residues with the addition of agricultural soil 2- Mixing the residues without adding any soil 3- Mixing the residues with the addition of previously prepared compost in Bani Zabyan 4- Mixing the residues with the addition of previously prepared compost in Nawan, Almakhwah. Bacteria isolated from environments other than compost such as mangroves may have low hydrolyzing activity for cellulose, reported by [15].

All the samples were used for isolation of bacteria on cellulose powder and CMC substrates as sole sources of carbon in the liquid or agar media at temperature of 45° C. Six isolates were obtained on cellulose powder agar medium, and the same number was obtained on CMC agar medium. Detection for exocellulase and endocellulase activities qualitatively by the isolates was recorded on cellulose powder and CMC agar media, respectively. The appearance of the decomposition zones around the growth of the isolates were recorded as a positive test.

The cultural characteristics were recorded on nutrient agar medium besides Gram's stain reaction. All the isolates were Gram positive, rod shaped, and spore former bacteria except one only, isolate A2. Mobility was also recorded for all isolates except the isolate A2. Growth of the isolates at different temperatures was also carried out. According to the recorded characteristics collectively for all isolates and by matching them with the characteristics of Gram-positive bacteria in [11], it appeared that these isolates may be largely like the following bacterial genera: *Bacillus*, *Amphibacillus*, *Paenibacillus* in case of all the spore forming isolates, and *Lactobacillus* in case of the non-spore forming isolate A2. In a similar study, cellulose hydrolyzing thermotolerant *Bacillus subtilis* was isolated from compost in large numbers [16]. Other similar studies with the related bacteria include the following works, *Bacillus licheniformis* and *Bacillus subtilis* [17], *Bacillus* and *Amphibacillus* [18], *Lactobacillus plantarum* [19], *Bacillus amyloliquefaciens* [20], *Paenibacillus terrae* [21], *Bacillus subtilis*, *Bacillus mojavensis* and *Bacillus cereus* [22], *Bacillus* and *Paenibacillus* [23], and *Bacillus* and *Paenibacillus* [24].

PCR detection *in-silico* for the cellulase genes of ß-glucosidase and 6-phospho-ß-glucosidase in bacteria like the isolates under study was performed using specific primers for Bacilli and Lactobacilli. These cellulase enzymes are important to complete the hydrolysis process by hydrolyzing cellobiose into glucose [25]. PCR was performed for all available species of *Bacillus*, *Amphibacillus*, *Paenibacillus*, and *Lactobacillus*. Only the representative species of all the genera that gave positive bands were selected. To ensure the accuracy of the program's work, it was also used to detect the well-known 16s rRNA gene for *Bacillus* and *Lactobacillus* using general primers F27 and R1492 that recorded positive bands and confirmed the accuracy of the PCR detection of the cellulase genes.

The isolates in each case, CMC or cellulose powder, were tested for their ability to grow at increasing temperatures included 30, 45, 50, and 55 °C. The results were recorded, and all isolates were able to grow in each case in temperatures 30 and 45 °C. Since no growth was recorded on both types of cellulose media at the rest of temperatures starting from 50, the isolates were described as thermotolerant. Many similar studies reported species of *Bacillus* and *Paenibacillus* as decomposers of cellulose with endo- and exocellulase activities, especially thermotolerant *Bacillus subtilis* and *Paenibacillus polymyxa*, including the studies of [26, 27, 28, 29, 30, 31, 32, 33].

The isolates were cultured separately with the same liquid medium on which they were first isolated, cellulose powder and CMC. Incubation was carried out at 45°C and growth was measured at intervals for each isolate for two days to construct a growth curve for each isolate. The curves showed that all isolates reach their maximum growth activity at 24 to 30 hours from the start of incubation except for isolate B1, which recorded the maximum growth at 24 hours from the start of incubation. This can also be considered as the maximum activity of enzymes as they are related to the primary growth of the isolates. These results agreed with those reported the maximum cellulase activity at temperature 45°C for *Bacillus* from 24 to 48 hours of the start of incubation [34, 35].

Previously, thermophilic *Bacillus subtilis* with endocellulase and exocellulase activities was isolated from composting by [36]. Also, thermophilic cellulolytic *Geobacillus pallidus* was isolated at 60°C by [37]. In another study, *Bacillus mycoides* recorded optimum growth and endocellulase activity at 20-25 °C by [38].

The ability of the isolates was tested under laboratory conditions to hydrolyze mixture of plant organic residues, cotton linters and filter papers. The isolates were cultured collectively, where all isolates growing on cellulose powder were inoculated in one treatment, and all isolates growing on CMC were inoculated a second treatment. The incubation was carried out at a temperature of 45 °C for all treatments and then followed up to one week. The results showed the efficiency of the isolates in the two treatments in consuming the mixture of plant fragments and decomposing it, as it shifted considerably to the form closest to maturity with a distinctive dark color and smell closest to the smell of soil. Similar study in agreement with ours was the work of [39].

A planting test was conducted for fenugreek seeds to test the quality of the compost and to ensure that it is free of any toxic substances for plants. The results showed the quality of the compost produced in both natural and laboratory conditions, where the seeds germinated, and the plant continued to grow naturally compared to the control grown in normal agricultural soil. A similar study recorded the absence of toxic substances in the compost through the germination of the seeds of some plants [40].

5. Conclusion

The current study showed the importance of exploring cellulose-degrading bacteria in natural environments and using them to improve and speed up the decomposition processes. In this regard, we find that thermotolerant Gram-positive bacteria, particularly spore-formers, have a great role notably in areas like Al-Baha. Also, the importance of developing some DNA probes can be deduced for the rapid detection of the presence of such bacterial isolates in different natural environments.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the Department of Biology, College of Science and Arts, Albaha University, Baljurashi, Saudi Arabia.

Disclosure of conflict of interest

The authors hereby acknowledge that there is no conflict of interest.

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