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# Screening of Aerobic Spore-Forming Soil Bacteria for Potential Biotechnological Applications

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**Abstract:** Soils represent a significant source of bacteria that produce enzymes and bioactive compounds; however, many microorganisms with high biotechnological potential remain insufficiently explored for isolation. The aim of this study was to isolate aerobic spore-forming bacteria from soil and to identify potential strains for biotechnological applications. Soil samples were collected from various locations in Artvin province, where olive trees are present. The morphological and biochemical characteristics of 11 bacterial isolates were evaluated, and their molecular identification was performed through 16S rRNA gene sequencing. The isolates were identified as belonging to *Priestia megaterium*, *Bacillus amyloliquefaciens*, and *Bacillus subtilis* species. All isolates were screened for protease, amylase, and cellulase production, with five isolates demonstrating the ability to produce all three enzymes simultaneously. Additionally, the bacteriocin production capacities of the isolates were qualitatively assessed, revealing that several isolates significantly inhibited the growth of other strains. The resistance of the isolates to UV radiation was also evaluated; while short-term UV exposure caused a reduction in growth for some isolates, most exhibited high tolerance. The obtained results indicate that the bacterial isolates possess promising potential for various industrial and biotechnological applications due to their multi-enzyme production, antibacterial activities, and resilience to environmental stressors. Furthermore, the potential of these isolates in other biotechnological fields has been discussed in light of the current literature.

**Keywords:** Aerobic Spore-Forming Bacteria, Enzymatic Activity, Bacteriocin, Antibacterial Effect, UV Resistance

**Öz:** Topraklar, enzim ve biyoaktif bileşikler üreten bakteriler için önemli bir kaynak olmakla birlikte, biyoteknolojik potansiyele sahip birçok mikroorganizmanın izolasyonu açısından henüz yeterince değerlendirilmemiştir. Bu çalışmanın amacı, topraktan aerobik spor oluşturan bakterilerin izolasyonunu gerçekleştirmek ve biyoteknolojik uygulamalar için potansiyel suşları belirlemektir. Toprak örnekleri, Artvin ilinde zeytin ağaçlarının bulunduğu çeşitli noktalardan alınmıştır. Elde edilen 11 bakteri izolatının morfolojik ve biyokimyasal özellikleri değerlendirilmiş ve moleküler identifikasyonları 16S rRNA gen dizileme yöntemiyle yapılmıştır. İzolatlar; *Priestia megaterium*, *Bacillus amyloliquefaciens* ve *Bacillus subtilis* türlerine ait bakteriler olarak tanımlanmıştır. Tüm izolatlar proteaz, amilaz ve selülaz üretimi açısından taranmış; beş izolatın üç enzimi birden üretebildiği belirlenmiştir. Ayrıca, izolatların bakteriyosin üretim kapasiteleri kalitatif olarak değerlendirilmiş ve bazı izolatların diğer suşların büyümesini anlamlı derecede inhibe ettiği saptanmıştır. İzolatların UV ışınına karşı dirençleri de test edilmiş olup, bazı izolatlarda kısa süreli UV maruziyeti büyümede azalmaya neden olurken, çoğu izolat yüksek dayanıklılık sergilemiştir. Elde edilen bulgular, bu bakteriyel izolatların çoklu enzim üretimi, antibakteriyel etkileri ve çevresel streslere karşı direnç gibi özellikleriyle çeşitli endüstriyel ve biyoteknolojik uygulamalar için potansiyel taşıdığını göstermektedir. Ayrıca, bu izolatların diğer biyoteknolojik alanlardaki potansiyelimevcut literatür ışığında tartışılmıştır.

**Anahtar Kelimeler:** Aerobik Sporlu Bakteri, Enzimatik Aktivite, Bakteriyosin, Antibakteriyel Etki, UV Direnci.

## 1. Introduction

Soil serves as a rich source of microorganisms with high enzymatic potential, particularly spore-forming bacteria from the genera *Bacillus* and *Priestia*. These bacteria are capable of producing industrially valuable enzymes such as proteases, amylases, and cellulases [1]. Notably, spore-forming aerobic bacteria have attracted considerable attention due to their exceptional resistance to environmental stress factors and their widespread applications in various biotechnological processes.

Proteases, amylases, and cellulases play pivotal roles across a diverse array of industrial sectors, including detergent formulation, food processing, and environmental waste management. Proteases, in particular, constitute a significant proportion of the global enzyme market and are extensively employed in the leather and cosmetic industries [2]. Amylases catalyze the hydrolysis of starch, thereby, enhancing biological degradation in wastewater treatment processes and contributing to environmentally sustainable solutions; they are also widely utilized in the textile industry [3, 4]. Concurrently, cellulases function as economically valuable biocatalysts in various industries such as textile, food and

beverage, animal feed, and biofuel production [5]. Their applications extend beyond the food and textile sectors to include the conversion of renewable biomass into fermentable sugars. In response to the depletion of fossil fuel reserves, global demand for these enzymes has markedly increased [6]. The biotechnological optimization of multifunctional enzymes enhances cost efficiency and promotes environmental sustainability within industrial applications.

The production of cellulase, alongside amylase and protease activities, has emerged as an increasingly important research area in biotechnology. Factors such as genetic engineering, cost, and purification play critical roles in optimizing cellulase production. Although numerous studies have been conducted on cellulose degradation, research on identifying the most suitable bacterial sources for industrial cellulase production remains limited. This has heightened interest in isolating aerobic bacterial strains exhibiting high cellulase activity [7].

Bacteriocins are ribosomally synthesized bacterial peptides that predominantly exert inhibitory or lethal effects on microorganisms phylogenetically related to the producing strain [8]. Furthermore, bacteria have the capability to produce bacteriocin-like antimicrobial peptides that impede the proliferation of pathogenic microorganisms within their ecological niche [9-11]. A thorough elucidation of the enzymatic profiles and bacteriocin biosynthesis in spore-forming soil bacteria is critical for optimizing biotechnological processes and advancing the development of novel biocatalysts.

The aim of this study was to isolate aerobic spore-forming bacteria from soil samples obtained from sites harboring olive trees, to perform molecular-level identification of the isolates, and to evaluate strains with potential biotechnological applications.

## 2. Material and Method

### Soil sample collection

Soil samples were collected in June and July of 2019 from areas containing olive trees in Artvin province, at a depth of approximately 5 cm from the surface, using a sterile spatula. The samples were placed in sterile containers and transported to the laboratory for analysis, where they were stored at 4°C.

### Bacterial isolation and storage

One gram of each soil sample was suspended in 9 mL of sterile Nutrient Broth (NB) and incubated at 30°C with shaking at 250 rpm for 2 minutes in a shaking incubator. The resulting soil suspensions were then incubated at 60°C for 60 minutes in a water bath. Subsequently, the suspensions were serially diluted in sterile NB, and the diluted samples were spread onto Nutrient Agar (NA) plates. The Petri dishes were incubated at 30°C for 24–48 hours, and colonies with distinct morphologies were selected, purified, and stored at -20°C.

### Morphological and biochemical characterization

The colony morphology, cell morphology, Gram staining, endospore formation, and motility of the bacterial isolates were examined. Colony morphology was observed on Nutrient Agar (NA), while endospore formation was detected using an endospore staining method. Motility was determined by the hanging drop method [12].

Biochemical tests, including catalase and oxidase tests, were performed. The catalase test was conducted using a 3% hydrogen peroxide solution, and the production of bubbles was considered a positive reaction. The oxidase test was carried out using test strips containing 1% tetramethyl-p-phenylenediamine dihydrochloride, and a bluish color change observed on the strips indicated the presence of oxidase activity.

### Molecular identification via 16S rRNA sequencing

Genomic DNA was extracted using the Promega (Germany) DNA isolation kit. For the amplification of the 16S rRNA gene, the primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Macrogen) were used. Polymerase chain reaction (PCR) reactions were set up in a total volume of 50 µl for each sample, with the following component distribution: 5 µl DNA, 5 µl 10x PCR buffer, 2.5 µl dNTPs (4 mM), 2 µl of 10 pM 27F primer, 2 µl of 10 pM 1492R primer, 3 µl $MgCl_2$, 0.5 µl Taq DNA polymerase (5 units/µl), and 30 µl sterile distilled water. The PCR cycle conditions were as follows: initial denaturation at 94°C for 2 minutes, followed by 35 cycles (denaturation at 94°C for 45 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute), with a final extension at 72°C for 10 minutes.

PCR products were subjected to electrophoresis on a 1% agarose gel in 1x TAE buffer to confirm the presence of the target gene. The amplicons were then sent to Macrogen (Netherlands) for bidirectional sequencing. The obtained sequences were compared with reference strains in the NCBI GenBank database for species identification. Sequences were edited using BioEdit (v7.09), aligned with the ClustalW algorithm, and phylogenetic analysis was performed using MEGA11 software [13,14,15].

**Bacterial enzymatic activity**

The amylase and protease enzyme activities of the isolates were evaluated quantitatively, while cellulase activity was assessed qualitatively on solid media.

Amylase activity was tested on starch agar medium (starch 5 g/L, tryptone 10 g/L, yeast extract 5 g/L, agar 15 g/L, NaCl 10 g/L). The isolates were inoculated onto the medium using the spot inoculation method and incubated at 30°C for 48–72 hours. After incubation, iodine solution was applied to the Petri dishes, and the diameter of the clear hydrolysis zones formed around the colonies was measured in millimeters. The amylase activity was then analyzed quantitatively [16].

Protease activity was determined on skim milk agar medium (skim milk powder 10 g/L, yeast extract 1 g/L, agar 26 g/L). The isolates were inoculated using the spot inoculation method and incubated at 30°C for 48 hours. After incubation, the diameter of the transparent zones around the colonies was measured, and protease activity was determined quantitatively [17].

Cellulase activity was tested on agar medium containing carboxymethylcellulose (CMC) (CMC 10 g/L, yeast extract 10 g/L, NaCl 10 g/L, tryptone 10 g/L, agar 15 g/L). The isolates were inoculated using the spot inoculation method and incubated at 30°C for 48 hours. After incubation, the Petri dishes were stained with 0.1% Congo red solution and washed with 1 M NaCl solution. The presence of clear hydrolysis zones around the colonies was considered a qualitative indicator of cellulase activity [18].

**Preparation of bacteriocin crude extract**

Bacterial isolates were incubated in 100 mL of Nutrient Broth (NB) medium at 35°C, with shaking at 180 rpm for 16 hours. After incubation, the cultures were centrifuged at 10,000 × g for 15 minutes to remove the cells. The resulting supernatant was filtered through a 0.22 µm pore-size sterile filter to remove any remaining cells and was stored in sterile containers at 4°C for antimicrobial activity testing. This supernatant was considered the bacteriocin crude extract [19].

**Determination of bacteriocin crude extract activity**

The antibacterial activity of the bacteriocin crude extracts obtained from the bacterial isolates was qualitatively evaluated using the agar well diffusion method. Fresh cultures of the isolates to be tested were incubated in Müller-Hinton Broth (MHB) for 18–24 hours, and the turbidity was adjusted to the 0.5 McFarland standard (~$1\times10^8$ cfu/ml). Subsequently, 100 µl of bacterial suspension was evenly spread over the surface of Müller-Hinton Agar (MHA) using a sterile swab. After the culture surface had dried, 20 µl of the bacteriocin crude extract was applied to sterile discs (6 mm in diameter), which were then placed onto the MHA plate inoculated with the isolates. The Petri dishes were incubated at 37°C for 24 hours. Antibacterial activity was determined by the presence of inhibition zones around the discs; the experiments were performed in duplicate, and the results are reported as mean values.

**Ultraviolet (UV) radiation resistance test**

The isolates were inoculated onto Petri dishes containing NA under sterile conditions. After inoculation, the plates were placed at a fixed distance from a UV-C radiation source with a wavelength of 254 nm and an intensity of 30 W, located in a Class II A2 biological safety cabinet (BİLSER BLF 2000). The plates were then exposed to UV radiation for 15, 30, and 60 minutes, respectively. Following UV exposure, the plates were incubated at 30°C for 24 hours for analysis. The temperature within the cabinet was maintained at 22 ± 1°C throughout the experiment. The responses of the isolates to UV radiation were categorized as normal growth (+), weak growth (wp), or no growth (–).

## 3. Result

**Isolation and identification of bacteria**

In this study, eleven aerobic spore-forming bacterial isolates were obtained from soil samples collected from sites harboring olive trees in Artvin province. Some of the morphological and biochemical characteristics of the isolates were determined, and their molecular identification was carried out using 16S rRNA gene sequencing.

All isolates were found to be Gram-positive and spore-forming, and morphologically, they exhibited rod-shaped structures. Molecular characterization revealed that the isolates belong to the genera *Bacillus* and *Priestia*. The Sb1 isolate formed white colonies, while the Sb2, Sb3, Sb6, and Sb7 isolates formed light cream-colored colonies, and the remaining isolates formed cream-colored colonies. Catalase activity was positive for all isolates, whereas oxidase activity was found to be negative in the Sb1 isolate (Table 1).

**Table 1.** Morphological and biochemical characteristics of bacterial isolates

| Isolates | Tests | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Shape** | **Gram** | **Spore** | **Motility** | **Colony colour** | **Catalase** | **Oxidase** |
| Sb1 | Rod | + | + | + | Whitish | + | - |
| Sb2 | Rod | + | + | + | Light Cream | + | + |
| Sb3 | Rod | + | + | + | Light Cream | + | + |
| Sb4 | Rod | + | + | + | Cream | + | nd |
| Sb5 | Rod | + | + | + | Cream | + | + |
| Sb6 | Rod | + | + | + | Light Cream | + | + |
| Sb7 | Rod | + | + | + | Light Cream | + | + |
| Sb8 | Rod | + | + | + | Cream | + | + |
| Sb9 | Rod | + | + | + | Cream | + | nd |
| Sb10 | Rod | + | + | + | Cream | + | + |
| Sb11 | Rod | + | + | + | Cream | + | + |

+: positive, −: negative, nd: not determined.

Based on 16S rRNA gene sequencing, the bacterial isolates were identified as *Priestia megaterium* (Sb1, Sb4, Sb8, Sb9, and Sb10), *Bacillus amyloliquefaciens* (Sb2, Sb5, Sb6, and Sb7), and *Bacillus subtilis* (Sb3 and Sb11). The 16S rRNA gene sequences of these isolates were submitted to the GenBank database, and accession numbers were assigned as follows: Sb1 (PV805444), Sb2 (PV805445), Sb3 (PV805446), Sb4 (PV805447), Sb5 (PV805448), Sb6 (PV805449), Sb7 (PV805450), Sb8 (PV805451), Sb9 (PV805452), Sb10 (PV805453), and Sb11 (PV805454). Phylogenetic analysis was performed using the Neighbor-Joining method with 1000 bootstrap replications in MEGA 11 software, and the evolutionary relationships among the isolates are illustrated in Figure 1.

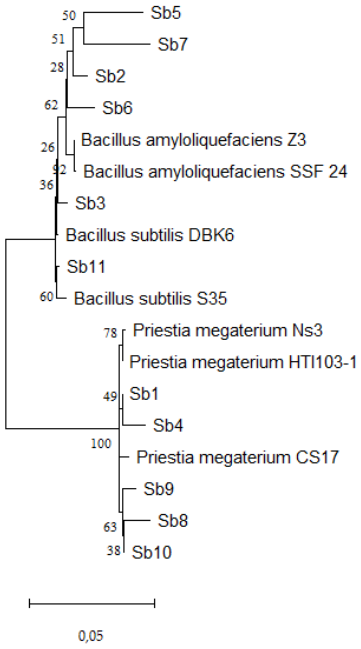


**Figure 1.** Neighbor-Joining tree of isolates based on 16S rRNA with 1000 bootstraps (MEGA 11).

**Enzymatic activities of bacterial isolates**

The enzymatic activities of the bacterial isolates, including amylase, protease, and cellulase, were determined on appropriate selective agar media, and were evaluated based on the presence and diameter of the hydrolysis zones. Amylase and protease activities were assessed quantitatively, whereas cellulase activity was analyzed qualitatively.

Amylase activity was determined by measuring the diameter of the transparent zones around the colonies on starch agar; all isolates exhibited amylase activity, with the highest and lowest activities observed in the Sb6 and Sb9 strains, respectively. Protease activity was evaluated by measuring the diameter of the hydrolysis zones around the inoculation area on skim milk agar; all isolates tested positive, with Sb6 and Sb9 exhibiting the highest and lowest activities, respectively.

Cellulase activity was examined by observing the presence of clear zones around the colonies after Congo red staining and NaCl washing. Sb1, Sb4, Sb5, Sb8, and Sb11 displayed significant cellulolytic potential, while Sb6 and Sb10 showed weak positive activity. The remaining isolates were found to be negative. These findings are summarized and compared in Table 2.

**Table 2.** Amylase, protease, and cellulase enzymatic activities of bacterial isolates

| Isolates | Zone diameter (mm) | | Cellulase |
|---|---|---|---|
| | Amylase | Protease | |
| Sb1 | 13 | 22 | + |
| Sb2 | 7 | 16 | - |
| Sb3 | 5 | 16 | - |
| Sb4 | 11 | 20 | + |
| Sb5 | 11 | 18 | + |
| Sb6 | 14 | 24 | wp |
| Sb7 | 11 | 23 | - |
| Sb8 | 12 | 19 | + |
| Sb9 | 4 | 4 | - |
| Sb10 | 6 | 8 | wp |
| Sb11 | 12 | 19 | + |

+: positive, −: negative, wp: weak positive.

**Antibacterial activity of bacteriocin crude extracts**

The antibacterial activities of bacteriocin crude extracts from various bacterial isolates were tested reciprocally in this study. Since no isolate was tested against its own extract, the phenomenon of autoinhibition was not evaluated. Antibacterial activity was qualitatively assessed using the agar well diffusion method.

It was found that all isolates exhibited high sensitivity against Sb6 and Sb11 extracts. Sb2 and Sb3 extracts formed inhibition zones in all isolates except Sb11. In contrast, the isolates showed resistance to Sb4, Sb7, Sb9, and Sb10 extracts. Sb8 and Sb5 extracts exhibited limited antibacterial activity, affecting only a few strains. Additional results are presented in Table 3.

**Table 3.** Activities of bacteriocin crude extracts

| | Isolates | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Crude extract** | Sb1 | Sb2 | Sb3 | Sb4 | Sb5 | Sb6 | Sb7 | Sb8 | Sb9 | Sb10 | Sb11 |
| Sb1 | **Nt** | + | - | + | + | - | - | + | + | - | - |
| Sb2 | + | **Nt** | + | + | + | + | + | + | + | + | - |
| Sb3 | + | + | **Nt** | + | + | + | + | + | + | + | - |
| Sb4 | - | - | - | **Nt** | - | - | - | - | - | - | - |
| Sb5 | - | - | - | - | **Nt** | - | - | + | - | + | - |
| Sb6 | + | + | + | + | + | **Nt** | + | + | + | + | + |
| Sb7 | - | - | - | - | - | - | **Nt** | - | - | - | - |
| Sb8 | - | - | - | + | - | - | - | **Nt** | - | + | - |
| Sb9 | - | - | - | - | - | - | - | - | **Nt** | - | - |
| Sb10 | - | - | - | - | - | - | - | - | - | **Nt** | - |
| Sb11 | + | + | + | + | + | + | + | + | + | + | **Nt** |

+: positive, −: negative, Nt: not tested.

**Resistance of isolates to UV-C radiation**

The resistance of bacterial isolates to UV-C radiation was assessed using a UV-C light source (254 nm, 30W) within a Class II A2 biosafety cabinet (BİLSER BLF 2000).

The resistance of the Sb3, Sb4, and Sb6 isolates to UV-C radiation varied based on the duration of exposure. The Sb3 isolate exhibited full growth (+) after 15 and 60 minutes of UV-C exposure, whereas partial growth (wp) was observed after 30 minutes. The Sb4 isolate demonstrated a partial decline in growth capacity following 15 minutes of UV-C exposure. The Sb6 isolate maintained full growth after 15 and 30 minutes of UV-C exposure; however, after 60 minutes,

it showed partial growth, accompanied by a noticeable decrease in its growth capacity. Exposure of the remaining isolates to UV-C radiation, across various exposure times, did not result in any significant inhibition of growth. The growth responses of the isolates to UV-C radiation are summarized in Table 4.

**Table 4.** Growth capacities of isolates under UV-C radiation

| Isolates | UV Radiation time (min) | | 60 |
|---|---|---|---|
| | 15 | 30 | |
| Sb1 | + | + | + |
| Sb2 | + | + | + |
| Sb3 | + | wp | + |
| Sb4 | wp | + | + |
| Sb5 | + | + | + |
| Sb6 | + | + | wp |
| Sb7 | + | + | + |
| Sb8 | + | + | + |
| Sb9 | + | + | + |
| Sb10 | + | + | + |
| Sb11 | + | + | + |

+: full growth, wp: weak growth.

## 4. Discussion and conclusion

In this study, aerobic spore-forming bacteria were isolated from soil samples collected from olive-growing areas in Artvin, and characterized using morphological, biochemical, and molecular (16S rRNA gene sequencing) techniques. Furthermore, the protease, amylase, and cellulase enzyme activities, bacteriocin production capacities, and resistance levels to UV radiation of the isolates were assessed. The findings aim to elucidate the biotechnological potential of these isolates for various industrial applications.

### *Priestia megaterium*

According to the 16S rRNA gene sequencing analysis, the isolates Sb1, Sb4, Sb8, Sb9, and Sb10 were identified as *Priestia megaterium*. While the name *Priestia megaterium* is used in current taxonomy, this strain is also known as *Bacillus megaterium* in the literature [20]. In this study, the current taxonomic nomenclature was preferred.

Previous studies have reported that *P. megaterium* can utilize various carbon sources and synthesize extracellular enzymes such as CMCase, cellulase, xylanase, amylase, protease, and chitinase effectively [21]. *P. megaterium* strains are considered important potential enzyme sources in biotechnological applications, especially for microbial waste degradation, due to their ability to secrete lysosomal enzymes [22]. Among the *P. megaterium* isolates in this study, Sb1 exhibited the highest amylase and protease activities and also demonstrated cellulase activity.

Bacteriocins are of great biotechnological importance as natural and safe antimicrobial agents and are considered as alternative antibiotics and preservatives in the food, agriculture, and healthcare sectors [23]. Among the *P. megaterium* isolates, Sb1 was identified as the strain with the highest bacteriocin activity and formed inhibition zones in all five other tested bacterial strains. Limited studies on bacteriocins produced by *Priestia* species have shown that these strains have the potential to produce bacteriocins. For example, *Priestia megaterium* and other *Priestia* spp. isolates have been reported to show antibacterial activity against pathogens such as *Streptococcus agalactiae* and *Aeromonas hydrophila* through cell-free extracellular products (CFECP) [24]. These findings indicate that Sb1 has the potential to produce effective antibacterial compounds against pathogens.

Except for Sb4, the *P. megaterium* isolates exhibited high resistance to UV-C radiation under different exposure times. In the Sb4 strain, although complete inactivation was not achieved after 15 minutes of UV-C exposure, a significant decrease in bacterial growth and reproductive capacity was observed. Interestingly, no reduction in growth capacity was recorded after 30 and 60 minutes of exposure. This suggests that Sb4 may have responded to short-term UV-C exposure with a temporary or adaptive response, effectively repairing DNA damage. Literature indicates that spore-forming bacteria can repair UV-C-induced DNA damage through mechanisms such as photoreactivation or nucleotide excision repair [25]. Moreover, these results suggest that different strains of the same species may exhibit varying resistance levels to UV-C radiation.

*P. megaterium* is an important microorganism in biotechnology, with broad applications in areas such as vitamin B12, polyhydroxybutyrate (PHB), and recombinant protein production. It is also widely used in biotechnological processes like plant protection and cytochrome P450 production [26]. Its ability to grow over a wide temperature range (3–45°C) and metabolize various carbon sources enhances its environmental resilience, making it a preferred candidate in industrial

applications [27]. Recent studies have shown that *P. megaterium* acts as an endophyte, promoting plant growth and enhancing stress tolerance in plants under abiotic stress conditions [28]. Additionally, it has potential applications as a probiotic in aquaculture [29].

In light of the biotechnological characteristics reported in the literature, the *P. megaterium* strains isolated in this study, particularly Sb1, exhibit significant potential for biotechnological applications due to their high enzymatic activity, bacteriocin production, and resistance to UV-C radiation.

### *Bacillus amyloliquefaciens* and *Bacillus subtilis*

According to 16S rRNA gene sequencing, isolates Sb2, Sb5, Sb6 and Sb7 were identified as *Bacillus amyloliquefaciens*, Sb3 and Sb11 as *Bacillus subtilis*. *Bacillus* species are distinguished not only by their ability to form spores but also by their capacity to produce various industrially important enzymes and antimicrobial compounds. These species are commonly utilized in the production of hydrolytic enzymes such as proteases, amylases, and cellulases [30,31].

Several soil-derived *Bacillus* species have demonstrated the ability to produce amylase, protease, and cellulase activities [32,33,34,35,36]. In this study, Sb6 isolate was selected for potential biotechnological applications due to its high amylase and protease activities. Furthermore, *Bacillus subtilis* strains are a significant group in protease production, with high collagenolytic activity, which can be utilized for the reduction of environmental pollution [37,38]. In this regard, Sb11 isolate may serve as a potential source for future studies aimed at these applications.

*Bacillus* species are rich in bacteriocins, bacteriocin-like substances, lipopeptides, and other inhibitory compounds [9]. Bacteriocins are small antimicrobial peptides that are ribosomally synthesized, and they inhibit the growth of target bacteria by forming pores in the cell membrane or by interfering with cell wall synthesis [39,40]. Antagonistic activities of *Bacillus subtilis* and *B. amyloliquefaciens* strains isolated from soil have been reported in the literature [41,42].

In this study, the bacteriocin crude extracts from the Sb6 and Sb11 isolates demonstrated antibacterial activity against isolates from both *Bacillus* and *Priestia* genera. These findings suggest that these bacteriocins could be utilized as natural and effective antimicrobial agents in food preservation, agriculture, and pharmaceutical sectors. However, for industrial applications, parameters such as production efficiency, stability, and toxicity must be optimized [43]. Therefore, further investigation into the characteristics of the Sb6 and Sb11 bacteriocins is crucial in future studies.

In contrast, the crude bacteriocin extracts from Sb4, Sb7, Sb9, and Sb10 isolates showed resistance, indicating that these bacteriocins were ineffective against these isolates. However, resistance to bacteriocins may confer a competitive advantage to these strains in their natural environment, where they interact with other bacterial species.

It is well-documented in the literature that *Bacillus* spores exhibit high resistance to UV radiation [25]. The thick protective layers of spores reduce UV absorption, preventing complete inactivation [44]. In the case of Sb3, a 30-minute exposure to UV-C radiation resulted in significant inhibition of bacterial viability, but complete inactivation was not observed. Similarly, Sb6 showed partial growth after 60 minutes of UV-C exposure, with no complete inactivation. These findings indicate that the isolates have developed partial resistance to UV-C radiation. The DNA and cellular damage caused by UV-C can be repaired through mechanisms such as photoreactivation or nucleotide excision repair [25]. Furthermore, differences in the protective layers of spores may account for variations in UV-C resistance levels. These findings underscore the importance of optimizing exposure time and dosage in UV-C disinfection protocols.

The significance of *Bacillus amyloliquefaciens* in other biotechnological applications is well-supported by various studies in the literature. *B. amyloliquefaciens* is an important bacterial species isolated from both soil and industrial amylase fermentations. Due to its wide ecological adaptability, it plays a key role in the biodegradation of various pollutants. Studies have demonstrated that it is particularly effective in the degradation of pollutants such as BTEX (benzene, toluene, ethylbenzene, and xylene) and phenols found in wastewater, originating from the petrochemical industry [45,46]. Furthermore, it promotes plant growth and enhances flocculation activity in water treatment processes [47,48]. The strain *B. amyloliquefaciens* RT7 is also noted for producing high levels of exopolysaccharides (EPS) when grown on a glucose-Tween 80 mixture, which have demonstrated antioxidant properties without cytotoxic effects. This indicates the potential use of EPS in bioremediation processes [49]. Additionally, the levan produced by this species serves as an effective source for the production of silver nanoparticles (AgNPs), which have potential applications in the biomedical and nanotechnology fields [50]. Given these versatile properties, the Sb6 strain, isolated in this study, holds significant potential not only in the context of the biotechnological properties observed here but also in terms of the diverse applications outlined in the literature.

Furthermore, future studies should investigate the optimization of biotechnological processes involving *B. amyloliquefaciens* strains, particularly focusing on improving production efficiency, stability, and scalability for industrial applications. This would enhance the applicability of this bacterium in various environmental and industrial settings.

*Bacillus subtilis* plays a significant role in various biotechnological processes. It is actively used in areas such as enzyme production, detoxification of explosives, and biodegradation of radioactive waste. Due to its fungicidal properties and suitability for genetic manipulation, it is widely employed as a biological control agent and laboratory model in agriculture

[51]. Additionally, it demonstrates significant potential in microbial waste degradation [22]. *B. subtilis* is used as a model organism in microbiological research, facilitating a better understanding of cellular processes through genomic and transcriptomic studies. It has broad application potential in fields such as agriculture, medicine, and bioremediation, exhibiting probiotic production capabilities and biological defense mechanisms against pathogens [52However, ethical and safety concerns may limit certain applications. Therefore, interdisciplinary approaches could offer more sustainable solutions. Furthermore, the use of this species as a probiotic in aquaculture holds considerable promise [29]. The Sb11 isolate should be further evaluated in depth, considering both the biotechnological characteristics observed in this study and the information reported in the literature.

This study focused on the isolation and identification of aerobic spore-forming bacteria from soil samples. The enzymatic activities, bacteriocin production capacities, and UV-C resistance of the isolates were comprehensively evaluated. The findings revealed the presence of various strains with high biotechnological potential. The Sb6 isolate exhibited the highest protease and amylase activities; its crude bacteriocin extract demonstrated antibacterial activity against all tested bacteria. Although its cellulase activity was lower compared to other enzymes, it showed partial resistance to 60 minutes of UV-C exposure. The Sb11 isolate inhibited all tested strains through bacteriocin production, exhibited full activity in three target enzymes, and displayed resistance to different durations of UV-C exposure. The Sb1 isolate showed complete enzymatic activity and partial bacteriocin activity, along with high resistance to UV-C radiation.

These results indicate that the isolates possess significant potential for various biotechnological applications. Future studies should focus on the production, purification, and characterization of these enzymes, as well as detailed investigation of their potential industrial applications.

**Conflict of Interest**

The authors declare that they have no competing interests.

**Ethics Committee Approval**

Ethics committee approval is not required.

**Author Contribution**

Conceptualization, methodology, laboratory analyses, drafting, proofreading, and editing were all performed by the author. The author has read and approved the final version of the manuscript.

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**5. References**

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