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Original article

Identification of bacteria species isolated from soil and investigation of optimum conditions: application in food industry and biotechnological fields

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Summary. In this study, soil samples were taken. Isolation of bacteria was carried out by dilutions technique. Identification and biochemical tests of the obtained bacteria were performed. Bacteria were identified to be *Bacillus cereus* WYS01, *Bacillus niacini* PRPB1, *Bacillus thuringiensis* VITLW1, *Bacillus thuringiensis* ES2I3P, *Bacillus cereus* TG16. As a result of biochemical tests, all bacteria showed gram (+), urease tests (-) and amylase activity (+) results. Bacteria reproduced in different feed-lots showed most reproduction in Nutrient Agar and MRS Agar. By determining the optimum reproducing conditions of the bacteria, all bacteria were found to be the most reproductive at 40°C, pH 7.0 within 24 hours.

Key words: bacteria, isolation, identification, food industry

Introduction

Microorganisms are spread out from the deepest part of the seas to the highest levels of the sky. The soil being one of these areas has water, air, oxygen, mineral matter, carbon and nitrogen resources that are necessary for the life of microorganisms (1). Soil is a complex and dynamic biological system. The number of bacteria in one gram of soil is estimated to be 10 billion and to have thousands of species (2). In addition to individual studies, global projects are also conducted to determine soil diversity (3, 4). As developing technology, microorganisms can be detected in laboratory by new generation 16S rRNA and 18S rRNA sequencing metagenomics studies. As a result of analyzes using new generation sequencing technologies, the maximum number calculated is 8.3 million (5). Physicochemical structure of the soil, climate changes and agricultural practices significantly affect the distribution and diversity of bacteria (2,3,6). This distribution and diversity offers a wide range of resources for biotechnological applications. Soil is generally poor and limited for microorganisms in terms of nutrients and energy sources compared to in vitro conditions (6). Besides, there shows up a competition between microorganisms both due to high number of microorganisms and variety. In this competition, microorganisms try to have factors such as larger area, organic matter and water for themselves by using the toxic substances they produce to take the advantage for life. An important part of the soil microflora is bacterium of the genus *Bacillus*. *Bacillus* species in the family Bacillaceae is aerop and facultative anaerope, gram positive endospore bacteria (7-9). *Bacillus* species is used to produce many industrially important enzymes. Because most of its species do not show pathogenic properties, they have a wide genetic diversity and are easy to develop (10). Typical enzyme production by fermentation can be carried out in a short time with *Bacillus* species and with much lower costs of nitrogen and carbon sources (11). About half of commercial enzyme production is produced by *Bacillus* species. These enzymes include enzymes such as proteases, α-amylases, glucose isomerases and pullunases. *Bacillus* species are in a significant group

among microbial rennet producing bacteria. Among these species, the enzyme secreted by the *Bacillus polymyxa* has very good properties (12). Most of the enzymes used in beer production, baking and textile industry are amylase and βglucanase enzymes produced by *Bacilllus* spp (13). Amylase produced by *Bacillus mojavensis* SO-10 is used in many fields (14). Alkaline polygalacturonase lyase (PGL) enzyme is the enzyme pectin depolymerase. PGL B, which is used in many fields such as tea and coffee fermentation, oil extraction, fruit juice and textile, is produced by *B. subtilis* (12). Proteases isolated from *Bacillus* are also used in detergent production and leather industry (13). It has been determined that the protease enzymes obtained from *Bacillus subtilis* and *Bacillus cereus* bacteria are an alternative to chemical use in the leather industry and thus it is an effective method for reducing environmental pollution (15). In addition, various foods are obtained from microorganisms (milk products such as yoghurt, kouz; all alcoholic beverages, vinegar, various products such as far eastern origin soy sauce, fermentation of bread, single cell protein).

Due to such effects of bacteria, in this study, soil samples were taken and bacterial isolation was performed by dilution technique. Bacterial identification and biochemical tests were performed. The optimum conditions of the identified bacteria were determined and it was aimed to be used both in biotechnological studies and in various industrial fields.

Material and Method

Biological material

Bacteria isolated from soil were used.

Isolation of microorganisms

To obtain microorganisms from the soil, 1 gram of sample was taken and 9 ml of sterile water was left on that sample. By that method, a suspension of 10^{-1} was made. By similar way, as sequential transfers were performed, and 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} dilutions were obtained. In order to obtain a single colony, smear sowing was done from the samples made of serial dilution instead of Nutrient Agar (NA) and left to incubation at 37°C for 1 night.

Identification of bacteria species

Isolated bacteria were partially subjected to 16S rRNA analysis by EpiGen.

Biochemical tests

Biochemical tests were performed in order to identify the selected bacterial samples. As biochemical tests such as gram staining, hemolysis, mobility, catalase, amylase, coagulase, urease tests were applied on bacteria separately.

The Effect of different medium on bacterial development

In order to determine the growing of bacteria on solid medium, medium such as Nurient Agar (NA), MRS Agar, Plate- Count Agar, EMB Agar, Endo Agar which were prepared on sterilized petris were experimented. Growing of bacteria was observed as being planted in these medium.

Effect of temperature, pH and incubation time on microorganism growing

25 ml of Nutrient Broth (NB) liquid medium were prepared and 100 ml of glass bottle were autoclaved and 2 ml of bacterial cultivation was performed from fresh overnight culture. It was incubated in shaking in the water bath at 120 rpm as starting from 30°C to 60°C in increments of 10°C.

The effects of pH on the development of bacteria were investigated. For that purpose, 25 ml NB liquid medium were autoclaved by adjusting the pH to be 4.0-11.0 and 2 ml of planting was done from fresh culture. The bacteria were incubated at 120 rpm in shaking in the water bath at the optimum temperature of the bacteria.

In order to investigate the effect of incubation time on the development of bacteria, bacteria was produced in shaking in water bath at 120 rpm at the optimum pH and temperature of the bacterium by 2 ml planting from the fresh culture instead of 25 ml of NB liquid medium. Absorbance of the bacteria samples taken at 12, 24, 36, 48, 60 and 72 hours at 460 nm by spectrophotometer was measured.

Results

Identification of microorganism and biochemical tests

5 bacteria species isolated from soil were subjected to partially 16S rRNA analysis by EpiGen. The results showed that the bacteria were *Bacillus cereus*

WYS01, *Bacillus niacini* PRPB1, *Bacillus thuringiensis* VITLW1, *Bacillus thuringiensis* ES2I3P, *Bacillus cereus* TG16. There found base strains as bacteria *Bacillus cereus* WYS01 891, *Bacillus niacini* PRPB1 900, *Bacillus thuringiensis* VITLW1 879, *Bacillus cereus* TG16 883, *Bacillus thuringiensis* ES2I3P 874. Some of base strains of bacteria are shown as follows.

CCCATGCGGCGTACTCCCAGGCGGAGTGCTTATGCGTTACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACT…(*Bacillus cereus* WYS01)

CATGCTGGCGTACTCCCAGGCGGAGTGCTTATGCGTTAGCTGCAGCACTAAAGGGGAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTTACAGACCAGAAAGCCCTTCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCGCTTTCCTCTTCTGTACTCAAGTCCCCCAGTTTCCAATGACCCTCCACGGTTAGCCGTGGGCTTTCACATCAGACTTAAAGGACCGCCTGCGCGCGCTTTACGCCCAATAATTCCGGACAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACCGGCAGTTACTCCGGTACTTGTTCTTCCCTAACAACAGAGCTTTACGACCCGAAGGCCTTCATCGCTCACGCGGCGTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGATCTGGGCCGTGTC…(*Bacillus niacini* PRPB1)

CATTGCGTCGTACTCCCAGGCGGAGTGCTTATGCGTTAACTTCAGCACTAAAGGCGGAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGGTTGCTCCGTCAGACTTTCG…(*Bacillus thuringiensis* VITLW1)

CCATGGCGCGTACTCCCAGGCGGAGTGCTTATGCGTTACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTACACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCC…(*Bacillus thuringiensis* ES2I3P)

CCTTGGCTGCCGTACTCCCCAGGCGGAGTGCTTATGCGTTACTTCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAAAGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATTTCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCACTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCGTGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGCGCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGGCGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCACTTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAGCCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCG…(*Bacillus cereus* TG16)

Various biochemical tests were applied to bacteria identified and which strain analysis had been conducted. It was determined that all bacteria were gram (+) during gram coloring. It is found that *Bacillus niacini* PRPB1 and *Bacillus thuringiensis* ES2I3P were inactive and the others were active. It was determined that all microorganisms performed hemolysis. All of the bacteria were found to have negative urease activity but positive amylase activity. Detailed information on biochemical tests and properties is shown at Table 1.

The effect of different medium on bacterial growing

In order to determine bacteria growing in different medium, medium such as Nurient Agar (NA),

Table 1. Biochemical Tests

Features	*Bacillus cereus* WYS01	*Bacillus niacini* PRPB 1	*Bacillus thuringiensis* VITLW1	*Bacillus thuringiensis* E S2I3P	*Bacillus cereus* TG16
Gram	+	+	+	+	+
Hemolysis	+	+	+	+	+
Mobility	+	-	+	-	+
Growth					
Temperature range	30-40°C	30-40°C	30-40°C	30-50°C	30-40°C
pH range	4.0-8.0	5.0-8.0	4.0-8.0	5.0-9.0	5.0-8.0
Activity					
Ureaz	-	-	-	-	-
Katalaz	+	-	+	+	+
Kuagülaz	+	-	-	-	-
Amylase	+	+	+	+	+

MRS Agar, Plate- Count Agar, EMB Agar, Endo Agar were examined. As a result of that study, it was found that *Bacillus cereus* WYS01 shows less growing in Endo and EMB Agars but its growing in other ones was good. It is determined that *Bacillus niacini* PRPB1 bacteria does not grow at all in Endo and EMG Agars, but contrary to those medium, it showed a great growing in Nutrient and MRS Agars. It is also found that *Bacillus thuringiensis* ES2I3P bacteria does not grow in Endo Agar. The growth of microorganisms in solid medium was shown in detail at Table 2.

Effect of temperature, pH and incubation time on microorganism growing

For the temperature effect on the bacterial reproduction experiment was conducted between 30°C and 60°C. In the analysis, it is found that *Bacillus cereus* WYS01 showed the highest growing among the other bacteria at 30 °C. It is also found that all bacteria showed a good reproductive growth from 30°C and 4°C but there showed decreases in the reproduction due to the increasing temperature. The growth of bacteria is shown at Figure 1.

The other important factor in the growth of microorganisms is pH. All of the bacteria produced at pH ranges between 4 and 11 were found to have the best growth rate at pH 7.0. It is found that the microorganism showing the best growing in bacteria was *Bacillus cereus* WYS01. Bacterial reproducing grows up respectively *Bacillus cereus* WYS01, *Bacillus cereus* TG16, *Bacillus thuringiensis* VITLW1, *Bacillus thuringiensis* ES2I3P and *Bacillus niacini* PRPB1. Incubation time has a great effect on bacterial growth like as the other parameters. The bacterial growth is observed between 12–72 hours. When checked the growing of bacteria at 12 hour, it is found that the most was *Bacillus cereus* WYS01. After that, it is found that there was respectively *Bacillus niacini* PRPB1, *Bacillus cereus* TG16, *Bacillus thuringiensis* VITLW1 and *Bacillus thuringiensis* ES2I3P as to their growing rate. As

Table 2. The effect of different medium on bacterial growing

Microorganisms	Nutrient Agar	Endo Agar	EMB Agar	Plate- Count Agar	MRS Agar
Bacillus cereus WYS01	+++	+	++	+++	+++
Bacillus niacini PRPB1	+++	-	-	+	+++
Bacillus thuringiensis VITLW1	+++	+	+	+++	+++
Bacillus thuringiensis ES2I3P	+++	-	+	+++	+++
Bacillus cereus TG16	+++	+	++	+++	+++

+, positive result or growth; -, negative result

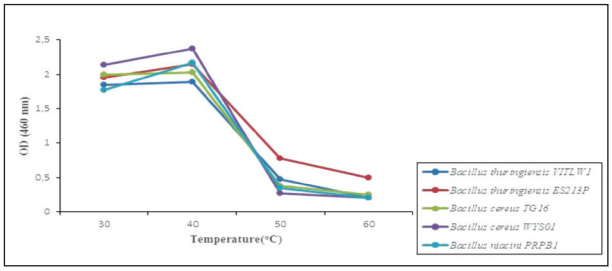

Figure 1. Effect of temperature on microorganism growing

the time that all microorganisms showed the most reproduction was found as the 24th hour. After that time, a proportional decrease was observed in bacterial growth. It is determined that *Bacillus cereus* WYS01 was the most growing microorganism at the 24th hour. The effect of time on the growth of microorganisms is shown at Figure 3 in detail.

Discussion and Conclusion

In our study, isolation of bacteria from soil was carried out. As a result of isolation, a partial 16S rRNA analysis was conducted and it was found that all bacteria were the *Bacillus* species. As a result of biochemical tests, bacteria was found as gram (+). Damodara Chari et al (16) detected that all bacteria they had obtained from soil was gram (+). As a similar result, Ortakaya et al (17) declared that *Bacillus simplex* microorganism was gram (+) in their study which was conducted as isolating bacteria from soil. The optimum conditions of bacteria identified in our study was found as 40°C, pH 7.0 and the 24th hour. Hamilton et al (18) found that the conditions of *Bacillus* sp. IMD 43 were 40°C and the 41st hour. Behal et al (19) conducted reproduction of *Bacillus* sp. AB 04 between 24 and 36 hours at 40°C. Asgher et al (20) detected that incubation time was 48 hours, temperature was 50°C and pH was 7.0 for thermophilic *Bacillus subtilis* JS-2004. Saxena et al (21) carried out reproduction of *Bacillus* sp. PN5 after incubation for 60 hours, at 60°C and pH 7.0. Hmidet et al (22) produced *Bacillus licheniformis* NH1 at 48 hours, 37°C and pH 7.0. Ozdemir et al (14) produced *Bacillus mojavensis* microorganism at 35°C, pH 7.0 and 36 hours for use in biotechnological studies. The temperature of the environment greatly affects the growth of microorganisms. Microorganisms can usually reproduce and grow within their specific temperature limits. These limits include the optimal temperature at which the growth occurs. On the other hand, pH of medium should be within the optimal limits for reproduction of microorganisms. Among the physical parameters, the pH of the growing environment plays an important role in the morphological changes in the microorganism and consequently in the increase of industrial and biotechnological production (20).

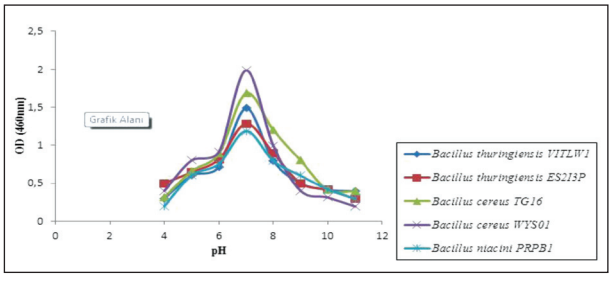

Figure 2. Effect of pH on microorganism growing

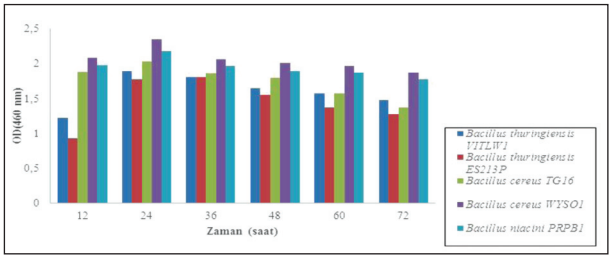

Figure 3. Effect of incubation time on microorganism growing

When we examine the properties of the bacteria obtained in our study, there are advantages both in terms of biochemical test characteristics and optimal conditions. Bacterial growth in a short term and providing low-temperature growth are the significant advantageous for industrially important issues, such as the short duration and electricity savings.

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