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Research Article

# Bacterial populations metabolizing barley straw from agricultural soils

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## ABSTRACT

The aim of this study was to determinate the bacterial communities that have potential to use and metabolize barley straw wastes at agricultural soils. Combination of different techniques; traditional isolation methods, metabolism-based methods such as Biolog Ecoplate and high-throughput sequencing of amplified 16S rRNA gene method were used for detailed analysis of samples taken from agricultural fields. Groups of bacteria isolated using barley straw-containing media belonged to the genus *Arthrobacter* and *Bacillus*. The Biolog Ecoplate analysis revealed the bacterial metabolic diversity. The AWCD, R, H index results showed that a great potential of substrate utilization ability of the barley straw metabolizing microbial community and greater functional diversity of samples.
The Illumina MiSeq sequencing was used to analyse diversity of microbial community associated with barley straw degradation. 16S rRNA gene amplicons indicated that samples showed great bacterial richness. At the phylum level, members of the phyla Proteobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Actinobacteria and Firmicutes were found to be intense. However, there were differences among the examples. At genus level *Luteimonas* and *Sphingomonas* were dominant in L1-30 and L2-15, in addition *Bacillus* was in high rate in L1-15 and *Brevundimonas*, *Flavisolibacter* and *Altererythrobacter* were detected in high rate in L2-30 sample. According to these results, the bacterial community can be used for properly decomposition of barley straw wastes which are common wastes in agricultural strategy.

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## INTRODUCTION

Due to the increasing food demand in the world, agricultural activities are also increasing, which causes an increase in agricultural wastes in the environment [1]. Many factors are effective in the formation and increase of agricultural waste, such as the increasing the amount of agricultural production, socioeconomic reasons, eating habits, geographical location and ecological conditions. Demand for new energy sources, the necessity to reduce the environmental problems and the increase in agriculture practices require the utilization of agricultural wastes [2]. With the increase in crop production, larger amounts

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of plant residue are also formed, including leaves, straw, grains, corn cobs [3].

Investigations about alternative energy sources are crucial and important issues in the world [4]. The agricultural crop residues especially lignocellulosic biomass have potential for biofuel production [5,6]. Barley straw is the one of the most produced lignocellosic wastes from agricultural activities [7]. In many countries including Türkiye, agriculture is mainly based on cereal cultivation, especially wheat and barley cultivation [8]. Especially a large amount of straw is produced because of agricultural activities. Straw is a residue obtained after the harvest of plants grown for their seeds and is a roughage with very low nutritional value. Straw, which is obtained in high amounts every year in Türkiye and in the world, consists of ripe stems and leaves of the plant [9].

Generally, most of these wastes is tried to be destroyed in farmlands. These kinds of agricultural wastes are among the causes of environmental pollution. Especially cellulose is the one of these substrates causes serious environmental pollution [10]. Farmers often burn agricultural waste in the agricultural lands. This method is economical and practical method of agricultural waste disposal, but this method affects the ecosystem negatively. This method creates serious negative effects such as air pollution, microbial population, deterioration of microflora and micro fauna and changes and damages in the physico-chemical and biological structure of the soil. Therefore, it becomes imperative to utilize crop residues resulting from agricultural production to ensure soil conditions, crop productivity and environmental sustainability [11]. For both economic and ecological reasons, biodegradation has become an increasingly popular alternative to the treatment of agricultural, organic and toxic wastes [12]. Most of the agricultural wastes is left in the agricultural areas to be decomposed by microorganisms [3]. There are many different types of bacteria in the natural ecosystem in the soil. Microorganisms play important roles in nutrient and geochemical cycles. Bacteria are also important and necessary for soil fertility and organic matter decomposition such as straw or leaves [13].

Bio-organisms in the use of agricultural wastes are studied and researched together with different types of decomposition processes. The main processes used for decomposition are anaerobic decomposition, compositing and fermentation processes. Microorganisms living in the soil play a fundamental role in all these decomposition processes. Different microorganisms play a role in each decomposition stage [14]. The use of microorganisms to improve agricultural waste treatment has been found to be highly efficient. Composting has gained significant popularity in the treatment of organic waste. It may be beneficial to use cellulolytic microorganisms to accelerate the rate of degradation of wastes, particularly lignocellulosic components [15]. Compost produced by the biological conversion of wastes contributes to the increase of agricultural productivity and biodiversity, reduction of ecological problems and the formation of healthier and more productive soil [16].

Cellulolytic bacteria can be found everywhere in nature and under suitable conditions, many of them can degrade the lignocellulosic structures. Cytophaga and Sporocytophaga are the dominant cellulolytic microorganisms involved in composting processes. In addition, more than half of *Bacillus* and *Cellulomonas* have been reported to cellulose degraders [16,17].

Biomass waste as raw material is very important to large-scale industries and community-level businesses and can improve the problems they cause when they turn into useful products. Biotransformation of agricultural waste is carried out by many microbial communities such as bacteria, fungi, actinomycetes. Many of these communities can degrade a wide range of agricultural compositions. Therefore, the biological content of microorganisms and the mechanisms they use for the degradation of agricultural waste biomass should be investigated [1].

Microorganisms have become very important agents for sorting and decomposing agricultural waste residues. They can use of the different components such as cellulose, hemicelluloses, lignin, pectin and so on. For these reasons, by detection of the agricultural high production level, barley straw wastes were selected in order to clearly reveal the potential of microorganisms in the breakdown of agricultural wastes and their microbial community structures. Main objective of this study was to characterize microbial populations capable of barley straw degradation. Specific objective includes: (1) to investigate the dominant bacterial populations associated with barley straw and (2) metabolic fingerprint of the microbial community that can be metabolizing the barley wastes in the soil as the only carbon source in the environment pre-enriched with barley. For this purpose, both traditional molecular methods and metabolic methods such as Biolog Ecoplate and next generation sequencing method were used to perform detailed analysis of microorganism groups degrading barley straw.

## MATERIALS AND METHODS

### Experımental Soil Samples and Properties

The soil samples sites were situated in the agricultural areas in Ayas, Ankara. (39°59’49.35”N 32°20’42.12” S). Ayaş district, which has 4% of the agricultural lands of Ankara, ranks ninth among Ankara districts in terms of agricultural area size, while the province meets 5% of grain and vegetable production. Approximately 49% of the area of the district is divided into agricultural areas. Ayaş district has the general climatic characteristics of the Central Anatolia Region and shows a continental climate feature [18]. When evaluated in terms of temperature; The lowest and highest monthly average temperatures in the district are observed in January and July and the annual average temperature

**Figure 1.** Sampling point on the map and working area

difference is 22 ° C. With these features, the district has the characteristics of the northern hemisphere temperature regime and displays terrestrial characteristics. Having an average annual rainfall of 374 mm, Ayaş district is an arid-semi-arid region according to its precipitation characteristics and there are two rainy periods, one in April-May and the other in December-January [19].

The soil and barley straw samples as shown in Figure 1 were collected from an agricultural field that had been in cereal agriculture for years including barley in May 2019. Samples were collected from 15 and 30 cm depth using a soil auger and 4 different points of area. The samples were brought to the laboratory in sterile bags and kept at 4 °C.

**Barley Straw Preparation and Analysis**

Tubitak Bursa Test and Analysis Laboratory determined the contents of crude protein, carbohydrate, total sugar, sugar types, nitrite, and nitrate in barley samples. Barley stalks were turned into flour in the plant mill for the analysis of the samples. For analysis HPLC-RID, IC Chromatography, Luff Scroll Method, Kjeldahl method and HPLC/IC equipment and methods were used by Tubitak.

**Isolation of Barley Straw Metabolizing Bacteria**

For determination of barley straw metabolizing bacteria, soil samples were strived to small pieces and 10 g soil samples was suspended with 90 ml Physiological Saline Solution (0.85%). Then, the samples were inoculated into Mineral salts medium (MSM) [20] was prepared containing barley straw as the sole carbon source and incubated. The medium was autoclaved by mixing 1.8 g $K_2HPO_4$, 4.0 g $NH_4$ CI, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$ in 1 L of distilled water. Then sterilized 100 ml of MSM and 5 gr barley stalk flour were mixed. 50 ml of suspended soil sample was added to the sterilized enrichment medium and incubated at 37 °C and 150 rpm for 14 days. Solid media were prepared for the isolation of microorganisms from samples. For the solid medium, 15 g of barley stalk flour was mixed with 300 ml of MSM and 4.5 g of agar and autoclaved. Samples grown in liquid enrichment were diluted up to $10^{-3}$ by serial dilution method and 100 µl samples were taken from $10^{-1}$ and $10^{-3}$ dilutions and inoculated into barley agar medium with the spread plate technique. Inoculated samples were incubated at 37 °C for 7 days. The colonies that developed as a result of incubation were selected and pure cultures were preserved at -85˚C in 20% glycerol stocks.

Extraction method based on boiling after homogenization in distilled water was used for DNA extraction from selected isolates. For this purpose, samples taken with sterile toothpicks from selected pure cultures were suspended in 200 µl sterile distilled water and kept at 96 °C for 10 minutes and the cells were lysed. After this process, the supernatant was transferred to new sterile tubes by centrifugation at 12000 rpm. It was stored at -20 °C to be used as a template for the PCR reaction [21, 22].

In the study, PCR reaction was applied using Bacteria specific primers for isolates that can use barley straw wastes as the only carbon source. For polymerase chain reaction; forward primer 27F (5'AGAGTTTGATCATGGCTCAG-3'), reverse primer 1492R (5'-GGTTACCTTGTTACGACTT3'), template and Biolabs OneTaq® Quick-Load®2X Master Mix (M0486S) and Bacter 50 programme were used [23]. 1% agarose gel was prepared to control the signals and quality of PCR products. Thermo Scientific Gene Ruler 1 kb Plus DNA Ladder was used to determine the size of the obtained PCR products. After loading into the wells, PCR products were run under 90 V for 40 minutes. When the run was finished, the gels were imaged with a transilluminator (Biolab Uvitec) at 312 nm wavelength.

The 16S rRNA region of the DNA obtained by DNA extraction of the isolates that can metabolizing barley straw wastes as the sole carbon source was amplified with PCR and positive results were sequenced. Reactions were established using primers 27F and 1492R. Sequences were analysed and deposited at Gene Bank System.

**Table 1.** 31 different carbon sources of Biolog Eco Plate

| C-source | Well | Group | C-source | Well | Group |
|---|---|---|---|---|---|
| Tween 40 | C1 | Polymers | D-Galactyronic acid | B3 | Carboxylic & Acetic acids |
| Tween 80 | D1 | | 2-Hydroxybenzoic acid | C3 | |
| α- Cyclodextrin | E1 | | 4-Hydroxybenzoic acid | D3 | |
| Glycogen | F1 | | γ-Hydroxybutyric acid | E3 | |
| Pyruvic acid methyl ester | B1 | Carbohydrates | Itactonic acid | F3 | |
| D-Cellobiose | G1 | | α-Ketobutyric acid | G3 | |
| α-D-Lactose | H1 | | D-Malic acid | H3 | |
| β-Methyl-D-glucoside | A2 | | L-Arginine | A4 | Amino acids |
| D-Xylose | B2 | | L-Asparagine | B4 | |
| i-Erythritol | C2 | | L-Phenyloalanine | C4 | |
| D-Mannitol | D2 | | L-Serine | D4 | |
| N-Acetyl-D-glucosamine | E2 | | L-Threonine | E4 | |
| Glucose-1-phosphate | G2 | | Glycyl-L-glutamin acid | F4 | |
| D,L-α-Glycerol phosphate | H2 | | Phenylethylamine | G4 | Amines & Amides |
| D-Glucosaminic acid | F2 | Carboxylic & Acetic acids | Putrescine | H4 | |
| D-Galactonic acid-γ-lactone | A3 | | | | |

**Substrate Utilisatıon Assays**

Biolog Eco Plate (Biolog Inc., Hayward, California) was used to determine the metabolic fingerprint of the microbial community that can use the barley wastes in the soil as the only carbon source in the environment pre-enriched with barley. 96 well- Biolog Ecoplate contain 31 different carbon sources (Table 1) and one control well in 3 replications. In each well, indicator dye was used. If substrates were utilized, tetrazolium violet redox dye become active, changes to purple colour [24,25].

For the analysis, after 14 days incubation of soil samples incubated in barley straw broth, the samples were serially diluted with sterile physiological saline. 150 µl of dilutions of $10^{-1}$ and $10^{-2}$ was added to the ecoplate wells. 150 µl of physiological saline was added to the control wells. At the end of this process, the ecoplates were left to incubate at 37 °C. Absorbance data was analysed at 590 nm at 24-hour intervals up to five days at Biolog Microstation [26].

Data from each well at 24-hour intervals were normalized using the readings from the OD values of the control wells. Other calculations were applied after the normalization step.

Microbial activity, incubation time effects and different parameters on the functional diversity were determined with average well-colour development (AWCD) and area under curve (AUC) values, these values were calculated with formulas in the Table 2 [27, 28].

The number of oxidized C substrates was used to calculate the richness values. In addition, OD 0.25 was chosen as a threshold value for positive reactions in the calculation of the Shannon–Weaver index values (H). This is the most commonly used index that shows the ability of the bacterial communities' types of carbon sources degradation and physiological diversity. The Shannon–Weaver index was calculated with formula in the Table 2 [29-32].

Calculating the utilization of 6 kinds of carbon sources, Amines/amides, Amino acids, Carbohydrates, Carboxylic acids, Polymers by different samples is a part of the content of static analysis, besides, calculating diversity index of carbon utilization and 31 carbons as variables are also included in statistical analysis.

**Analysis of Sequencing-Derived Data**

For determination of barley metabolizing bacterial community, Metagenomics DNA was extracted from barley straw enrichment culture using the Power Soil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) and phenol chloroform extraction method. Quality of DNA was controlled both on 1% agarose gel and Nanodrop for determining A260/280 ratio.

Primers for the amplification of the V3–V4 hypervariable region of 16S rDNA gene were designed for amplicon libraries. Nextera XT Index Kit (Illumina Inc., USA) was used for preparation of amplicon libraries. The concentration of the libraries was measured and normalized by diluting to 4nM. Normalized samples were combined by pooling method. After the preparation, the samples were loaded to MiSeq for Paired-end sequencing. In this type of sequencing, fragments were sequenced in both the forward

**Table 2.** Formulas of Biolog Ecoplate calculations

| Index Name | Formula | Explanations |
|---|---|---|
| **The average well colour development** | $AWCD = \sum_{n=1}^{31} \left(\frac{\text{ODi}}{31}\right)$ | **ODi:** the normalized optical density of each well |
| **Area Under the Curve** | $AUC = \sum_{n=1}^{31} \frac{A_n + A_{n+1}}{2x(t_{n+1} - t)}$ | **An and An+ 1:** absorbance value of each well in two consecutive time intervals<br>**tn and tn + 1:** two consecutive times |
| **Shannon-Wiener functional diversity index** | $H = -\sum_{n=1}^{31}(\text{pi}(\text{Inpi})$ | **pi:** the ratio of the absorbance of each substrate (ODi) to sum of the absorbance for the all substrates ( ΣODi) |
| **Substrate/catabolic richness** | S= the total number of oxidized substrate (C) | **The total number of oxidized substrate (C):** Wells in the absorbance value over 0.25. |
| **Shannon Evenness index** | $E = \frac{H}{lnS}$ | **H:** Shannon-Wiener functional diversity index values<br>**S:** Substrate/catabolic richness value |

and reverse. The data produced after sequencing was converted into raw data (FASTA format) for analysis.

The sequence data were analysed using EzBioCloud 16S rRNA gene-based microbiome taxonomic profiling (MTP) (ChunLab, Inc.) [33] with the following parameters: "Bacteria" as a target taxon and the prokaryotic 16S rRNA gene database PKSSU4.0. Sequences processed in the EzBioCloud 16S rRNA gene-based MTP pipeline were subjected to taxonomic assignment using the sequence identity thresholds proposed previously [34]. Taxonomic assignment was performed using the USEARCH program to detect and calculate the sequence similarities of the query paired-end reads against the EzBioCloud 16S database. EzBioCloud sequencing reads were clustered into OTUs at 97% sequence similarity using the UPARSE algorithm. Paired-end reads from each sample were clustered into many OTUs using the UCLUST tool with the above-noted cut-off values**.**

## RESULTS AND DISCUSSION

### Barley Straw Content Analysis

According to the results obtained from Tubitak Bursa Test and Analysis Laboratory, crude protein, carbohydrate, total sugar, sugar types, nitrite and nitrate determination results of barley stalk were given in Table 3. The carbohydrate content was found to be quite high.

### Identification of Microorganisms Using Barley Straw Waste as a Carbon Source

9 isolates that were thought to be different from the mixed colonies developed in the barley agar media were selected and isolated as pure colonies and positive results were obtained from 9 isolates as a result of 16S rRNA amplification.

**Table 3.** Content of Barley straw

| Parameter | Unit | Test result mean ± s |
|---|---|---|
| **Crude Protein** | g/100g | 3,66 ± 0,12 |
| **Carbohydrate** | g/100g | 79,1 ± 1,0 |
| **Total Sugar** | g/100g | 0,45 ± 0,11 |
| **Glucose** | g/100g | <0,03 |
| **Fructose** | g/100g | <0,01 |
| **Saccharose** | g/100g | <0,01 |
| **Maltose** | g/100g | <0,03 |
| **Nitrate** | mg/kg | <50 |
| **Nitrite** | mg/kg | <2,5 |

Sequence analysis of isolates whose DNA was extracted and 16S rRNA gene amplified with PCR were performed. After the sequence analysis, the samples were analysed by comparing them with reference samples in the NCBI database. Similarity rates were given in Table 4 *Bacillus* and *Arthrobacter* genus members were detected in high rate.

### Microbial Community Substrate Utilization Profiles

The values of average well colour development index indicate the high microbial community metabolic activity for the analysed carbon substrates. The AWCD values showed the increasing results with incubation time and varied for different soil samples (Table 5). The L1-15 and L2-15 soil samples had the higher AWCD values than L1-30 and L2-30. Especially L-1 15 soil sample had the highest AWCD values. Higher AWCD values indicate that

**Table 4.** Identified isolates, closest relatives and accession numbers

| Isolate | Closest Relative in Gene Bank | Similarity % | Accession Number (GenBank) |
|---|---|---|---|
| **S27** | *Bacillus megaterium* strain A6-Y 16S ribosomal RNA gene, partial sequence (MT588721.1) | 1052/1069(98%) | MW445145 |
| **S28** | *Arthrobacter koreensis* strain AFF1 16S ribosomal RNA gene, partial sequence (MN969917.1) | 1321/1323(99%) | MW445146 |
| **S29** | *Ornithinibacillus sp.* GL1-1 gene for 16S rRNA, partial sequence (AB489106.1) | 1035/1049(99%) | MW445147 |
| **S30** | *Bacillus sp*. 6A-8 partial 16S rRNA gene, strain 6A-8 (AM900499.1) | 900/901(99%) | MW445148 |
| **S31** | *Arthrobacter koreensis* strain 96A20 16S ribosomal RNA gene, partial sequence (MT012066.1) | 811/816(99%) | MW445149 |
| **S32** | *Microbacterium arborescens* strain MFB-39 16S ribosomal RNA gene, partial sequence (MN220635.1) | 875/882(99%) | MW445150 |
| **S33** | *Bacillus megaterium* strain LWEC5 16S ribosomal RNA gene, partial sequence (KU340993.1) | 717/718(99%) | MW445151 |
| **S34** | *Arthrobacter koreensis* strain 96A20 16S ribosomal RNA gene, partial sequence (MT012066.1) | 684/684(100%) | MW445152 |
| **S35** | *Lysinibacillus sp.* strain PF111X 16S ribosomal RNA gene, partial sequence (MK575035.1) | 629/639(98%) | MW445153 |

**Table 5.** Biolog Ecoplate analyse results of soil samples

| Soil Samples (Depth)(cm) | Incubation Time (Hours) | Indices | | | | |
|---|---|---|---|---|---|---|
| | | AWCD | H | E | S | AUC |
| **L-1 15** | 24 | 2,038±0,114 | 3,420± 0,013 | 0,996 ± 0,004 | 31 | |
| | 48 | 2,332± 0,100 | 3,413± 0,014 | 0,993 ± 0,004 | 31 | |
| | 72 | 2,512± 0,051 | 3,414± 0,011 | 0,994 ± 0,003 | 31 | |
| | 96 | 2,600 ± 0,069 | 3,417± 0,008 | 0,995 ± 0,002 | 31 | 171,914 |
| **L-1 30** | 24 | 0,564± 0,019 | 3,260 ± 0,015 | 0,949 ± 0,0004 | 30,333 ± 1,555 | |
| | 48 | 1,120 ± 0,034 | 3,257± 0,056 | 0,948 ± 0,016 | 30,333 ± 1,555 | |
| | 72 | 1,384 ± 0,133 | 3,299± 0,010 | 0,960 ± 0,003 | 30,333 ± 1,555 | |
| | 96 | 1,462 ± 0,130 | 3,312± 0,004 | 0,964 ± 0,004 | 30,333 ± 1,555 | 84,398 |
| **L-2 15** | 24 | 1,232±0,044 | 3,414 ± 0,014 | 0,994 ± 0,004 | 31 | |
| | 48 | 1,458 ± 0,178 | 3,376 ± 0,019 | 0,983 ± 0,055 | 31 | |
| | 72 | 1,651 ± 0,078 | 3,379 ± 0,018 | 0,984 ± 0,005 | 31 | |
| | 96 | 1,662 ± 0,095 | 3,379 ± 0,020 | 0,984 ± 0,006 | 31 | 109,362 |
| **L-2 30** | 24 | 0,826 ± 0,051 | 3,406 ± 0,010 | 0,992 ± 0,003 | 31 | |
| | 48 | 1,058 ± 0,152 | 3,286 ± 0,012 | 0,957 ± 0,003 | 31 | |
| | 72 | 1,184 ± 0,237 | 3,288± 0,036 | 0,957 ± 0,010 | 31 | |
| | 96 | 1,316 ± 0,269 | 3,304± 0,040 | 0,962 ± 0,011 | 31 | 79,509 |

microbial communities have a stronger metabolic activity to use substrates. The order of AWCD values for soil samples was L-1 15 > L-2 15 > L-1 30 > L-2 30. Values such as substrate richness (S) and substrate evenness (E) were calculated to further compare catabolic diversity. These types of values were higher for L-1 15 and L-2 15 soil samples. For the L-1 30 and L-2 30 samples there were some changes in diversity, richness, and evenness values. The higher diversity and richness were calculated in the L-1 15 soil sample and lower values were obtained for L-1 30 sample. The lowest S value showing the number of substrates consumed by the community microorganisms was determined in sample L-1 30. So, there was correlation between AWCD and S value.

Substrates were grouped as carbohydrates, polymers, carboxylic & acetic acids, amino acids, amines and amides to compare the substrate consumption status of the microbiomes in the samples analysed.

As a result of the analysis of the usage rate of the substrates in the specified groups, it was determined that microorganisms actively use all types of substrates in most samples. In L1-30 sample, low utilization level of some substrates especially carboxylic & acetic acids and amino acids groups were detected. Shannon index (H) was measurement of alpha diversity. In all samples these values were nearly same. In samples L-1 30 and L2-30 these values were calculated slightly lower.

**Analysis of Sequencing-Derived Data**

When the incoming raw data were analysed, it was determined that the number of reads for L1-15, L1-30, L2-15 and L2-30 were 47664, 20266, 36282 and 57847, respectively. When the taxonomic diversity at the phylum level was examined, members of the phyla Proteobacteria, Bacteroidetes, Gemmatimonadetes, Planctomycetes, Actinobacteria and Firmicutes were found to be intense. However, as can be seen in the Figure 2 and 3, there were differences among the examples. While there were 60% Proteobacteria members in L1-30, L2-15 and L2-30 coded samples, 32.99% Proteobacteria and 29.56% Firmicutes members were determined in L1-15 coded samples. High and low-richness values of phyla were combined, and a heat map was generated. The similarities and differences of the samples with the colour gradient were observed (Figure 4).

When the data were analysed at the genus level, 28.59% and 21.67% *Luteimonas* species were found to be intense in L1-30 and L2-15 samples, respectively. It was very low density in L1-15 and L2-30 (0.40% and 1.80%) samples. There were 18.85% *Sphingomonas* species in L1-30 sample, L1-15 0.45%, L2-15 7.74%, L2-30 7.96%. While 19.17% *Brevundimonas*, 18.24% *Flavisolibacter*, 12.30% *Altererythrobacter* were determined in L2-30 sample, it was observed that they were quite low in other samples. In the L1-15 sample, rather dense (18.10%) *Bacillus* genus members were observed compared to the other samples.

As a result of alpha diversity analysis, the diversity index values were obtained in the table 6 below. When the data and index values were analyzed, it was determined that the samples with the highest OTU values were L1-15 and L2-15. Considering the diversity indexes, it was determined that the sample with the richest microbial content was L1-15. The Good's coverage values of the samples are between 98.97% and 99.52%.

When the results are evaluated in general, there are differences between the soil samples in terms of microbial community content and density, and it is seen that all samples have a dense and rich diversity.

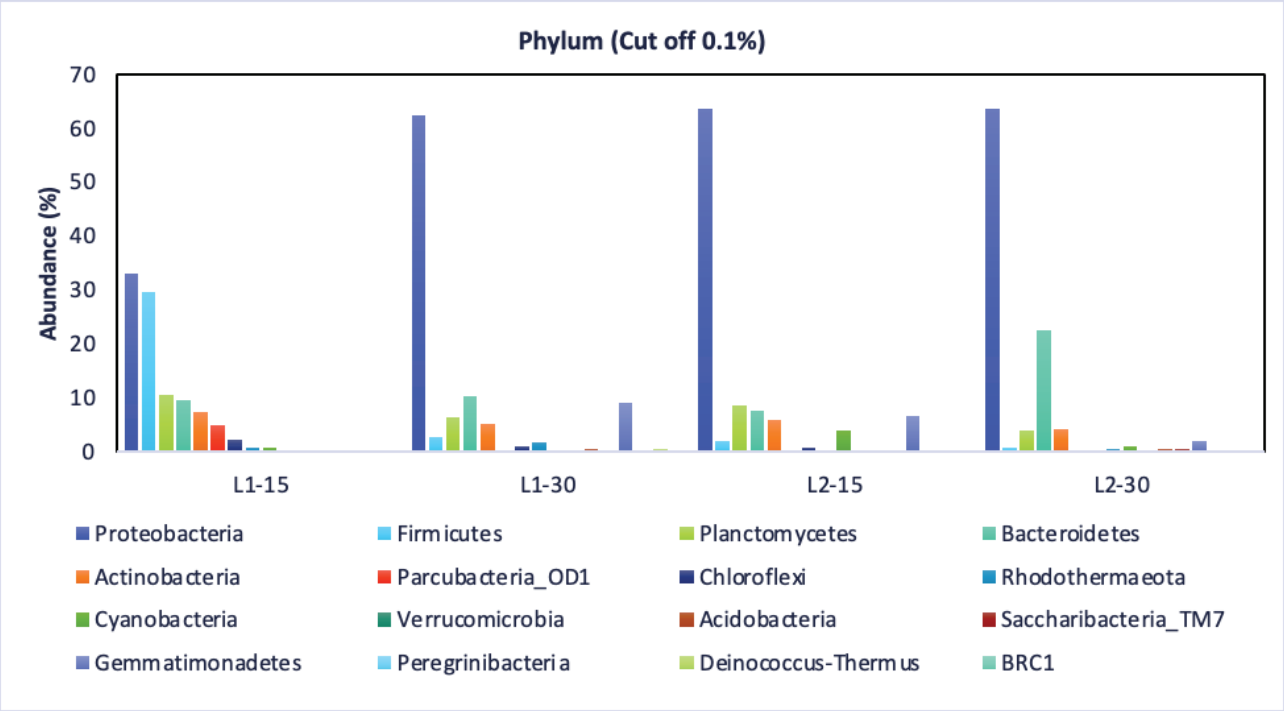


**Figure 2.** Relative abundance of microbial community composition of soil samples at the phylum level.

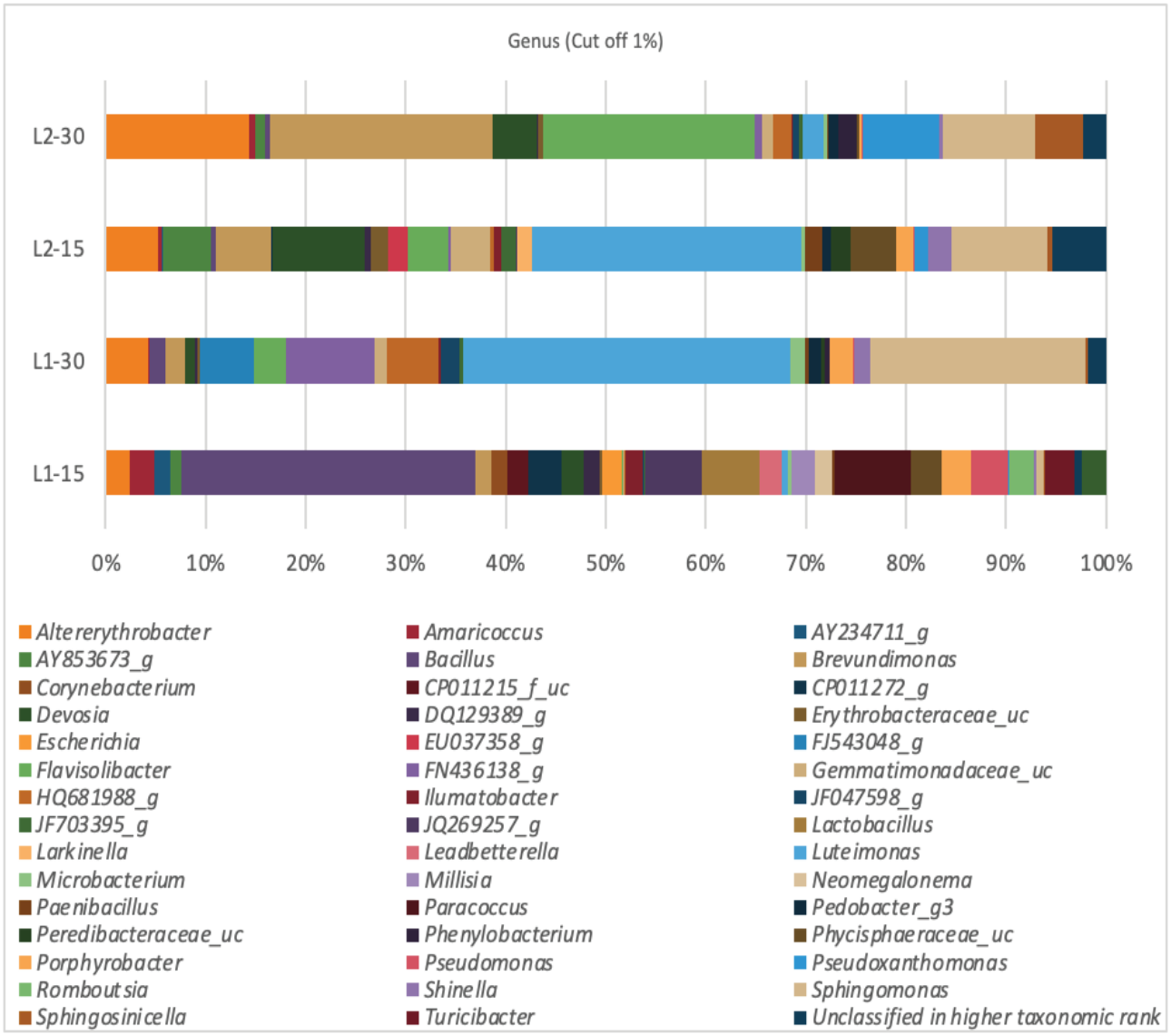


**Figure 3.** Relative abundance of bacterial community composition in three samples at the genus level.

**Table 6.** Diversity indices of the microbial communities of the soil samples

| Sample name | Target reads | OTUs | ACE | CHAO | Jackknife | NPShannon | Shannon | Simpson | Phylogenetic Diversity | Good's coverage of library (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| **L1-15** | 47664 | 2364 | 2577.14 | 2448.42 | 2726.00 | 5.88 | 5.80 | 0.01 | 2321 | 99.24 |
| **L1-30** | 20266 | 697 | 885.07 | 809.13 | 905.00 | 3.47 | 3.40 | 0.12 | 935 | 98.97 |
| **L2-15** | 36282 | 1429 | 1662.08 | 1542.09 | 1751.00 | 4.52 | 4.45 | 0.06 | 1529 | 99.11 |
| **L2-30** | 57847 | 1603 | 1771.20 | 1674.90 | 1878.00 | 4.18 | 4.13 | 0.06 | 1375 | 99.52 |


**Figure 4.** Bacterial community heat map analysis at phylum level.

In many countries, grain production forms the basis of the general economy. Grain is important not only in human nutrition, but also in meeting the feed needs of animals. Türkiye is also the highest share of cereal crops in agricul-tural areas. Therefore, sampling done Ayas district in the state shows the same features with Türkiye in general. 90% of the agricultural lands of Ayaş district are for grains and legumes; 8% is reserved for vegetables, mainly tomatoes, and 2% for fruit areas, mainly mulberry and cherry. 55.5% of the products grown in the district are wheat, 30.2% bar-ley, 5.5% chickpea and the remaining 7.8% are reserved for corn and fodder crops [19]. Since agricultural production is high, agricultural waste is also generated in this region. For this reason, a study was carried out on microorganisms that break down agricultural waste by sampling from the barley cultivation land in the district.

Although the Biolog method has been criticized for being a culture-dependent technique [35], it is a fast and useful method for analysing microbial structure and metabolic diversity in soil. The AWCD, R, H index results showed that a great potential of substrate utilization ability of the barley straw metabolizing microbial community and greater functional diversity of samples. There were some minor differences between samples. These differences can be due to the presence of more complex or different organic material in sampling sites and depth of samples. The substrate utilization profiles of samples which were enriched with barley straw were similar in the two samples (L-1 15, L-2 15). These results suggested that bacterial communities in same depth were similar. The samples from 30 cm

depth differed from the 15-cm depth and in particular L-1 30 sample showed a clearly negative difference for substrate utilization potential. In the upper soil sample, the activity of the microbial community was greater than in deep soil. This indicates that microorganisms metabolizing barley straw in less depth soil were more active and more soil C was lost through microbial respiration.

Microbial growth is limited when available carbon amounts and types are low [36]. In our study with Biolog Eco-Plate analysis, we determined both the bacterial metabolic diversity using barley straw as a carbon source and the substrate usage profiles. The highest AWCD, H, E, S and AUC values were determined in samples L1-15 and L2-15. These values had positive correlation with depth and also indicated the number of substrates consumed by the barley straw associated community or metabolic richness. In most of samples all substrates were consumed. Only in L1-30 sample, the low levels of 2-Hydroxybenzoic acid, Itactonic acid, α-Ketobutyric acid and Phenylethylamine utilization profiles were analyzed. The reason for these results can be complexity and chemical structures of substrates. Utilization process for these substrates requires more time [37].

Isolates indicated that barley straw degrading bacteria was belong to *Arthrobacter* and *Bacillus* genera. In analysis *Bacillus megaterium, Arthrobacter koreensis, Microbacterium arborescens, Ornithininbacillus sp.* and *Lysinibacillus sp.* species were identified. The bioconversions of agricultural wastes are very crucial for ecological and economical. Bioconversion carried out by many microorganisms. Most of the wastes generated by agricultural activities are plant residues. Plant residues contains lignocellulosic structures [38]. Some soil bacteria such as *Streptomyces*, *Micromonospora*, *Bacillus*, *Cellulomonas* and *Cytophaga* can degrade cellulose [39].

In a study twenty bacterial strains were isolated from different agriculture wastes and biotechnologically most important strain was identified as *Bacillus flexus* [40]. Afzal et al. 2012 [41] studied about higher cellulase producing *Bacillus cereus* from waste. Venkata *et al*. 2013[42] reported that the *Bacillus* genus produces cellulase from wastes. Literatures are limited for degradation of lignocellulosic and agricultural wastes by microorganisms so, in our study we reported *Bacillus megaterium*, *Arthrobacter koreensis*, *Microbacterium arborescens*, *Ornithininbacillus sp.* and *Lysinibacillus sp.* species as a barley straw using and agricultural waste decompositier.

Apart from these properties, the isolated bacteria are useful and applicable for different purposes in agricultural applications. For example, genome sequence of *Arthrobacter koreensis*, a plant growth promoting, and desiccation-tolerant strain was published [43]. Growth-promoting 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophore-producing proteins, and tryptophan biosynthesis proteins were reported from the whole genome of *Microbacterium sp.* This bacterium was isolated from date palm orchards with high soil salinity [44]. A *Bacillus megaterium* strain was identified as among plant growth promoting rhizobacteria (PGPR) by production of cytokinin [45]. Plant growth promoting bacteria play an important role in nitrogen and phosphorus cycling. They can fix nitrogen, and nitrify, and solubilize phosphorus, in this way, they increase the soil nutrients for plant growth [46].

In addition to isolation methods, to provide a more in-depth view, Illumina MiSeq sequencing technology was used. There were many unclassified OTUs and phyla with a relative abundance <1%. Sequencing-Derived Data from barley straw enrichment soils indicated that microbial communities using barley straw were rich and diverse. These communities were diverse in different soil samples. Agricultural applications and soil chemical parameters are important factors for bacteria Since soil samples are taken and analyzed from selective pre-enrichment medium with barley straws, the results show only microorganisms that grow and survive intensively in barley straws.

MiSeq sequencing indicated some differences in the microbial taxonomic composition between barley straw enrichment soil samples. Totally, 10 phyla (> 1%) were detected and Proteobacteria, Firmicutes, Bacteroidetes and Gemmatimonadetes were widely analysed as the predominantly. In all samples, Probacteria members were dominant but, in L1-15 sample rate was in low density than others. 32.99% Proteobacteria and 29.56 % Firmucutes was identified. In other samples Firmicutes members were in low rates. Then analysis was made at genus level for detection richness and diversity. *Luteimonas* and *Sphingomonas* were dominant in L1-30 and L2-15, in addition *Bacillus* was in high rate in L1-15 and *Brevundimonas*, *Flavisolibacter* and *Altererythrobacter* were detected in high rate in L2-30 sample.

Further analysis was made to obtain statistical values about richness and diversity. Ace and Chao index shows the richness and Shannon indicates the microbial diversity. Index results indicated that L1-15 had higher values. The species composition of the sample L1-30 and L2-15 were more similar. L1-15 sample was different.

There are not many such studies like ours. In particular, there is little if any regarding the analysis of microbial community by next generation sequencing in the presence of agricultural waste. So, it is difficult to analyse and compare the data with other studies. In a study that can be associated with this study was carried out on chernozem. Barley straw was used for cellulose decomposition in chernozem (a kind of soil). In the study cellulose destructors Chitinophaga and representatives of the families Streptosporangiaceae and Micromonosporaceae and Chthoniobacter was reported [47].

In this study, agricultural soil samples were obtained and enriched with barley straws. Bacteria using barley straw were isolated and identified as *Bacillus megaterium*, *Arthrobacter koreensis*, *Microbacterium arborescens*, *Ornithininbacillus sp*. and *Lysinibacillus sp.* species. For deep and detailed analysis Biolog Ecoplate and Illumina Sequencing methods were applied.

## CONCLUSION

Results concluded that microbial community using barley straw from agricultural soils had a great substrate utilization potential and greater functional diversity and richness. However also new genus members using barley straw were reported in agricultural soils. As the need for food increases in the world, agricultural activities will increase, so the amount of waste generated will also increase. Therefore, alternative solutions are required to reduce environmental pollution. Microorganisms are a strategically advantageous option for the degradation of agricultural waste. These microorganisms can decompose different components of agricultural wastes from plant residues. The study clearly showed the efficiency of bacteria to degrade barley straw wastes so these results can be used properly decomposition of barley straw wastes which are common wastes in our agricultural strategy.

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## AUTHORSHIP CONTRIBUTIONS

Authors equally contributed to this work.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

## CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## ETHICS

There are no ethical issues with the publication of this manuscript.

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