

Isolation and identification of bacteria with cellulolytic activity from the microflora of animal juice

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Abstract

According to current modern concepts, cellulose complexes produced by various microorganisms are polyfermentative systems composed of enzymes that differ in molecular structure and the way they act on cellulose. Microbiological processing is one of the most economical, promising and ecologically safe ways of processing cellulose raw materials. In the study, 27 spore-forming microorganism isolates were isolated from goat stomach juice, goat stomach, rabbit stomach juice, chicken stomach, bark beetle (*Dendroctonus ponderosae*) and termites (*Coptotermes formosanus*). Morphological characteristics of bacterial isolates were studied and identified using matrix laser desorption/ionization mass spectrometry (MALDI-TOF) method and genetic method. Based on the primary screening, the cellulolytic activity of the isolated bacteria was evaluated by reducing the mass of #10 100% cotton thread from 0.05 g to 0.035 g after 96 hours. Freshly obtained goat gastric juice was used as a control. The cellulolytic activity of the bacterial strains was determined by the application of Congo red to the agar medium. 1-Bacillus isolated from the termite (*Coptotermes formosanus*) when cellulolytic activity was carried out according to the modified method of Mendels and Weber. sp. and isolated from goat gastric juice 4- *B. subtilis* sp. the highest activity of 0.233 and 0.193 TslS/cm³ was determined in the strains. Also 1-Bacillus. sp. and 2- Bacillus. sp. bacterial strains produce up to 0.34 mg/ml of protein and the highest activity was isolated from goat gastric juice 3- *B. subtilis* sp. strain and was 0.38 mg/ml. In our research, we determined the cellulolytic activity of Bacillus strains isolated from the bark beetle (*Dendroctonus ponderosae*) and termites (*Coptotermes formosanus*), which feed on roughage consisting of cellulose. serves to create.

Keywords: *Bacillus subtilis*, microflora, gastric juice, animal husbandry, cellulose

Introduction

According to modern concepts, cellulose complexes produced by various microorganisms are polyenzymatic systems consisting of enzymes that differ in their molecular structure and mode of action on cellulose. Microbiological processing is one of the most economical, promising and environmentally friendly methods of processing cellulose raw materials. At present, the growing interest in the use of cellulolytic enzymes of microorganisms for important technical and economic purposes in different countries of the world shows how important is the selection of active producers of these enzymes.

In particular, the production of amylase, protease, cellulase, glucose isomerase, and pullulanase enzymes by bacteria belonging to different genera is considered a commercial source. Bacteria are an important source of cellulose for various industrial and biotechnological applications. Preparations created based on the microflora of the gastric juice of small ruminants (goats) and regulating the beneficial microflora of the digestive system are called probiotics. It is known that the lack of quality feed and the abundance of concentrates in the feed used for consumption reduce the activity of cellulose in the stomach of the animal. The use of preparations based on bacteria with cellulolytic activity improves the digestive processes in highly productive cows. It has been established that "Cellobacterin", created on the basis of bacteria with cellulolytic activity, when introduced into the diet of dairy cows, increases milk yield, protein and fat content of milk. Bacteria with cellulose activity improve the digestion of cellulose in the animal's digestive system; and contribute to the complete assimilation of protein and other nutrients. The use of preparations based on cellulolytic bacteria leads not only to an increase in milk productivity but also to a reduction in the cost of milk production. Agnieszka Wita et al. (2019) isolated *Bacillus sp.* SV1 strain from the stomach of the cattle, which has high cellulolytic (7.89) activity.

The activity of cellulolytic enzymes - endoglucanase and β -glucosidase, which are synthesized outside the cell, was determined. It was found that with the addition of CMC, the synthesis of cellulose increased by 2.9 times, and the endoglucanase enzyme - by 2.1 times (Agnieszka Wita et al., 2019). Also, 214 bacterial strains were isolated and their cellulose-producing properties were determined by diffusion method. The highest cellulolytic activity was observed in isolates of *Bacillus subtilis*, *Bacillus licheniformis* at a temperature of 32°C. Researchers have conducted studies aimed at increasing the activity of medicinal cellulose by cloning the cel8A and cel48S genes in *Bacillus licheniformis* 24 and *B. velezensis* 5RB

strains. The activity of cellobiohydrolase (GH48) and endocellulase EglS (GH5) was found in the Cel 8A B. *B. Licheniformis* strain, endocellulase Cel A (GH9) in the bacterial strain *B. Velezensis* Cel48S. Also, the method of genetic complementation was used to increase the activity of cellulase in strains belonging to the genus *Bacillus* (Syazwan Ngalimat *et al.*, 2021). Bacteria of the genus *Bacillus amyloliquefaciens* are able to fix free atmospheric nitrogen, dissolve phosphorus, produce phytohormones, and synthesize the enzymes amylase, protease, lipase, cellulase, and pectinase enzymes. This strain is also known to synthesize commercial enzymes and antibiotics (Mohd Huzairi Mohd Zainudin *et al.*, 2022). In the course of the research, 23 isolates of cellulolytic bacteria were isolated at different stages of compost preparation, strains of *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus aerius*, *Bacillus haynesii* involved in the decomposition of lignocellulose were identified by 16S RNA sequencing. *Bacillus licheniformis*, a facultative anaerobe, has been found to produce large amounts of the cellulase enzyme (Waseem Ayoub Malik *et al.*, 2021). Martina Aulitto *et al.* (2017) found that the culture fluid of *Bacillus coagulans* MA-13 strain when treated with 30% lignocellulose hydrolyzate, the treatment time was reduced, the average volume of productivity increased by 50%, and the average specific productivity increased by 115% compared to the control. When studying the cellulolytic microbiome at different stages of the fermentation process, bacterial and fungal strains from the genera *Bacillus*, *Aspergillus* and *Mucor* were identified in the early stages of fermentation. The enzymatic activity of amylase and glucoamylase of bacteria of the genus *Bacillus* was determined, which increased within 7 days and decreased on subsequent days (Pan Li *et al.*, 2015). Lactic acid bacteria have been found to synthesize proteolytics, b-glucosidase, b-mannosidase and b-mannase outside the cell. Proteolytic, cellulolytic and hemicellulose activity of *Lactobacillus plantarum* strain 7 was determined. *Lactobacillus plantarum* strains produce many extracellular hydrolytic enzymes. The highest total activity of hydrolytic enzymes was determined in the strains *L. plantarum* RI11, *L. plantarum* RG11, and *L. plantarum* RG14. Studies were also carried out to determine the activity of cellulolytic and hemicellulolytic enzymes of the *Lactobacillus plantarum* RI 11 strain. The strain *Lactobacillus plantarum* RI 11 showed an increase in biomass (10^{10} HHB/ml) on a nutrient medium with the addition of molasses and yeast extract. The enzymatic activity was endoglucanase (11.70 $\mu\text{g}/\text{min}$), exoglucanase (9.99 $\mu\text{g}/\text{min}$), β -glucosidic and mannose (8.03 $\mu\text{g}/\text{min}$).

In a nutrient medium with the addition of glucose syrup and sesame oil waste, the activity of endoglucanase increased 4 times, the activity of exoglucanase - 2.6 times, and mannose - 2.6 times (Nursyafiqah *et al.*, 2020). *Bacillus licheniformis* 2D55 strain 3 showed the highest cellulolytic activity (CMC 0.33 U/ml and 0.09 U/ml) when cultivated in MCT for 18-24 hours. It showed (0.160 Ed/ml) when grown on untreated sugar cane. The cellulolytic activity was significantly increased (2.5 and 11.5 times) on substrates obtained in the form of raw sugar cane and processed rice waste (Muinat Olanike Kazeem *et al.*, 2017).

Bacillus vallismortis RG-07, a cellulase producer resistant to thermoerythritol, was isolated and identified from the soil. During the study, it was found that the *Bacillus vallismortis* RG-07 strain synthesizes the cellulase enzyme in an amount (41.05 units/ml) using a nutrient medium with crushed sugar cane as a carbon source. The molecular weight of the purified cellulase enzyme was 80 kDa. The enzymatic activity also increased up to 7 days with the addition of 30% organic solvents from toluene, hexane, butanol and cyclohexane (Rajeeva Gaur and Soni Tiwari, 2015) The enzymatic activity of bacterial strains is characterized by the inhibition of pathogenic microorganisms. When using concentrations of 1.25 2.5; 5 and 10 units/ml, cellulase enzyme, it was observed a decrease in the biomass of pathogenic bacteria and a decrease in the pathogenic biomass of *P. aeruginosa* from 32 to 128 times (Esmat Kamali *et al.*, 2021). In order to increase the activity of the cellulase enzyme, it was found that the cellulolytic activity of the *Bacillus subtilis* VS15 strain was increased by 128, 148 and 167% with ethyl methylsulfonate, N-Methyl-N' nitro-N-nitrosoguanidine, and then the combination of protoplasts (Soujanya Lakshmi Ega *et al.*, 2021). *Conella sp.* the thermophilic bacterial isolate was found to synthesize the CelC 307 protein at 40°C and undergo purification. Activation of the endoglucanase enzyme was observed with trace elements Na⁺, Li⁺, Ca²⁺, as well as 2-mercaptoethanol (2-ME) and glycerol substances (Shima Mohammadi *et al.*, 2022). In this work, a quantitative and qualitative analysis of the cellulase enzyme was carried out in the study of the intestinal part of the silkworm *C. longicaudata* and the gray moth. The activity of endoglucanase, xylanase, and pectinase enzymes was found to be high in insects. Endo-1,6- β -glucosidase containing glycan in insects is consistent with bacterial enzymes among the enzymes detected by a mass spectrometer (Ratnasri *et al.*, 2019). The *R. stolonifer* strain was found to be induced by cellobiose synthesis. Based on this strain, it can be used to produce cellulase enzymes in a short period of time using a modified carbon source (Yingying Zhang *et al.*, 2017). When cultivated *Candidatus, Reconcilibacillus cellulovorans* a

bacterial association in 300 l, the activity of soluble cellulase was determined depending on the amount of Ca (Sebastian Kolinko et al., 2018).

Strains with cellulolytic activity were isolated from natural humus and wheat straw from the low-temperature region of China. The *Pseudomonas mendocina* strain was identified as a result of the morphological, physiological, biochemical and molecular biological characteristics of the strain. The optimal growth temperature of *Pseudomonas mendocina* is 28°C, pH 7.4-7.8, the maximum cellulase reaction rate is 0.3261 g/l and 0.1525 mg/(min.l), molecular weight is 42.4 kD. and amounted to 20.4 kD (Ianfeng Zhang *et al.*, 2016). As a result of the experiments, a cellulase-producing strain of *Bacillus amyloliquefaciens* NBRC15535 was isolated. The strain is a Gram-positive aerobe with an optimal growth temperature of 37°C, pH 7.0 and an optimal NaCl concentration of 2.0%. The strain was found to have endoglucanase, exoglucanase, beta-glucosidase and cellulase activity (Cheng Fan et al., 2012). The decomposition of cellulose in nature involves microorganisms belonging to different taxonomic groups: bacteria, actinomycetes, micromycetes and yeasts. Some researchers believe that the use of bacteria with cellulase activity is effective for the hydrolysis of cellulose substrates (Maki M. et al., 2009; Rabinovich M.L, 2000; Dabhi B.K. et al., 2014). In particular, the bacteria *Bacillus licheniformis* (Seo J.K et al., 2013) and *Paracoccus pantotrophus* (Faridha Begum. et al., 2023) isolated from the stomach of goats were found to be able to synthesize active cellulase. Cellulase is a complex enzyme complex with endo and exo 1,4- β -glucanase - and β -glucosidase activity (Pothiraj C. et al., 2020). According to current scientific data, cellulase complex enzymes mainly include three enzymes (Cristica M. et al., 2012; Kaur J. *et al.*, 2006; Thongekkaew J. et al., 2008). Some sources state that the main enzymes of group II) exoglucanases consist of cellobiohydrolases and glucanohydrolases (Lynd L.R et al., 2010). The enzyme complex catalyzing the hydrolytic decomposition of cellulose consists of four different carbohydrates (Klesov A.A.*et al.*, 1980). According to them, the set of cellulolytic enzymes includes: endo-1,4-b-glucanase (1,4-b-D-glucan-4-glucanohydrolase, KF 3.2.1.4.), exo-1,4-b- glucanase (exo-cellobiohydrolase or 1,4- β -D-glucan-cellobiohydrolase, KF 3.2.1.91), exo- 1,4- β -glucosidase (1,4- β -D-glucan-glucohydrolase, KF 3.2.1.74) and cellobiase (β -glucosidase or β -D-glucoside glucohydrolase, KF 3.2.1.21). The synergistic action of these enzymes is necessary for the complete decomposition of cellulose.

The function of complex enzymes can be described as follows: endoglucanases randomly hydrolyze internal β -1,4-glycosidic bonds in the cellulose chain and form new ends of the chain. Cellobiohydrolase, on the other hand, cleaves the ends of cellulose chains and converts them into cellobiose or glucose (Karmakar M. et al., 2011). β -glucosidase hydrolyzes only cellobiose and forms glucose (Zhang Y.H. et al., 2006; Kumar R. et al., 2008).

Materials and methods

Isolating pure cultures of bacterial strains with cellulolytic activity

With the help of veterinarians and physiologists, samples were taken from the stomachs of goats, rabbits and chickens, as well as gastric juices and large stomachs of goats, rabbits and chickens grown in a vivarium at the Department of Human and Animal Physiology. and Biochemistry" Samarkand State University. Also, the "Bark" beetles, which feed on cellulose and live in the bark of trees, and "Termites" which cause great damage to buildings and ancient monuments were brought and cleaned with 3% hydrogen peroxide, alcohol and distilled water. Samples were opened using crushed glass in the laboratory and cultured on sterile prepared meat-peptone agar media.

Isolating pure cultures of aerobic bacteria

The isolation of pure cultures of aerobic bacteria is divided into several stages. At the first stage, a smear is prepared from the stomach of a rabbit or bird, inoculated on pre-prepared agar and liquid nutrient media. The pure isolate is isolated by the generally accepted method of recultivation in microbiology. It is stained by Gram or other methods and analyzed under a microscope. A sterile 0.85% physiological solution is placed in test tubes. 0.01 ml of the prepared diluted suspension is taken and poured onto the surface of the agar nutrient medium in a Petri dish in the form of a ring and gently inoculated with a spatula, the planted material should be evenly distributed over the entire surface of the nutrient medium. After inoculation, the Petri dish is turned over, fixed and incubated in a thermostat for 18-24 hours at 37°C.

Second phase. Individual colonies that have developed in Petri dishes are studied, their shape, size, color, density and other characteristics are recorded. To determine the morphology of cells and their characteristics, a smear is prepared from a part of the colony under study. Gram-stained and analyzed under a microscope. To isolate a pure culture, one isolated colony or several different isolated colonies are plated several times on agar medium.

Third stage: attention is paid to the growth characteristics of the isolated pure culture. A visually clean colony is characterized by uniform growth. Microscopic examination of a stained smear prepared from such a culture reveals morphologically and tinctorially homogeneous cells. Samples of gastric juice of a goat, rabbit and chicken stomach were placed in sterile flasks containing 50 ml of 0.85% physiological solution, and placed on a shaker for 30 minutes. The suspension prepared on the basis of the samples (100 µl) was poured into ready-made solutions in the amount of 10^3 - 10^8 into test tubes. The prepared solutions were sown in Petri dishes on the MPC nutrient medium with the addition of 0.0025% violet-red indicator. Incubation was continued for 48 hours in a thermostat at 37°C. In the course of the studies, yellow-coloured bacterial colonies formed on the agar medium were purified by replanting.

Determining and identifying morphological features of spore-forming bacteria

Morphological characteristics of pure strains of bacteria isolated from the gastric juice of goat, rabbit, chicken stomach, Bark beetle and Termite, incubated in MRS and MPA (meat peptone agar) media (Hi Media, India) at 35-37°C, (in vitro) as an inoculum-inoculation, a suspension of strains of spore-forming bacteria grown during for 3 days was used at a concentration of 10^6 spores/ml. The microscopic structure of the isolated bacterial strains was studied under light microscopes XSP-136 B and OLYMPUS BX41 (400 times magnification) and identified by the conventional method using a Bergey detector. Bacteria were also identified by the method of matrix laser desorption/ionization mass spectrometry (MALDI-TOF) in the sanitary and hygienic laboratory of the Ministry of Health of the Republic of Uzbekistan (Kazakov V.S. 2017).

Determining the cellulolytic activity of isolated bacterial strains

The selected bacterial strain is incubated for 2-3 days in an MRS medium at 37°C. In 10 sterilized tubes, 10 ml of bacterial strains are mixed with a concentration of 10^{8-9} spores/ml and 0.3 ml of 16% glucose solution is added. In the first control variant, 10 ml of distilled water is taken, and in the second, 10 ml of freshly obtained goat gastric juice and 0.3 ml of 16% glucose solution are added. Glass beads are tied to the ends of the cotton (100%) thread №10 to the experimental samples, and 0.3 ml of 16% glucose solution is added to 10 ml of the experimental and control options. It is placed into a thermostat at a temperature of 37 ° C. The first result was obtained after 12 hours, and the overall result of the study was obtained

after 96 hours. Cellulolytic activity is measured by the reduction in fibre weight (Kondrakhin I.P. *et al.*, 2004).

Screening methods for bacterial strains with cellulolytic activity

Isolated pure strains of bacteria Peptone - 10 g, (SM-Cellulose) - 10 g, K_2HPO_4 - 2 g, $MgSO_4$ - 0,3 g, $(NH_3)SO_4$ - 2,5 g, Gelatin - 2 g, Agar - 15 g, pH-6.8-7.2, the nutrient medium is incubated at a temperature of 37 °C. The inoculum is used as a suspension at a concentration of 10^6 spores/ml, incubated in MPC nutrient medium for 24 hours. 0.1 ml of a suspension with a concentration of 10^6 spores/ml has been taken and inoculated in the form of drops and left in a thermostat at 37 °C for 72 hours. After 72 hours, a 5% alcohol solution of iodine is applied to the prototypes and left for 15 minutes at room temperature. At the next stage of the study, it is washed with 1 M NaCl solution (Mohammed *et al.*, 2008).

Determining the activity of the cellulase enzyme in individual bacterial strains

Determination of cellulase enzyme activity has been carried out according to the modified method by Mendels and Weber. The activity of the cellulase enzyme has been determined in the supernatant of a suspension of the culture liquid of bacterial strains at a concentration of 10^{8-9} spores/ml.

Cotton fibre has been used as a substrate for the determination of exoglucanase activity. 1 ml of the supernatant of bacteria was taken and placed in a test tube with 50 mg of fat-free cotton wool, and 1 ml of 0.2 M acetate buffer pH 5.5 was added. The reaction mixture was incubated at 50°C for 1 hour. The amount of reducing sugars was determined by the Somoji-Nelson method. 1 ml of Somoji solution was added to the incubation mixture and boiled for 20 min on a water bath, quickly cooled by immersion in cold water, and 1 ml of Nelson's solution was added. After the addition of Nelson's reagent, the mixture was shaken and the solution was made up to 25 ml in a volumetric flask with distilled water. The optical density of the samples was measured on a Shimadzu UV-1800 spectrophotometer at a wavelength $\lambda = 610$ nm. When constructing a calibration curve for determining the concentration of glucose in a glucose solution, D (optical density) values are entered on the X-axis, and concentration values are entered on the Y-axis.

Evolutionary relationships of taxa

The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown above the branches. The tree is drawn to scale, with

branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 12 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1588 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar R. *et al.*, 2018).

Determining the amount of total protein produced by isolated bacterial strains

The amount of total protein in the supernatant of a suspension of isolated bacterial strains at a concentration of 10^{8-9} spores/ml was determined by the Lowry method. The determination of total protein was carried out as follows. After the solution flowed out of the column, 2 ml of liquid was taken from each obtained fraction with a graduated pipette.

The supernatant was transferred into previously prepared test tubes, 1 ml of reagent B was added, mixed, and left at room temperature for 10 min. After 10 minutes, 0.1 ml of Folin's reagent was added to the tube. After 30 minutes, the yellow colour of the liquid turned blue. After 30 minutes, the colour intensity (blue) was determined on a spectrophotometer at a wavelength of 750 nm. All experiments were performed in triplicate, and the mean value and standard error (SE) of the values were calculated in Microsoft Excel (Microsoft Corporation, USA). A significant difference between the obtained results and the control value at $P \leq 0.05$ was analyzed using the ANOVA program.

Results

Bacteria isolated from the gastric juice of small ruminants and the stomachs of rabbits and chickens

Samples were taken from goat, rabbit and chicken stomachs, stomachs, gastric juice and large stomachs. The samples were also taken of Bark beetles, which feed on cellulose and live in the bark of trees, and termites, which cause great damage to buildings and ancient monuments. (Fig. 1). At the next stage of the study, it was purified with 3% hydrogen peroxide, alcohol, and distilled water. Purified samples were dissected in the laboratory using crushed glass and cultured on sterile prepared meat-peptone agar media.



Figure 1. Isolation of microorganisms from the gastric juice of a goat, rabbit and stomach From rabbit gastric juice 3 pieces; From chicken stomach 1 piece; From chicken crop 2 pieces; From goat stomach juice 2 pieces; From the large belly of a goat 1 piece; From termites 3 pieces; From Bark beetle 2 pieces; A total of 14 pure bacterial isolates were taken.

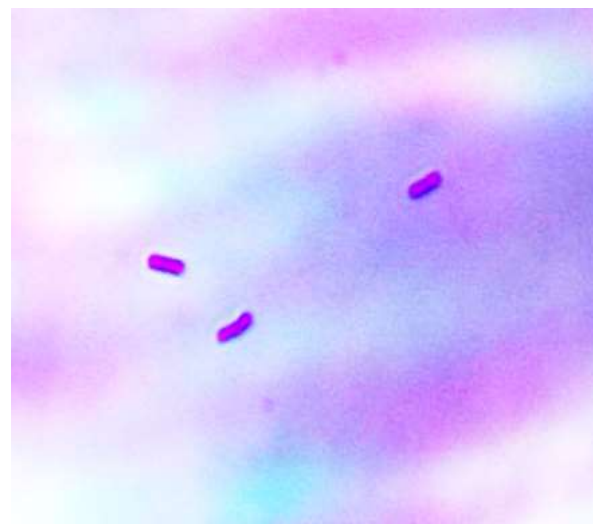
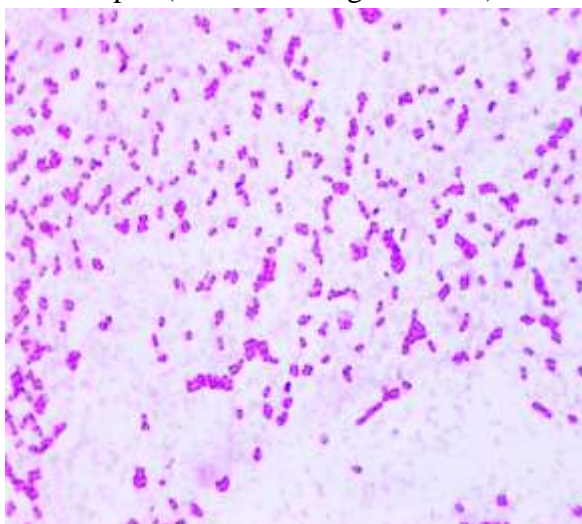


Figure 2. Isolation of pure cultures of microorganisms isolated from gastric juice and stomachs of animals

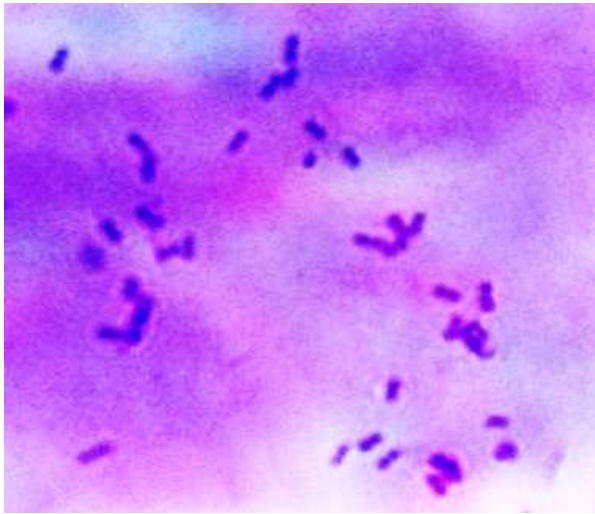
In order to isolate pure isolates from isolated colonies of microorganisms, the recultivation method was used by generally accepted in microbiology. In the course of the study, the morphological characteristics of isolated isolates of microorganisms were studied.

Definition and identification of morphological features of spore-forming bacteria

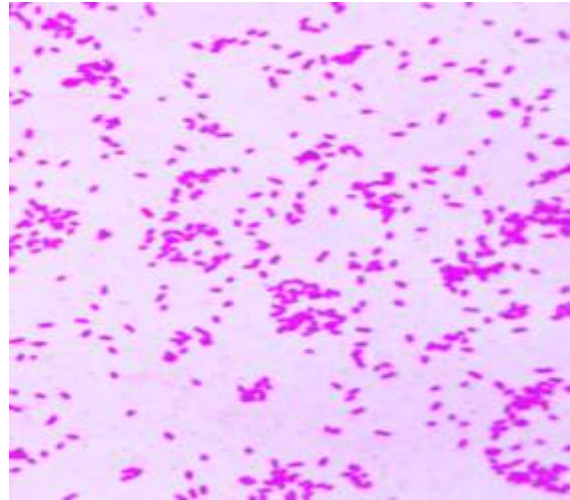
Pure bacterial isolates were incubated on MRS and MPA (meat peptone agar) media (Hi Media, India) at 35-37°C. A suspension of isolates grown for 3 days (in a test tube) at a concentration of 10^6 spores/mL was used as an inoculum. The microscopic structure of the isolated bacterial strains was studied using XSP-136 B and OLYMPUS BX41 light microscopes (400 times magnification).



Bacterial isolate isolated from termite



Bacterial isolate isolated from the Borax beetle



Bacterial isolate isolated from rabbit gastric juice



Bacterial isolate isolated from goat gastric juice



Bacterial isolate isolated from goat rumen

Bacterial isolate isolated from goat gastric juice

Figure 3. Microscopic view of pure bacterial isolates

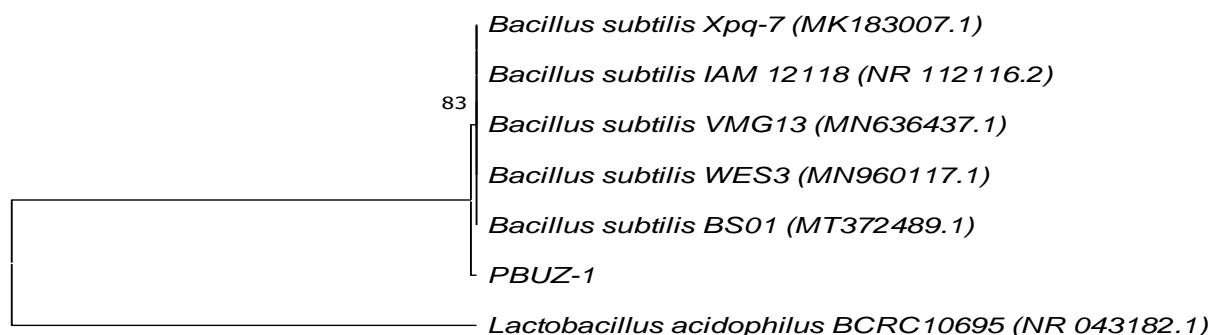
The bacterial isolate which is isolated from termite morphologically belongs to the group of rod-shaped bacteria. A gram-positive bacterium with a rod thickness of 0.7 (μ , μ) and a length of 2-8 (μ , μ) with a colourless and flat appearance. A spore-forming aerobic or facultative anaerobic bacterium. These features are characteristic morphological features of bacteria belonging to the genus *Bacillus*. Therefore, this isolate is identified as *Bacillus sp.* A *Bacillus* isolate has also been isolated from the Bark beetle. Spore-forming aerobic or facultative anaerobic Gram-positive bacteria. Only one sporulation was observed from each cell. The resistance of spores to various external influences was noted. *Bacillus* thickness 0.9 (μ , μ), length 2-8 (μ , μ), was identified as *Bacillus sp.*

Similar morphological features were found in a bacterial isolate isolated from rabbit gastric juice. A strain of Gram-positive bacteria of spore-forming, rod-shaped form. *Bacillus* thickness was 0.9 (μ , μ), length was 2-6 (μ , μ), and was identified as *Bacillus sp.*

It was revealed that the isolate of microorganism isolated from goat gastric juice is a strain of gram-positive bacteria producing rod-shaped spores 0.5 (μ , μ) thick and 2-5 (μ , μ) long, and was identified as *Bacillus sp.* It has been established that the cells of the bacterial isolate, which is isolated from the large stomach of a goat are round sticks with straight ends, single or paired, sometimes forming a chain. The thickness of the stick can be up to 0.5-1.5 (μ , μ), and the length - up to 3-6 (μ , μ). It was found that it forms spores that are resistant to external influences. Based on these morphological features, *Bacillus megaterium* was identified as a bacterial strain.

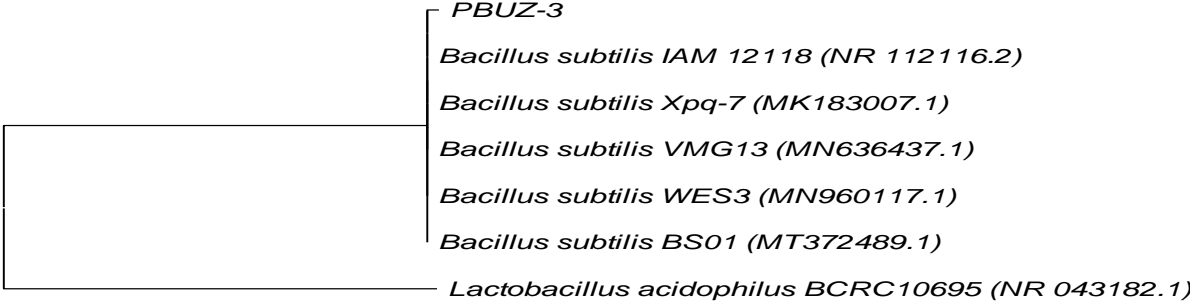
A bacterial isolate isolated from goat gastric juice was identified as a Gram-positive, aerobic or facultative anaerobic bacterial isolate 0.9-1.3 (μ , μ) thick and 1.2-1.5 (μ , μ) long, and *Bacillus pumilis* has been identified as a bacterial strain. Also, in order to achieve high accuracy in identification of bacterial strains, the isolates were identified by the method of matrix laser desorption/ionization mass spectrometry (MALDI-TOF) in the sanitary laboratory of the Ministry of Health of the Republic of Uzbekistan and according to our studies, bacterial strains of *Bacillus sp.*, *Bacillus megaterium* and *Bacillus pumilis* were identified. Also, bacterial strains belonging to the genus *Bacillus* were genetically identified and included in the NCBI database (figure 4). Bacteria belonging to the genus *Bacillus* are characterized in that their spores are generally highly resistant to environmental influences, including exposure to UV rays, desiccation, and oxidizing agents such as hydrogen peroxide. In our studies, it was found that the bacterial strain *Bacillus pumilis* is resistant to hydrogen peroxide. *Bacillus sp.* isolated from termites. the resistance of the bacterial strain to high salt concentrations was determined. These bacteria form polysaccharide chains in the cell wall. Their spore-forming properties are explained by the fact that they retain their viability under various adverse abiotic influences.

Bacillus subtilis PBUZ-1



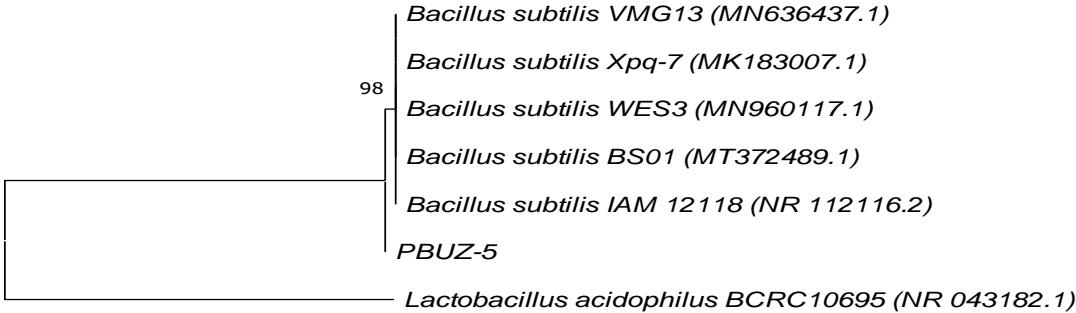
0.020

Bacillus subtilis PBUZ-3



0.020

Bacillus subtilis PBUZ-5



0.020

Bacillus subtilis PBUZ-7

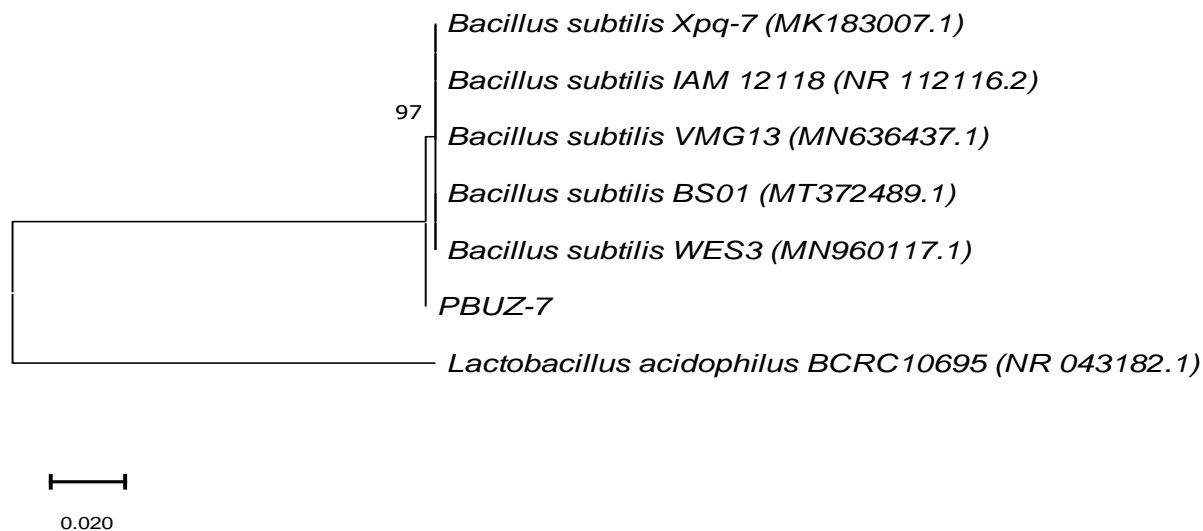


Figure 4. Phylogeny of new indigenous strains of *Bacillus subtilis*.

Determination of cellulolytic activity of selected bacterial strains

Bacterial strains belonging to the genus *Bacillus* were incubated for 2-3 days in MRS medium at 37°C. 10 ml of a suspension of bacterial strains with a concentration of 10^{8-9} spores/ml were placed in 10 sterilized test tubes, and 0.3 ml of 16% glucose solution was added to it. In the first control variant, 10 ml of distilled water was taken, in the second - 10 ml of freshly obtained goat gastric juice and 0.3 ml of 16% glucose solution was added.

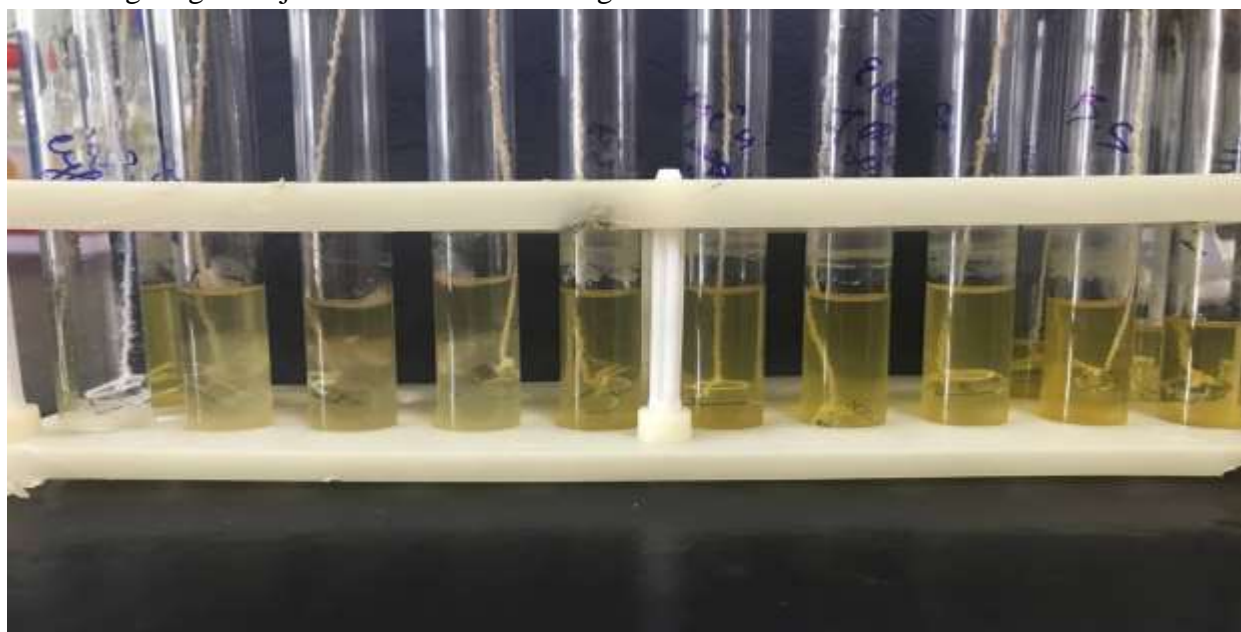


Figure 4. Determination of cellulase activity of selected bacterial strains

Glass balls were tied to the ends of the cotton thread №10 (100) to the experimental samples and 0.3 ml of 16% glucose solution was poured into 10 ml of the experimental and control

Table 1. Determination of cellulase activity of selected bacterial strains

№	Bacterial strains	Weight of string and glass ball before experiment(g)	Weight of string and glass ball after 96 hours (g)
1	<i>Bacillus sp.</i>	0,425	0,375
2	<i>Enterococcus faecalis</i>	0,300	0,265
3	<i>Bacillus sp.</i>	0,470	0,465
4	<i>Bacillus sp.</i>	0,515	0,510
5	<i>Pediococcus acidilactici</i>	0,485	0,480
6	<i>Bacillus sp.</i>	0,460	0,455
7	<i>Bacillus sp.</i>	0,445	0,440
8	<i>Bacillus sp.</i>	0,385	0,380
9	<i>Enterococcus gallinarum</i>	0,365	0,360
10	<i>Bacillus sp.</i>	0,335	0,330
11	Control (water)	0,395	0,395
12	Control (ГИБ nutrient medium)	0,380	0,380
13	Control goat gastric juice	0,380	0,380

variants. It was placed into a thermostat at a temperature of 37°C. The first result was obtained after 12 hours, and the overall result of the study after 96 hours.

According to the results of studies of *Bacillus sp.*, *Enterococcus faecalis*, *Bacillus sp.*, *Bacillus sp.*, *Pediococcus acidilactici*, *Bacillus sp.*, *Bacillus sp.*, *Bacillus sp.*, *Enterococcus gallinarum*, *Bacillus sp.* suspensions of bacterial strains at a concentration of 10⁸⁻⁹ spores/ml, the highest activity in comparison with control-1 (water), control-2 (MPB nutrient medium) and control-3 with goat gastric juice was determined in variants using *Bacillus sp.*, strains of *Enterococcus faecalis*. *Bacillus sp.* In the variant where a suspension of strains with a concentration of 10⁸⁻⁹ spores/ml was used, the initial weight of the thread was 0.425 g, but after 96 hours it turned out to be 0.375 g, i.e. 0.05 g less. A suspension of *Enterococcus faecalis* at a concentration of 10⁸⁻⁹ spores/ml, initially had a thread weight of 0.300 g, after 96 hours it was 0.265 g and, as it was found, decreased to 0.035 g. In the variants of control-1 (water), control-2 (nutrient medium MPB) and control-3 with goat gastric juice, there was no decrease in thread weight (Table 1). The cellulolytic activity was assessed by the reduction in fiber mass.

Screening of bacterial strains with cellulolytic activity

The selected strains of bacteria peptone-10g, (CM-Cellulose)-10g, K₂ HPO₄ -2 g, MgSO₄ - 0,3 g, (NH₃)₂ SO₄ -2,5 g, gelatin-2g, agar-15g, grown under pH conditions of 6.8-7.2 nutrient medium in a thermostat at 37°C for 72 hours. A suspension at a concentration of 10⁶ spores/ml was used as an inoculum, which was incubated in MPC nutrient medium for 24 hours. 0.1 ml of a suspension with a concentration of 10⁶ spores/ml was taken, sown in drops on an agar medium, and left in a thermostat at 37°C for 72 hours. The formation of a clear zone of hydrolysis around bacterial strains seeded on nutrient media indicates the degradation of colony cellulose. The clear zone was measured from the diameter to the diameter of the bacterial colony. To select the bacterial strain with the highest cellulose activity, the bacterial strain producing the zone of the highest capacity was determined.

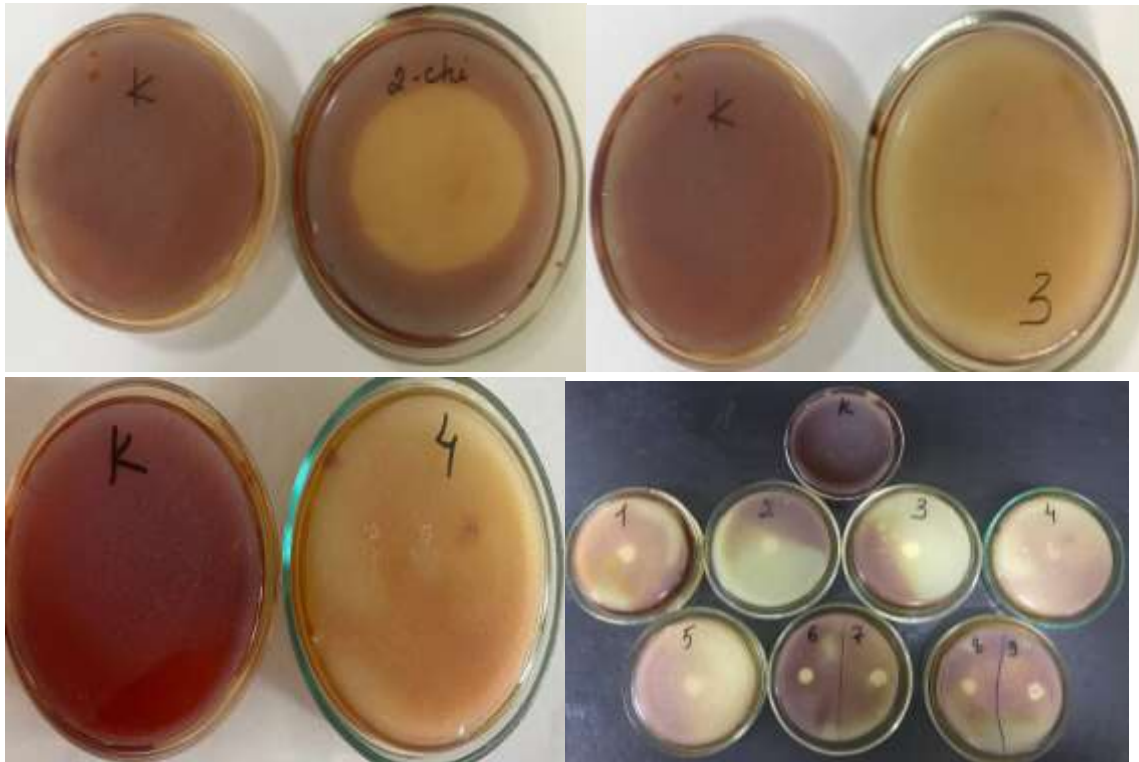


Figure 5. Identification of bacterial strains with cellulolytic activity using iodine solution

It was found that the cellulose activity of the isolated bacterial strains forms the highest zones in 4 strains. It was noted that the cellulose activity of bacteria of strains 6-7 and 8-9 was somewhat lower and formed small zones (Fig. 5).

Bacillus sp. isolated from termites and goat gastric juice strains showed the highest activity and formed rings ranging in size from 21 to 43 mm. It was also found that some strains of bacteria form rings with a diameter of 0.15 mm and 0.21 mm.

Determination of the activity of cellulose enzymes in selected bacterial strains

Cellulose enzyme activity was determined by the modified method of Mendel and Weber. Bacterial strains for determining the activity of cellulose enzymes Sodium citrate - 1.29 g/l; $(\text{NH}_4)_2 \text{HPO}_4$ -4,75 g/l; $\text{KH}_2 \text{PO}_4$ -9,6 g/l; $\text{MgSO}_4 \cdot 7\text{H}_2 \text{O}$ -0.18 g/l, grown for 2 days on a nutrient medium. 0.5% wheat bran and 0.5% carboxymethyl cellulose (SM-cellulose) were used as carbohydrate sources. Separate culture media were prepared for each source of carbohydrates. For the study, the cultured bacterial liquid was filtered on the 2nd day.

The cotton fiber was used as a substrate for the determination of exoglycanase activity. 1 ml of the supernatant of the bacterial liquid was taken and placed in a test tube with 50 mg of fat-free cotton wool, 1 ml of 0.2 M acetate buffer pH 5.5 was added. The reaction mixture was incubated at 50°C for 1 hour. The amount of reducing sugars was determined by the Somoji-Nelson method. 1 ml of Somoji solution was added to the incubation mixture and boiled on a water bath for 20 min, cooling by immersion in cold water. 1 ml of Nelson's solution was added.

After the addition of Nelson's reagent, the mixture was shaken and the solution was made up to 25 ml in a volumetric flask with distilled water. The optical density of the samples was

measured on a Shimadzu UV-1800 spectrophotometer at a wavelength $\lambda = 610$ nm. When constructing a calibration curve for determining the concentration of glucose in a glucose solution, D values (optical density) are entered along the X axis, and concentration values are entered along the Y axis. A study was carried out based on the quantitative determination of reducing (reducing) sugars formed as a result of the action of the cellulase enzyme on the substrate of the sodium salt of carboxymethylcellulose (Na-CMC) at a temperature of 50 °C. The determination of the amount of reducing sugars was carried out according to the Somogyi-Nelson method.

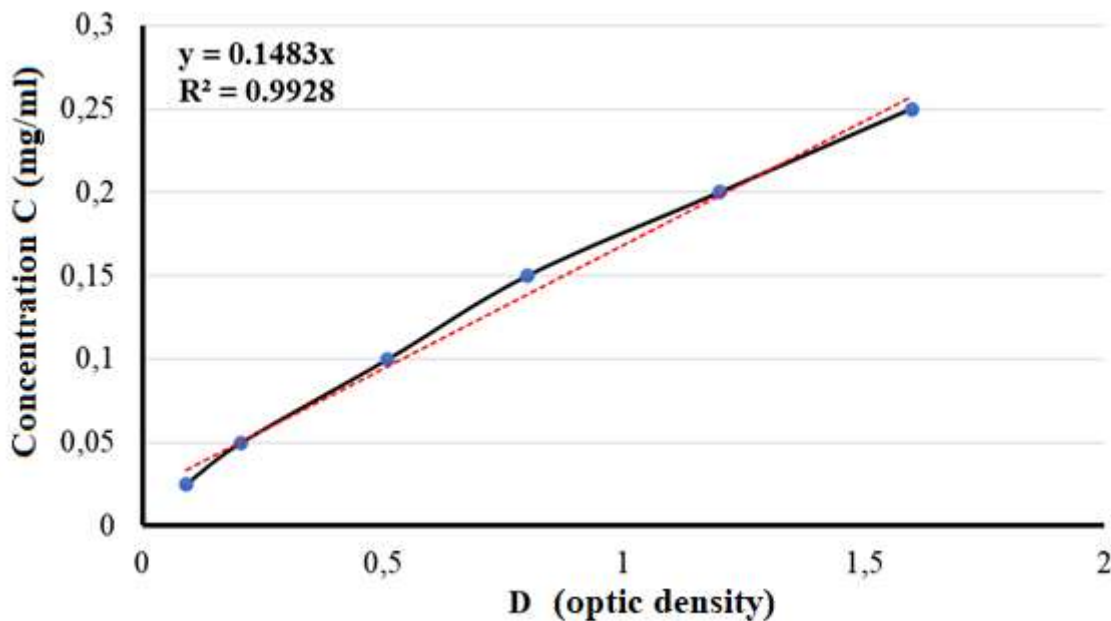


Figure 6. Calibration curve for the determination of reducing sugars

Calculations were carried out according to the calibration curve. When creating a calibration curve, D (optical density) values were entered on the X-axis and concentration values were entered on the Y-axis. The optical density of the samples was measured on a Shimadzu UV-1800 spectrophotometer at a wavelength $\lambda = 610$ nm.

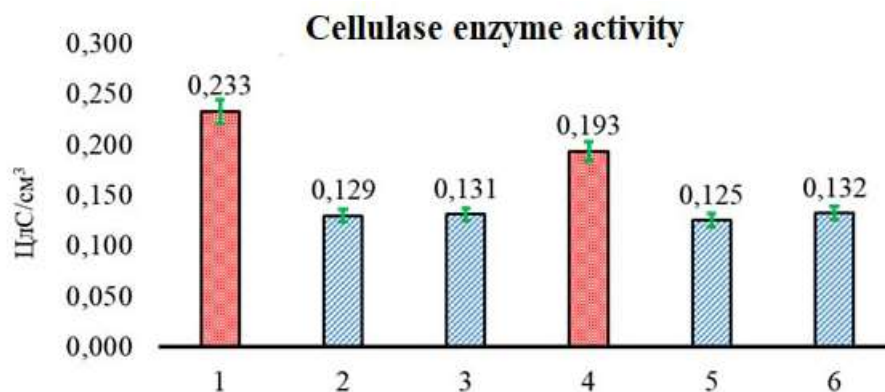


Figure 7. Cellulase activity of bacterial strains

During the studies, when the cellulosic activity of bacterial strains was determined based on the change in the value of the optical density based on the Lambert-Bouger-Ber conunity by the Shomody-Nelson method, 1-*Bacillus*. sp. Which isolated from a termite. sp. and isolated from goat gastric juice 4- *B. subtilis* sp. the highest activity was found in strains. It was 0.233 and 0.193 ClC/cm³. Positive results were obtained on the basis of catalase and gelatinase tests of 2 strains. The results obtained were processed using the Excel program. Arithmetic mean (M), standard deviation ($\pm m$) and statistical significance (R) were studied. Results less than $R < 0.05$ were considered statistically significant.

The amount of total protein produced by isolated bacterial strains

Bacterial strains selected to determine the activity of cellulose enzymes Sodium citrate - 1.29 g/l; $(\text{NH}_4)_2 \text{HPO}_4$ - 4,75 g/l; $\text{KH}_2 \text{PO}_4$ - 9,6 g/l; $\text{MgSO}_4 \cdot 7\text{H}_2 \text{O}$ - 0,18 g/l, were grown for 2 days on a nutrient medium. Wheat bran 0,5% was used as carbohydrate source. For research, the culture bacterial liquid was filtered on the 2nd day.

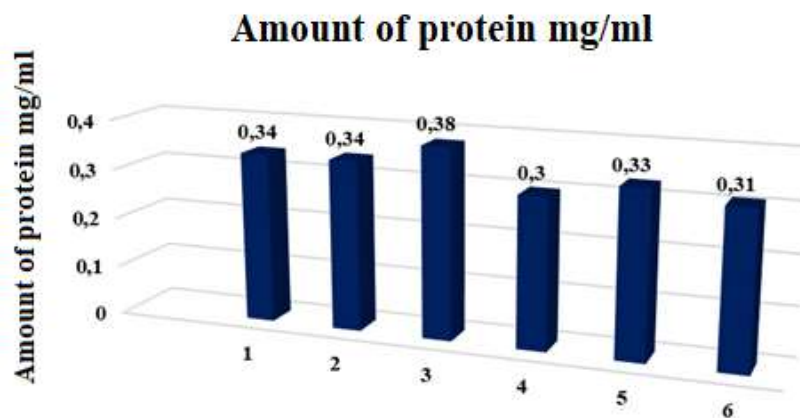


Figure 8. The amount of protein produced by selected bacterial strains

The determination of the total protein is based on the reaction of proteins with copper (II) salts in an alkaline solution and staining with a phosphorus-molybdenum-tungsten reagent (Folin's reagent) with the formation of colored products. Reagent C was added to the bacterial supernatant prepared for the study and mixed well. Left at room temperature for 10 minutes. Then 0.5 ml of Folin's reagent was added. After 30 minutes, it was detected by a spectrometer at a wavelength of 750 nm. The molding curve has been prepared.

1-*Bacillus*. sp., 2- *Bacillus*. sp. strains which isolated from termites and bark beetles produce active protein. 1-*Bacillus*. sp., and 2- *Bacillus*. sp. the bacterial strains were found to produce 0.34 mg/ml of protein. The highest activity was isolated from the gastric juice of a goat 3- *B. subtilis* sp. strain and was 0.38 mg/ml.

Discussion

Bacterial strains with cellulolytic activity were isolated from the gastric juices of goats fed on yogurt, chicken stomachs, termites and bark beetles (*Dendroctonus ponderosae*) and termites (*Coptotermes formosanus*). Cellulolytic activity was determined by reducing the mass of 100% cotton thread and forming bright rings in the nutrient medium (cellulose Congo-Red

agar). In the research of Mohamad Syazwan Ngalimat and others, the cellulolytic activity of bacteria was studied and the highest activity was determined in the isolates of *Bacillus subtilis*, *Bacillus licheniformis* at a temperature of 32°C. Bacterial strains isolated in our research were incubated in a thermostat at a temperature of 37 °C and cellulolytic activity was determined. Based on the morphological characteristics of each isolated strain, it was determined that they are spore-forming rod-shaped aerobic or facultative anaerobic, Gram-positive bacterial strains. Also, the cellulase-producing *Bacillus amyloliquefaciens* NBRC15535 strain was isolated in the experiments. The strain is gram-positive aerobic, the optimal growth temperature is 37°C, pH 7.0, and the optimal NaCl concentration is 2.0%. The strain was found to have endoglucanase, exoglucanase, beta-glucosidase and cellulase activities (Cheng Fan. *et al.*, 2018). In line with our research, Seo J.K. and others. *Bacillus licheniformis* bacterial strain was isolated from goat stomach (Seo J.K et al., 2013). Faridha Begum et al. (2013) *Paracoccus pantotrophus* bacterial strain was isolated and active cellulase synthesizing properties were determined. To determine the cellulase enzyme activity, the bacterial strains were grown in nutrient media enriched with 0.5% wheat bran and 0.5% Carboxymethyl-cellulose (SM-Cellulose) as substrate. Consistent with our research, Satheesh Kumar et al. *Bacillus* sp. used filter paper and cellulose powder as substrates to increase the cellulolytic activity of a bacterial strain. In our research, *Bacillus subtilis* PBUZ-1 bacterial strain isolated from goat gastric juice and *Bacillus subtilis* PBUZ-3 strain isolated from termites had the highest activity of 0.233 and 0.193 TsIS/cm³. The cellulolytic microbiome at different stages of the fermentation process was studied by Pan Li et al., bacterial strains belonging to the genus *Bacillus* were identified in the early stages of fermentation. Agnieszka Wita et al. *Bacillus* sp. The activity of cellulolytic enzymes - endoglucanase and β-glucosidase, synthesized in the culture fluid of the strain SV1 with high cellulolytic activity (7.89) was studied. During the research, when *Bacillus subtilis* PBUZ-3 isolated from termites and *Vacillus subtilis* PBUZ-1 isolated from goat gastric juice were inoculated into agar nutrient medium by drop method, a hydrolysis zone of 21 to 43 mm was formed around the bacterial strains and the cellulose degradation of the bacterial colonies was shown. It was also found that some bacterial strains form rings of 0.15 mm and 0.21 mm. Pratima Gupta et al. isolated bacterial isolates from termites and explained their cellulolytic activity by ring formation on cellulose Congo-Red agar medium.

Conclusion

27 pure bacterial isolates were isolated from the gastric juice of small ruminant goats, rabbits, chicken stomachs, cellulose-eating termites and tailed beetles and their cellulolytic activity was studied. Morphological characteristics of bacterial isolates were studied and identified by the method of matrix laser desorption/ionization mass spectrometry (MALDI-TOF). The primary screening of the cellulase activity of bacterial strains isolated from animals was carried out, and the cellulolytic activity of bacterial strains belonging to the genus *Bacillus* was determined. 4- *B. subtilis* sp. which is isolated from the gastric juice of a goat. and 1- *Bacillus*. sp. strains isolated from termites were found to have the highest activity and made up 0.233 and 0.193 ClC/cm³. Selected 1-*Bacillus*. sp. and 2- *Bacillus*. sp. bacterial strains produce protein up to 0.34 mg/ml, and the highest activity was isolated from the gastric juice

of goat strain 3- *B. subtilis* sp. and was 0.38 mg/ml. It serves to identify bacterial strains with cellulolytic activity isolated from the digestive system of animals, to convert roughage into an easily digestible form in animal husbandry and to enrich them with enzymes. In the studies carried out, pure strains of bacterial strains with cellulolytic activity were isolated and identified from the gastric juice of small ruminant goats, rabbits, chicken stomachs, Bark beetles and termites. *Bacillus* bacteria were screened for cellulase activity. The morphological characteristics of bacterial strains with high cellulase activity were studied. In our research, the determination of the cellulolytic activity of microorganisms isolated from ruminants and termites, which feed on roughage cellulose, serves to create a new type of microbiological enzyme feed additives that are involved in the easy digestion of roughage in the veterinary field.

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