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Research Article

CELLULOLYTIC ACTIVITY OF BACILLUS SUBTILIS STRAINS OBTAINED FROM DOMESTIC ANIMALS

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ABSTRACT

Ruminants have a varied population of bacteria, fungus, and protozoa in their rumens. Ruminants may digest plant nutrients thanks to a mechanism of symbiosis with microbes. Due to their high cost, many commercial enzymes for the bioconversion of cellulose-containing materials into simple, easily digestible sugars are hard to come by, which limits their usefulness. Finding bacterial strains that can produce different hydrolytic enzymes - among which the complex of cellulolytic enzymes is of special interest - seems pertinent in this regard.

In the course of research, more than 20 strains of bacteria have been found from the rumen of domestic animals. To identify producers of cellulolytic enzymes, we screened obtained aerobic spore-forming bacteria of the genus Bacillus. As a result, we discovered that 6 strains of Bacillus subtilis have the ability to degrade carboxymethylcellulose.

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KEYWORDS

Microorganisms, bacilli, cellulose, cellulolytic activeness, enzyme, carboxymethylcellulose, glucose, endoglucanases.

INTRODUCTION

To date, a lot of progress has been made in the field of animal husbandry in breeding highly productive breeds, including the development of enriched feed rations and the breeding of highly productive breeds. Nevertheless, there are numerous issues with increasing productivity that stem from the physiological and biological traits of animals. The management of comprehensive high-quality nutrition for farm animals contributes to an increase in the security and output of these animals. There are numerous symbiotic bacteria in the rumen and caecum that work to break down fiber into substances that are simple to digest in the digestive tract. For every milliliter of rumen fluid, there are 1010 different species of rumen bacteria. Up to 50% of the rumen's total microbial biomass can be found in their volume. Bacteria account for 75–80% of the metabolizable protein in ruminants, making them a significant source of microbial protein. Enzymes that break down fiber (cellulose, hemicellulose), starch, and sugars are also produced by bacteria. In comparison to bacteria, ciliary protozoa (ciliates) are bigger. Additionally, even though they make up no more than 106 organisms per milliliter of ruminal fluid, their overall volume can account for up to 50% of the microbial biomass. Cellulolytic and hemicellulolytic protozoa consume plant particles, which is how protozoa affect cicatricial digestion. Some species digest starch (slower than bacteria). Many protozoa absorb lactic acid, lowering the possibility of acidosis. Some species can utilize oxygen, which encourages the development of anaerobic conditions.

The majority of ciliary protozoa are adept at degrading proteins and releasing ammonia. Protozoan cells are essential for the stability of the rumen microbial population since they can consume thousands of bacteria every hour. Rumen fungi are exclusively anaerobic and make up up to 8–10% of the total microbial biomass. Because they include filamentous rhizoids that enter plant tissues, they are crucial in the initial breakdown of cellulose. Additionally, fungi have a high enzyme activity with regard to fiber, allowing bacteria access to plant tissues that are easier to digest and assisting in the release of polysaccharides linked to lignin [1;2].

The creation of probiotic feed additives is a potential area of microbial biotechnology. In connection with the issues with Russian feed production, probiotics with cellulolytic activities are receiving a lot of attention. Since the country's feed raw materials have undergone substantial changes recently, indigestible and low-calorie components (bran, rye, oats, and barley) have been compelled to be added to feed. The substitution of vegetable protein (soybean meal, corn gluten) for meat and bone meal, which is used in feed for farm animals but contains fiber contaminants, is of utmost importance. Since fiber has a considerable impact on how well dietary nutrients are utilized by animals, this presents the issue of boosting its absorption and results in an increase in the proportion of indigestible fiber in feed. The buildup of fiber-rich plant waste (beer grains, different types of meal, bagasse, etc.) leads to efforts to utilize them by including them in the diet of farm animals, which also

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forces the creation of medications that promote fiber digestion. To obtain cellulolytic and other symbiotic bacteria involved in digestion from herbivorous animals with high levels of fiber digestibility, as well as to develop a biotechnology for the commercial production and use of such microorganisms, it is important to study the internal food chains of these animals. Oats, rye, barley, wheat, and other cereal grains are frequently used in the manufacturing of animal feed. Non-starch polysaccharides (NPS), which are present in several food sources, have a negative impact on feed digestion. When soluble NPS is consumed with feed, it causes the development of viscous jelly-like substances in the gastrointestinal tract of monogastric animals (poultry), which worsens the nutrients' digestibility [2, 3, 4]. In this aspect, cellulose and polysaccharides are difficult to assimilate, compared to feed.

To optimize digestion and fiber assimilation in ruminants, as well as for the physiological justification of their nutrition, it is crucial to research the features of the cellulolytic activity of rumen symbiotic microorganisms [10,15,17].

The primary issue is that cellulose is extremely resilient to several stimuli. In order to utilize cellulose and, first of all, crop waste that contains cellulose, biotechnological methods are being developed. New strains of microorganisms with a higher level of cellulase production are therefore always being sought out. Without the hunt for new strains expressing cellulolytic enzymes, such discoveries are not possible [16, 18]. Finding microorganisms with cellulolytic activity from the rumen of some domestic animals is the goal of our research (rabbits, domestic goats and birds). Therefore, the goal of this work is to obtain microorganisms and bacteria from animal rumens and to choose strains with cellulolytic activity.

More than 20 isolates of various bacteria have been obtained from animals. The list of microbes under study did not include opportunistic bacteria. The genus Bacillus contains the majority of all isolated microbes. The chosen strains were recognized as being Bacillus subtilis, Bacillus megaterium, and Bacillus pumilis species representatives. A Brucker MicroFlex LR MALDI-TOF mass spectrometer and the customized program Maldi Biotyper (Brucker) were used to identify the species of bacteria.

Cellulolytic bacteria of the genus Bacillus, which form a vital component of the ecosystem and a crucial link in the natural carbon cycle, are of great interest. In this context, investigating the potential for their use as a foundation for finding a new cellulolytic enzyme production seems interesting.

Research methods. We used strains of microorganisms capable of biodegrading cellulose isolated (obtained) from the rumen of domestic animals.

The MPB (meat-peptone broth) medium, which contains meat extract, dry enzymatic peptone, and sodium chloride, was used to cultivate bacteria in flasks on rocking chairs for two days at a temperature of 37°C. As a carbon source, 0.5% sodium salt of carboxymethyl cellulose (Na-CMC) was added. The generation and activity of the cellulase complex's enzymes were evaluated by how they affected endoglucanases on Na-CMC. Two levels of bacterial activity screening were completed. Various bacterial species were directly selected from their inoculations on the surface of an agar medium using a variety of cellulose-containing substrates as a carbon source in the first stage. After staining the plates with Congo red dye, the diameter of the color clearing zones surrounding the growing colonies was used to determine the activity of the enzymes produced by the cultures [11, 13]. The capacity of first screening strains to hydrolyze soluble, medium

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viscosity carboxymethyl cellulose served as a measure of their enzyme activity. Amount of All Reducing Sugars Calculated (RR) The Somodi-Nelson methods and the approach using 3,4-dinitrosalicylic acid (DPS) have found the most widespread application among the many methods for quantitative detection of RR. The Somogyi-Nelson approach was used in our research. [5,7,14].

Results and discussion.

On the basis of the Department of Animal Physiology at Samarkand University, strains of microorganisms that show promise for use as a source of cellulolytic enzymes were isolated from the rumen of domestic animals. Microorganism cultures were isolated by inoculation as soon as possible from the materials that were provided. The storage media for the isolation of microorganisms was a liquid medium. The strains were chosen based on how well they could break down CM cellulose. 10 bacterial strains with cellulase activity were as a consequence discovered. According to the Bergey's determinant [13] and the information gathered from the study of the physiological and biochemical characteristics of isolated cellulolytic microorganisms [12], 6 strains were chosen for further study and identified as members of the genus Bacillus (gram-positive straight rods that form endospores, motile, aerobes or facultative anaerobes, and 2 catalase-positive cultures).

When cultures were sown on the surface of appropriate composition agar media, the selection of spore-forming bacterial strains as potential producers of cellulases revealed that cultures that are able to form active cellulases produced clear zones around the colonies, which were clearly visible after staining with the dye (Fig. 3).

Fig.1. Obtained isolates of microorganisms from animal rumen

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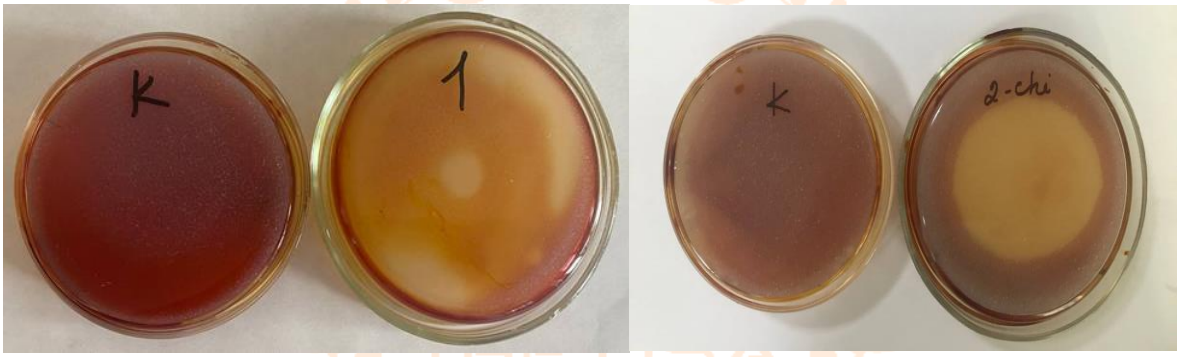
Fig.2. Obtained cultures of bacteria of the genus Bacillus subtilis



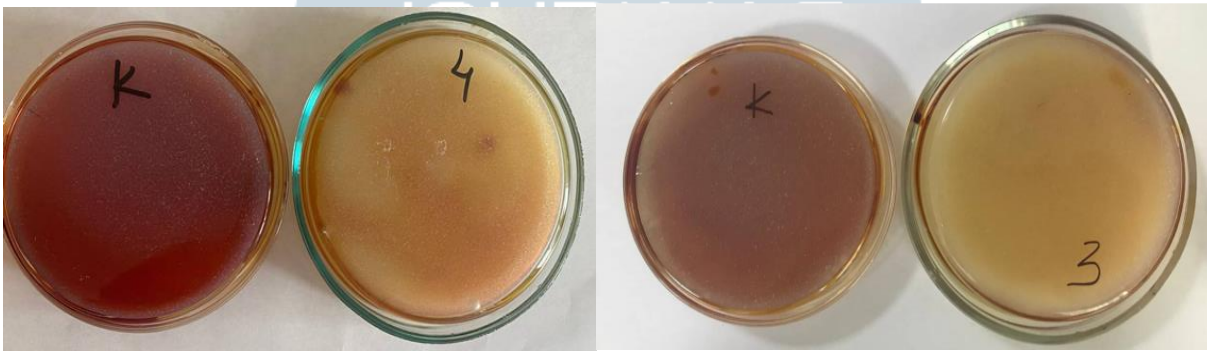


Fig. 3. Zones of hydrolysis of Na-CMC by cultures of bacilli.

C (control) - no zone, plates with clarification show cellulose hydrolysis.

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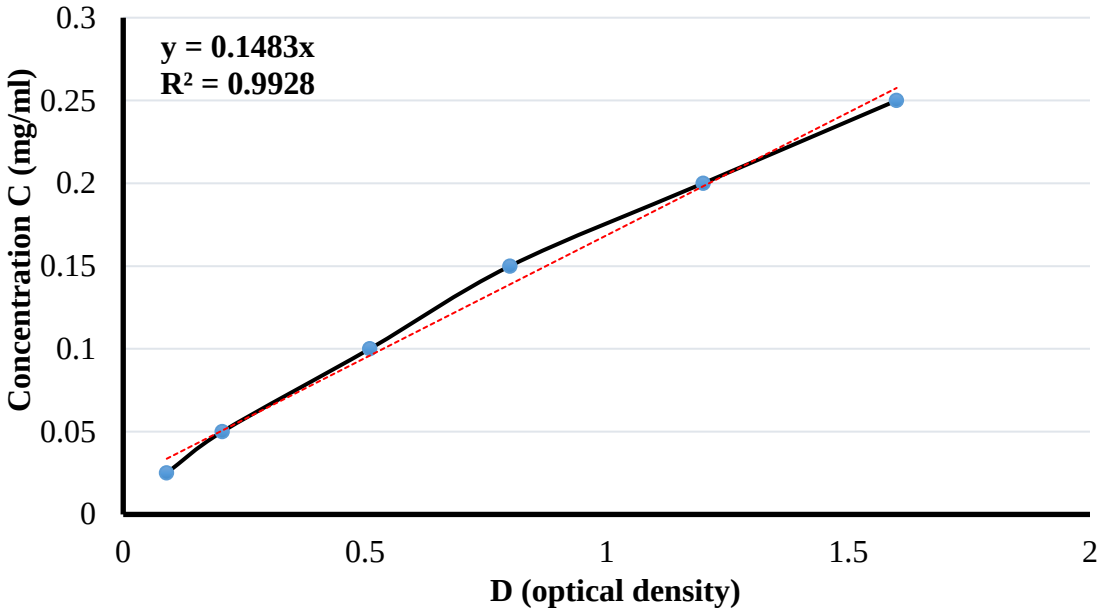
Primary screening showed that in most of the studied strains, by hydrolyzing soluble CMC, they have the ability to form a complex of extracellular cellulases. Thus, strains of the species Bacillus subtilis, Bacillus megaterium, Bacillus pumilis had the ability to form extracellular enzymes that cleave Na-CMC.

Cellulase activity was determined by a calorimetric method based on the determination of reducing sugars (RS) formed by the action of enzymes of the cellulolytic complex on the substrate, Na-CMC. The method is based on the quantitative determination of reducing sugars formed as a result of the action of the cellulase enzyme on the substrate of the sodium salt of carboxymethyl cellulose (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 [6-9].



Calibration curve for determining the amount of reducing sugars

Calculations are performed according to the calibration curve. D (absorbance) values are entered on the x-axis and concentration values are entered on the y-axis when building a calibration curve. On a Shimadzu UV-1800 spectrophotometer, the samples' absorbance was determined at a wavelength of A = 610 nm. The Excel application was used to process the results. We investigated the arithmetic mean (M), standard deviation (m), and statistical significance (R). Results were deemed statistically significant if R 0.05.

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Samples №	Cellulase activity (ClS/cm3)
1	0.233±0.0025
2	0.129±0.0015
3	0.131±0.017
4	0.193±0.0018
5	0.125±0.0040
6	0.132±0.0021

Findings. The first screening stage showed that most of the studied strains hydrolyze soluble CMC. The majority of the bacilli strains under investigation produced endoglucanase. The maximum endoglucanase synthesis was found in cultures No. 1 and No. 4, which were isolated from the rumens of a domestic goat and a rabbit. Both 0.2330.0025 (ClS/cm3) and 0.1930.0018 (ClS/cm3) of cellulase activity were present. The examined sample's cellulase capacity (ClS) is estimated in units of ClS/g or ClS/cm3.

As a consequence, 6 strains of Bacillus bacteria from the families Bacillus subtilis, Bacillus megaterium, and Bacillus pumilis were screened. Endoglucanase-cellulolytically active strains have been identified and researched. The chosen strains can be used in future biotechnological studies to create biological preparations that are used as feed additives for farm animals and to treat organic plant waste. The potential probiotic qualities of the isolated microorganisms—the native microflora of animals-will be researched and analyzed in later investigations. Additionally, it will make it possible to use novel probiotic strains as cost-efficient, efficient topical biologics for agricultural animals.

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