Cellulase activity of a strain of Bacillus subtilis isolated from termite Anacanthotermes turkestanicus

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Abstract. Termites are the most primitive insects. They play an important role in the degradation of cellulose materials in nature. Their main food is cellulose contained in wood, grass and tree leaves, so termites can cause economic damage, damaging wooden structures and woody species. Particular attention is paid to the activity of thermite cellulases. The purpose of the scientific research is to identify and study the cellulolytic enzymatic activity of bacteria isolated from local termites Anacanthotermes turkestanicus**.**

Keywords: Anacanthotermes turkestanicus, cellulose, cellulase, endoglucanases, Bacillus subtilis.

Termites (Isoptera) are herbivorous insects. Termites do not have deadly poisons, but, nevertheless, they carry a certain danger to humans: eating wood, they cause irreparable damage to buildings; their bites are quite painful; a bite can cause a severe allergic reaction. Their highly developed social organization is based on the various functions of the three main castes - producers, soldiers and workers. Most termites are found in the tropics, although they are also found in areas with a temperate warm climate. The damage caused by them is significant in tropical and warm temperate regions, although it is also observed in southern Canada, central France, Korea and Japan [1;2].

Termites were first discovered in Uzbekistan at the beginning of the last century at a depth of 16 meters. When people began to actively develop the land for arable land, then the deep inhabitants, fleeing from the rising groundwater, rose to the surface. This is where the problems started for the people. Termites cause serious damage to wooden structures of cultural and historical monuments, strategic facilities, hydraulic structures, settlements and administrative buildings. One termite family of 25 thousand individuals, living in 100 cm3 of volume, consumes on average up to 50 thousand cm3 of various types of cellulose per year. At the same time, all this leads to a global carbon cycle and an increase in the concentration of a greenhouse gas, carbon dioxide, in the atmosphere.

Scientists of Uzbekistan have been studying the types, structure and habits of termites for several years. It was determined that two species are dangerous in Uzbekistan - Trans-Caspian and Turkestan. According to the latest data, termites have been found in Uzbekistan in 30,000 residential buildings, 14 Uzelectroset

substations, 135 historical sites, the Termez airport and railway, and thousands of telegraph poles between Beruni and Nukus. Previously, destructive insects were found only in Surkhandarya and Kashkadarya, but now they are mastering region after region. If in 1992 3,200 infected households were identified, then in fifteen years already 20,000 [3].

Fig.1. termites (*Anacanthotermes turkestanicus)*

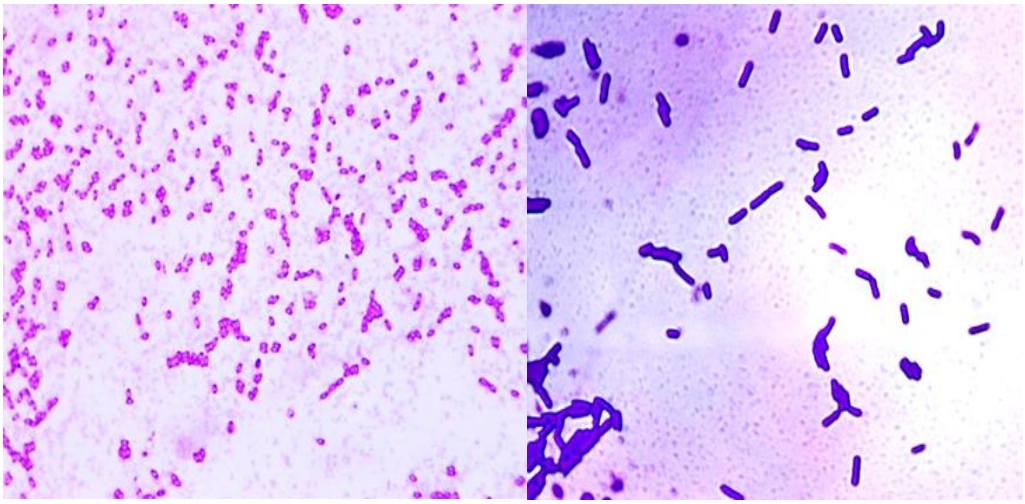
The main food of almost all termites is cellulose or its derivatives. Usually termites eat dead branches and rotting parts of tree trunks, only occasionally attacking their living tissue, although some primitive tropical species have been reported to damage tea bushes and tree stems. Representatives of the subfamily Hodotermitinae damage forage crops in Africa and Asia [4]. A number of species feed on cereals, collecting their dry shoots in the storage chambers of their underground nests or hill-shaped termite mounds. Some termites feed on dead leaves and quite a few on the humus of tropical soils. Representatives of the subfamily Macrotermitinae breed the so-called. mushroom gardens, populating their excrement or plant debris with mushroom mycelium, and then eating it. Thus, dry-wood (Kalotermitidae), wet-wood (many Rhinotermitidae), humus-eating termites (many Termitidae) and reapers (Hodotermitidae) are distinguished. A few species of termites have adapted to feeding on living plants, but most of them consume dead, more often woody, plant tissues at various stages of decomposition and can be classified as xylophages. Xylophagy, which is quite widespread among several orders of insects, is always associated with the use of different microflora. The nutrition of termites and the assimilation of wood by them is also not complete without the help of a large number of various microorganisms. These processes involve fungi, bacteria, and specialized protozoa [4;5].

In Uzbekistan, studies have been carried out to study the microflora of termite mounds in the zone of Karakalpakstan. It has been established that ammonifying,

nitrogen-fixing, yeast and cellulose-decomposing microorganisms are widely represented in termite mounds, which are distinguished by a huge variety of enzyme systems and high metabolic lability. Populating the substrate, they carry out the mineralization of protein substances, supplying insects with the necessary enzymes, biologically active substances [3;6]. Termites have their own specialized cellulose digestion system. Various cellulases are involved in the degradation of cellulose in termites and their symbionts. An important line of research is the study of the cellulase activity of termites [7;15]. Recently, the distribution of different cellulase activity in each segment of the termite gut has been mainly studied, and it has been found that the expression of endogenous cellulase genes has shifted from the salivary glands of lower termites to the midgut of higher termites [8;9]. Recent studies show that the distribution of cellulase activity in termites is related to their evolutionary levels [9;10;11]. In the studies of Nazarov K. [16], the cellulolytic enzymatic activity of termites was revealed. At the same time, the activity of enzymes from different parts of the body of termites was studied. The data obtained by them showed that suspensions prepared from different parts of the body of termites after filtration hydrolyze cellulose in the form of filter paper more than suspensions without filtration. This proves that the presence of various substances in the composition of the substrate negatively affects the biodegradation of cellulose samples (in the form of filter paper). The purpose of our research is the isolation of microorganisms from the local termites Anacanthotermes turkestanicus, their identification and detection of cellulase activity.

Materials and methods. In Uzbekistan, there is a representative of termites of the genus Anacanthotermes turkestanicus. The object of our research was termites of this genus. Termites were brought from the Khorezm region. To isolate microorganisms from termites (workers or soldiers), they were washed with pre-chilled 0.09% normal saline. Microbiological studies were performed according to the classical methods adopted in microbiology "Workshop on Microbiology" [13]. Microorganism isolates in the termite digestive tract content samples were studied by inoculation on the liquid medium of the MPB meat-peptone broth and subcultured on dense nutrient media glucose-peptone-yeast (GPD) under aerobic and microaerobic conditions (using anaerobic balloons, desiccators). Pure culture was identified by morphological and physiological-biochemical characteristics (Key to Bacteria Bergey, 2003). To identify the species of microorganisms, a Brucker MicroFlex LR MALDI-TOF mass spectrometer and specialized software Maldi Biotyper (Brucker) were used. Of great interest are cellulolytic bacteria of the genus Bacillus, which are an important link in the carbon cycle in nature and an essential part of the ecosystem. In this regard, it seems promising and interesting to study the possibility of isolating

microorganisms from termites and other woody insects and using them as a basis for obtaining cellulolytic enzymes. Cellulase activity of the Bacillus subtilis bacterium strain isolated from termites was carried out at the Institute of Microbiology of the Academy of Sciences of the Republic of Uzbekistan.



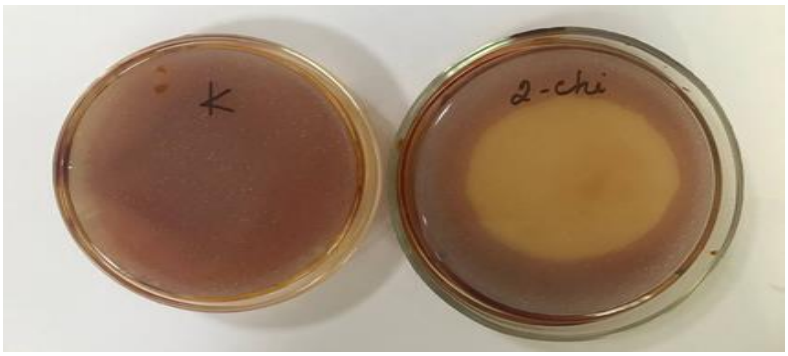
Rice. 2. Microbiological preparations of isolated strains of Bacillus subtilis from termites.

We used strains of microorganisms capable of biodegrading cellulose isolated from the thoracic-abdominal parts of termites. The cultivation of bacteria was carried out in flasks on rocking chairs for 2 days at a temperature of 37°C on the MPB (meat-peptone broth) medium containing: meat extract, dry enzymatic peptone, sodium chloride. 0.5% sodium salt of carboxymethyl cellulose (Na-CMC) was added as a carbon source. The formation and activity of enzymes of the cellulase complex was assessed by their effect on substrates: on Na-CMC - endoglucanases. Screening for bacterial activity was carried out in two stages. The first stage consisted of direct selection of cultures of various bacterial species from their inoculations on the surface of an agar medium with various cellulose-containing substrates as a carbon source. The activity of the enzymes produced by the cultures was judged by the diameter of the color clearing zones around the grown colonies after staining the plates with the Congo red dye [12;14]. Enzyme activity in strains selected as a result of primary screening was assessed by the ability to hydrolyze soluble, medium viscosity carboxymethyl cellulose. Determination of total reducing sugars (SR). The cellulase activity of the strain was determined by the formation of glucose and reducing sugars by the Somogyi–Nelson method [14]. Cellulase capacity (CLC) is calculated in the analyzed sample in units of CL C/g or CL C/cm3.

Results and its discussion. The isolated and identified strains of Bacillus subtilis from native termites are Gram-positive straight rods that form endospores, motile, aerobes or facultative anaerobes, catalase-positive. Strains of Candida krusei and

Sphingobacterium thalpopium, also isolated from termites, were excluded from the list of studied crops, as they are possibly pathogenic.

The selection of strains of spore-forming bacteria as potential producers of cellulases when cultures were sown on the surface of agar media of appropriate composition showed that cultures that are capable of forming active cellulases produced clear zones around the colonies, which were clearly visible after staining with the dye (Fig. 3). Primary screening showed that the studied strains, by hydrolyzing soluble CMC, have the ability to form a complex of extracellular cellulases. Thus, strains of Bacillus subtilis species isolated from termites had the ability to form extracellular enzymes that cleave Na-CMC.



Rice. 3. Zones of hydrolysis of Na-CMC by a bacillus culture isolated from thermite.
K (control) - no zone, plates with clarification show cellulose hydrolysis.

Studies of the synthesis of cellulase enzyme according to the Somogyi-Nelson method showed the presence of endoglucanase activity in strains No. 1 and No. 2 (table-1).

Table 1.

Samples №	Cellulase activity (ClS/cm3)
1	0.243±0.0025 (peritoneal region)
2	0.198±0.0018 (thoracic region)

Calculations were performed according to the calibration curve. When creating a calibration curve, D (absorbance) values are entered on the x-axis and concentration values are entered on the y-axis. The absorbance of the samples was measured on a Shimadzu UV-1800 spectrophotometer at a wavelength of A = 610 nm. The results obtained were processed using the Excel program. Arithmetic mean (M), standard deviation (±m) and statistical significance (R) were studied. Results less than $R<0.05$ were considered statistically significant.

Findings. Thus, as a result of the research, strains with endoglucanase-cellulose-lytic activity were isolated and studied. These are strains of bacteria of the genus Bacillus subtilis isolated from local termites Anacanthotermes turkestanicus. The synthesis of endoglucanase was possessed by cultures No. 1 and No. 2, which were

isolated from the thoracic-abdominal sections of termites. The activity of endoglucanase was 0.243±0.0025 (ClS/cm3) and 0.198 ±0.0018 (ClS/cm3). The strain of Bacillus subtilis isolated from the peritoneal region has the highest synthesis of endogluconase in comparison with the strain isolated from the thoracic region of the termite.

The selected strains can be used in further biotechnological research as part of biological preparations used for the treatment of organic, plant waste. Further studies are planned to optimize the conditions for cultivating Bacillus subtilis bacteria in order to increase cellulase activity. Toxicological studies will also be carried out to determine the safety of the isolated new strains.

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