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USE OF SOME MICROBIAL ENZYMES AND THEIR COMPOSITIONS AGAINST PHYTOPATHOGENES IN AGRICULTURAL AREAS

ИСПОЛЬЗОВАНИЕ НЕКОТОРЫХ МИКРОБНЫХ ФЕРМЕНТОВ И ИХ КОМПОЗИЦИЙ ПРОТИВ ФИТОПАТОГЕНОВ В СЕЛЬСКОХОЗЯЙСТВЕННЫХ РАЙОНАХ

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Abstract. Enzymatic hydrolysis the lignocellulose substrates (wood sawdust, wheat bran, bard), brought in a nutrient medium are given as a carbon source for cultivation of fungus and actinomycetes, producers of making components of a bio stimulator which is used in agriculture for cultivation of cotton plant. It was established that among examinees of substrat spirit grain the bard in experimentally picked up concentration (10–50%) it appeared a full–fledged substratum for the active growth developments and antibiotic enzymatic making with use to which can be prepared rather cheap and effective preparation is providing for increase in productivity and decrease in incidence of crops in agriculture.

At the current time using biological methods of cultivation and protection of environment is the most important link in cultivation of ecologically safe agricultural production. The biological methods of protection of plants is recognized as the most resource–saving reception, allowing to protect plants from diseases, wreckers and to increase their efficiency without expenses of irreplaceable natural resources and without harmful emissions in environment [1, 2]. The microorganisms used for production of valuable biological products, promote supply of plants not only elements of mineral food, but also physiologically active agents (enzymes, phytohormons, vitamins, etc.) [3].

Studying of enzymatic activity and efficiency of culture of fungus of *Pleurotus ostreatus* UZBI-I 105, *Aspergillus terreus* 9, and actinomycete *Streptomyces sp.* 166 on cheap the lignocellulose substrat for development of effective biotechnology of a biological product for

increase in productivity and protection of crops against wreckers of phytopathogenes was the purpose of the real work.

Аннотация. В работе рассматривается процесс действия микробиоты на фитопатогены. Ферментативный гидролиз лигноцеллюлозных субстратов (древесные опилки, пшеничные отруби, барды), вводимые в питательную среду, приведены в качестве источника углерода для выращивания грибов и актиномицетов, производителей компонентов биохимического средства, которые используются в сельском хозяйстве для выращивания хлопка растение. Было установлено, что среди испытуемых субстратов в экспериментально подобранной концентрации (10–50%) оказался полноценным субстратом для активных процессов роста и создания антибиотических ферментов с использованием которых можно получить довольно дешево и эффективно подготовка предусматривает увеличение производительности и снижение заболеваемости сельскохозяйственных культур в сельском хозяйстве.

Биологические методы защиты растений признаны наиболее ресурсосберегающим приемом, позволяющим защитить растения от болезней, вредителей и повысить их эффективность без затрат незаменимых природных ресурсов и без вредных выбросов в окружающую среду. Микроорганизмы, используемые для производства ценных биологических продуктов, способствуют обеспечению растений не только элементами минерального питания, но и физиологически активными средствами (ферментами, фитогормонами, витаминами и т. д.).

Изучение ферментативной активности и эффективности культуры гриба *Pleurotus ostreatus* UZBI-I 105, *Aspergillus terreus* 9 и *Streptomyces sp.* 166 лигноцеллюлозных субстратов позволяет предложить лучшие технологии защиты культур в сельском хозяйстве.

Keywords: waste materials, fungus, actinomycete, optimization, fungi cultivation, cellulolytic enzymes, phytopathogens, hydrolysis, activity, preparation, Microzym-1, Microzym-2, treatmevent wheat and cotton seeds.

Ключевые слова: отходы, грибы, актиномицеты, оптимизация, культивирование грибов, целлюлолитические ферменты, фитопатогены, гидролиз, активность, препарат, микрозим-1, микрозим-2, семена пшеницы и хлопчатника.

Materials and methods

Producers: *Pleurotus ostreatus* UZBI-I 105, *Aspergillus terreus* 9 and actinomycete *Streptomyces sp.* 166 which re provided from Institute of Microbiology of Science Academy of Uzbekistan.

Cultivation conditions: room temperature (20 °C), shake–flask propagator, under sterility.

Medium: For bottom cultivation of *Pleurotus ostreatus* UZBI-I 105, *Aspergillus terreus* 9 and actinomycete *Streptomyces* sp.166 were taken rigid on character and structure substrates, such as wood sawdust, further a xylan a containing substrate — wheat bran and bard, with inclusion of the specified substrata in structure of the modified circle of Capek's–Doks where the main carbohydrate source (sucrose) was replaced with wood sawdust and junctures (1–3%) and bard (10–50% which data are provided in the table.

Methods

Determination of cellulase activity defined at hydrolysis 1,0 solutions of the item CMS, Sigma firms (USA). Xylanase activity — determine modified by Somogyi–Nelson method in Feniksova R. This is based on hydrolysis of xylose by xylanase and determination of enzymatic

products with use of reactants 1 and 2 as it is described above where as a substrate solution of a xylan of oats of Sigma firm (USA) served 1,0%. For enzyme quantification applied the Lowry method [4].

Results and discussion

Taking into account the greatest distribution in agriculture gains enzymatic organic fertilizing biostimulator developed by using the way, containing enzymes, antibiotic substances and the phytohormones, being formed cultures of fungus of *Aspergillus terreus*, *Pleurotus ostreatus*, and also antibiotically active liquids soil actinomycete *Streptomyces sp.* 166 [5].

In order to make Microzym-1 and Microzym-2, bellowed microbial producers cultivated in three types of mediums like waste materials. For example, wood granules, wheaten bran and 40% of the bard. Every medium's details are given individually.

Apparently given to the table xylotrophic *Pleurotus ostreatus* UZBI-I 105 possesses bigger cellulase and xylanase activity, than, soil saprotrophic *Aspergillus terreus* 9 and actinomycete *Streptomyces* sp. 166. Similar data were obtained for these cultures with bran where polysaccharide wheat the xylan (hemicelluloses) prevailed before other polysaccharides (Figure 1–2).

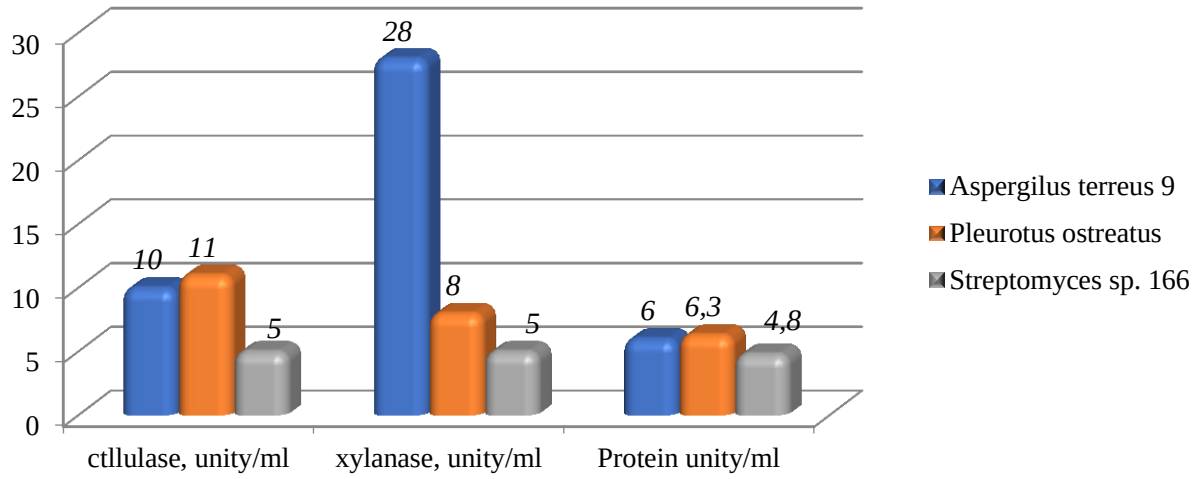

Figure 1. Formation of enzymes on the medium with wood granules (3,0%)

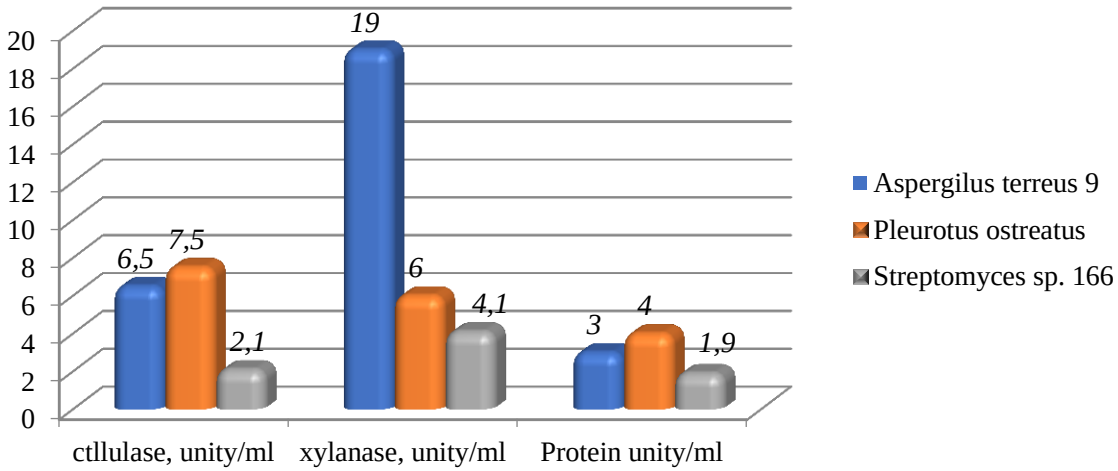

Figure 2. Formation of enzymes on the medium with wheten bran

It is obvious that, maximum quantity of cellulase and xylanase is formed with 40% of bard's medium. Moreover, synthesis of protein is showed the highest quantity with this medium than others, respectively (Figure 3).

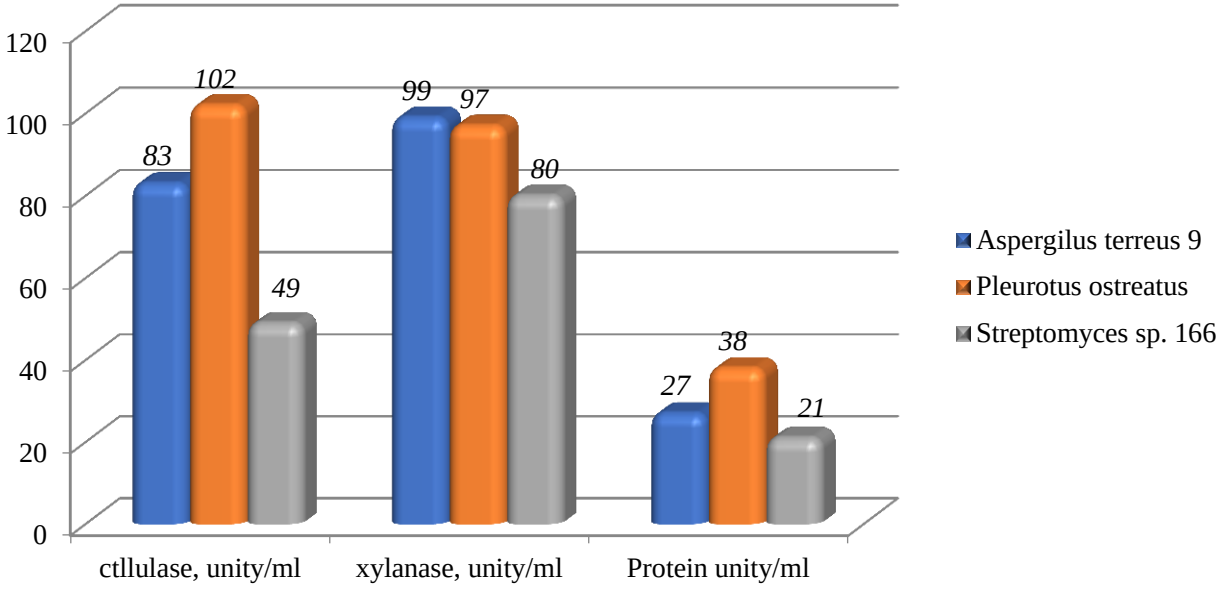

Figure 3. Formation of enzymes on the medium with bardy 40%

Thus, uses the lignocellulose of production wastes with the greatest content of cellulase and xylanase separately and in a combination in various combinations for the active growth, development and a enzymatic making showed that for examinees of three cultures it was favorable a nutrient medium with introduction of 40% grain bards. The maximum quantity of cellulase and xylanase is formed for the 10th days of growth of fungus.

Antibiotic activity of cultures (Figure 4–5) defined against widespread phytopathogenic fungus of *Fusarium oxysporum* and *Verticillium dahliae*.

1 2 3

Figure 4. Antibiotic action of culture of S*treptomyces*. sp.166 (1), *Aspergillus terreus* 9 (2) and *Pleurotus ostreatus* UZBI-I 105 (3) on growth and *Fusarium oxysporum* development

Apparently from Figure 1 actinomycete and *Aspergillus terreus* 9 possessed bigger pernicious force, than *Pleurotus ostreatus* UZBI-I 105 to what testifies zones of inhibition of growth of fungus.

1 2 3

Figure 5. Antibiotic action of culture of S*treptomyces sp.* 166 (1), *Aspergillus terreus* 9 (2) and *Pleurotus ostreatus* UZBI-I 105 (3) on growth and *Verticillium dahlia* development

Sort *Fusarium* fungus as causative agents of diseases of a set of the highest plants, cotton and the leguminous cultures, striking plants most often in field conditions and at the time of their fructification.

After application of enzyme preparations "Microzym-1" in various sowing areas of Namangan region the harvest increase equal to 10–12 centners/hectare in cultivation of such wheat varieties as "Polovchanka", "Chillaki", "Kroshka" and analogous "Microzym-2" increase equal to 4.2–5.7 centners/hectare in cultivation of such cotton varieties as Omad, Namangan-34, Namangan-77, Buchara-108 have been achieved. The enzyme preparations promote to increase of germination energy and seed germination at relatively lower temperatures, active growth and development of sprouts, stalks, also reduction of time of vegetative growth of cultivated plants and maturation of their harvest.

The norm of enzyme compositions expenditure for pre-seeding treatment of seeds of various wheat varieties comes to 30 litres/ton, for the bared cotton seeds — 30 litres/ton, for the pussy–coated cotton seeds — 35 litres/ton. The noted volumes and componental structure of enzymes can be increased, reduced and excluded depending on kinds, varieties and densities of seeds, enzyme activities and ways of processing.

Application of these enzyme technology allows to receive the additional income at a rate of 400–500 sums at the expense of each spent 100 sums. Cost of enzyme preparations and their compositions depends on their structure, activity, varieties of seeds intended for processing, and comprises in average 7.5–8.0 thousand sums/litre.

There are collection of active cultures, growing ways, optimum a condition and parameters enzyme formation, methods of isolation, clearing of enzymes both their active forms and corresponding industrial rules for wide introduction of preparations in an agriculture and various branches of manufacture.

Conclusions

Various intensity of biosynthesis of cellulase, xylanase and antibiotic substances with bran, wood sawdust or the grain bard can, will be explained by the general nonspecific influence of carbohydrates on growth and developments of microorganisms.

As a result of this experiment showed that, biotechnological companies are using from waste materials (like bard, wheaten bran and wood granules) as essential ready materials of agriculture. It enables to protect our environment and create new non-waste technologies of industry.

With use of the above optimized structure of a nutrient medium and conditions the laboratory regulations of preparation enzymatic and antibiotically active cultural liquids from examinees of cultures for creation of enzymatic composition of Microzym-1 and Microzym-2 were developed.

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