NOVATEUR PUBLICATIONS
JournalNX- A Multidisciplinary Peer Reviewed Journal
ISSN No: 2581 - 4230
VOLUME 6, ISSUE 6, June -2020

THE USE OF BACTERIAL STRAINS BELONGING TO THE RHODOCOCCUS GENERATION

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ABSTRACT:

The article provides theoretical information about the amidase enzyme, which belongs to the group of hydrolase enzymes, and its bio transformational properties. Soil cultures of actinobacteria of the genus Rhodococcus with thermostable amidase activity were studied. Gram staining showed that they belonged to a group of gram-positive bacteria. Given preliminary results of experimental work.

KEYWORDS: enzymes, exoenzymes, endoenzymes, location, Rhodococcus ruber - 8/4/1, amidase, purification, properties, substrate specificity.

INTRODUCTION:

The use of microorganisms and their enzymes for the sustainable and environmentally friendly production of various chemical compounds is one of the priority areas for the development of microbiology and biotechnology. According to experts, over the current decade, the share of biotechnological products in the total volume of chemical products will increase 10 times. Particularly fast pace (more than 15 times) will increase the production of polymers from monomers obtained using biotechnology.

All biological catalysts are substances of protein nature and are called fermentes (hereinafter referred to as F) or enzymes (E). Enzymes are not components of reactions, they only accelerate the achievement of equilibrium by increasing the rate of both direct and reverse transformations. Enzymes accelerate the most diverse reactions in the body. Thus, the reaction of the removal of water from carbonic acid with the formation of CO_2, which is quite simple from the point of view of traditional chemistry, requires the participation of an enzyme, because without it, it is too slow to regulate the pH of the blood. Due to the catalytic action of enzymes in the body, it becomes possible for such reactions to occur that would go hundreds or thousands of times slower without a catalyst. The enzyme composition of any microorganism is determined by its genome and is a fairly constant sign. The main part of the enzymes is localized in the cytoplasm. Enzymes are produced by the microbial cell itself and are divided into endoenzymes and exoenzymes according to the place they perform.

Endozymes - bacterial enzymes that act on substrates within the cell (splitting amino acids, monosugar, etc.). By appointment, exoenzymes should be divided into the following groups:

- Enzymes that ensure the fulfillment of their physiological processes associated with the growth and reproduction of microbial culture.
- Enzymes providing the microbial cell with protective properties. For example, enzymes that inactivate antibiotics.
- Pathogenicity enzymes. This group of enzymes is produced, as a rule, by pathogenic microorganisms. Enzymes are isolated that provide protection to the microbial cell against nonspecific macroorganism protection factors

(invasion enzymes - neuraminidase, hyaluronidase, collagenase), as well as enzymes that activate the work of biologically active compounds of the cells of the macroorganism and lead to its death. These are aggression enzymes (exfoliatins can modify hormones or proteases that destroy the structure of cells of an infected organism, etc.).

Exoenzymes are bacterial enzymes secreted into the environment and acting on the substrate outside the cell (proteases, polysaccharidases, oligosaccharidases). Exoenzymes play a large role in providing a bacterial cell with sources of carbon and energy accessible for penetration. Most hydrolases are exoenzymes that, released into the environment, break down large molecules of peptides, polysaccharides, lipids to monomers and dimers that can penetrate into the cell. A number of exoenzymes, for example hyaluronidase, collagenase and others, are enzymes of aggression. Some enzymes are localized in the periplasmic space of a bacterial cell. They participate in the processes of transfer of substances into the bacterial cell.

One of the most important properties of enzymes is enzyme activity. Enzyme activity - the ability to accelerate the reaction rate to varying degrees. Activity is expressed in:

1) International units of activity - (IU) the amount of enzyme that catalyzes the conversion of 1 μM substrate in 1 min.

2) Katalakh (kat) - the amount of catalyst (enzyme) capable of converting 1 mol of substrate in 1 s.

3) Specific activity - the number of units of activity (any of the above) in the test sample to the total mass of protein in this sample.

4) Less commonly used molar activity is the number of substrate molecules converted by one enzyme molecule per minute.

Activity primarily depends on temperature. The enzyme is most active at the optimum temperature. For Φ of a living organism, this value is in the range +37.0 - +39.0 ^{0}C, depending on the type of animal. As the temperature decreases, the Brownian motion slows down, the diffusion rate decreases, and therefore, the formation of the complex between the enzyme and the reaction components (substrates) is slowed down.

If the temperature rises above +40 - +50 ^{0}C, the enzyme molecule, which is a protein, undergoes a denaturation process. In this case, the rate of a chemical reaction decreases markedly. The activity of enzymes also depends on the pH of the medium. For most of them, there is a certain optimal pH value at which their activity is maximum. Since hundreds of enzymes are contained in a cell and each of them has its own limits of optimal pH, a change in pH is one of the important factors in the regulation of enzymatic activity. So, as a result of one chemical reaction with the participation of a certain enzyme, pH opt of which lies in the limits 7.0 - 7.2, a product is formed, which is an acid.

In this case, the pH value shifts to the region of 5.5 - 6.0. The activity of the enzyme decreases sharply, the rate of product formation slows down, but another enzyme is activated, for which these pH values are optimal and the product of the first reaction undergoes further chemical conversion. (Another example about pepsin and trypsin).

The biotransformation of nitriles of carboxylic acids into compatible amides using micro organisms, especially in the synthesis of acrylamide, has been studied by Japanese scientists since the 1990s and scientists from Russia, the USA, Korea, China and India in the 21st century.

Successful use of microorganisms in biocatalytic technologies depends on the properties of biocatalysts: substrate hydrolysis rate, pH and temperature optimums of enzyme activity, substrate selectivity, and optimal composition of the working medium. Different

enzymes differ significantly in these properties, so a similar characteristic of the enzyme helps to assess the prospects for the use of producers. In connection with the foregoing, the selection and study of bacteria, the active producers of amidases, is an important area of microbiological research.

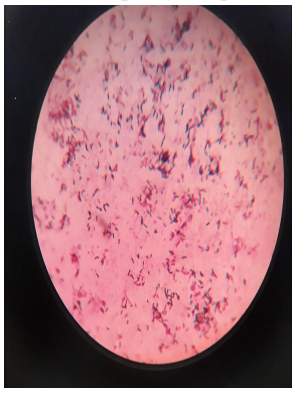
METHODS:

Objects of research - R. rhodochrous actinobacteria isolated from soils, actively transforming carboxylic acid amides. The cultures were isolated from direct seeding soil and grown on a nitrogen-free mineral salt medium N, the carbon source was glucose, and the nitrogen source NH_4Cl.

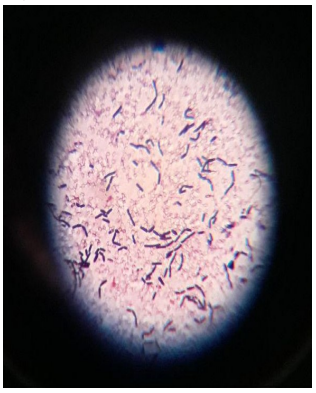
Prepared 1 l of peptone growth medium for the growth of bacteria belonging to the genus R. rhodochrous. Pour 100 ml into 250 ml volumetric flasks. $CoCl_2$ was added using a small dispenser. Sterilized at 1 ATM pressure for 17 minutes. The cell suspension was inoculated into sterilized flasks inside the laminabox using sterile pipettes. Put on a shaker at 28 °C 120 rpm. After 96 hours of bacteria growth, the pH and enzyme activity were analyzed.

RESULTS:

The strain of the bacterium Rhodococcus was stained with a Gramm method and was seen under an immersion microscope (1-2 pictures):



1-picture. Rhodococcus ruber - 8/4/1 (10-12 h.)



2-picture. Rhodococcus ruber - 8/4/1 (30-40h.)

According to morphological characteristics, the strain Rhodococcus ruber - 8/4/1 - gram-positive aerobic, immobile, not acid resistant, not spore-forming. At different stages of the life cycle, the cells of the isolated strain differed in morphology. In the initial stage of growth, the germination and branching of the initial coccoid and short rod-shaped cells begins with the formation of one to three growth tubes. At an exponential stage of development (10-12 h), cells 6.0-12.0 x 0.6-0.9 μm long with abundantly branching mycelium are detected, which are fragmented into shortened uneven rod-shaped and coccoid elements. In 30-40-hour cultures, branching cells 4.0-5.0 μm long are present, a small amount of shorter fragments. There is a three-stage morphogenetic cycle of development (cocci - rod-shaped, filiform or branching cells - cocci).

The cultural properties of the strain on standard laboratory media at a temperature of 28-30 °C, pH 6.5-7.5 form colonies of soft consistency without air mycelium with a pinkish-red color.

CONCLUSION:

Amidases (EC 3.5.1.4) - enzymes that catalyze the hydrolysis of amides with the formation of the corresponding carboxylic acids and ammonium, are involved in the metabolism of nitrogen in cells and are widespread in nature. In bacteria, the appearance of amidase activity is often associated with the metabolism of nitriles. Amidases isolated from various sources are characterized by different substrate specificity. Some of them catalyze the hydrolysis of aliphatic acid amides, others break down aromatic amide compounds, and others hydrolyze amino acid amides. Some amidases have stereoselectivity.

The amidase Rhodococcus ruber - 8/4/1 is involved in the metabolism of nitrile, which

is used as a biocatalyst in the production of acrylamide. Unlike the well-studied nitrile hydratase of this strain, the properties of amidase remain unstudied.

In this regard, the aim of our research is to study the biosynthesis of amidase with the strain Rhodococcus ruber - 8/4/1 and its use for the preparation of amides and carboxylic acids of nitriles. It was established that the strain Rhodococcus ruber - 8/4/1 showed pronounced activity against acrylonitrile, as a result of biotransformation, acrylamide and acrylic acid were found in the medium. It should be noted that acrylic acid is formed from amides as a result of amidase activity. This suggests that the strain has a nitrile hydratase-amidase pathway for nitrile metabolism.

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