BIOCHEMICAL COMPOSITION OF CULTURE FILTRATE OF THE FUNGUS *COLLETOTRICHUM LINI* - ANTHRACNOSE PATHOGEN FLAX

Proliotova N.V. Institut of Flex, Lunacharskogo, 35, 172002, Torzhok, Rassia (nataljaprljotva@rambler.ru)

Abstract: Anthracnose occurs in flax seedlings annually and everywhere. Fungus Colletotrichum lini acts on the plant by the virtue of toxins. For the development of selective media in the process of anthracnose resistant cell selection in vitro anthracnose pathogen culture filtrate is used. The diversity and specificity of the existing proteins in nature are determined by amino acid composition and genetically precisely defined sequence of amino acids in the polypeptide chain. Qualitative analysis of free amino acids revealed that there are the following free amino acids in the culture filtrate strains 419 and 639: on the 7th day culture filtrate cysteine Rf = 0,07 presented in both strains; on the 14th day serine Rf = 0,22 was defined in both strains ; on the 21st day - glutamine Rf = 0,17; on the 28th day - asparagine Rf = 0,12; on the 35th day - arginine Rf = 0,14; on the 42nd day - tyrosine Rf = 0,44.

Key words: Anthracnose, flax, culture filtrate, existing proteins, amino acid, cysteine, serine, glutamine, asparagines, arginine, tyrosine.

Introduction

Anthracnose is found in flax seedlings annually and everywhere. This disease can cause severe thinning of crops, and sometimes their complete destruction. Currently, practically all seed undergoes etching, but despite this, anthracnose flax has significant damage is a common disease and sprouting of flax. Resistant to anthracnose fiber flax varieties currently in production is not. Therefore, the use of cell selection, as a tool for rapid development of new varieties with desired traits is an important biotechnological reception.

The fungus Colletotrichum lini by acting on the plant toxins. Toxins, waste products of pathogenic organisms or remnants of the decay products of their food, are also in the culture filtrate. To create the selective media at a cell in vitro selection for resistance to the anthracnose pathogen culture filtrate used anthracnose. Currently, the literature is very little data on the biochemical composition of the culture filtrate. Therefore, the current study is the change in the chemical composition of the culture filtrates during the growth and development of the fungus Colletotrichum lini.

Material and methods

Character of accumulation of proteins in the culture filtrate was unknown to us, and such data in the literature could not be found. In this connection we have used two

methods to determine the amount of proteins with varying degrees of sensitivity. In studies using culture filtrate of the fungus Colletotrichum Lini highly virulent strain 639 and strain 419 moderately virulent.

In the first step the optical density of culture filtrates of strains 419 and 639 were determined by biuret reaction method, Lowry method and every 7 days.

Calculation of the content of proteins in the culture filtrate of strains 419 and 639 were found from the equation of the calibration curve obtained with standard solutions of the crystalline protein.

Qualitative analysis of free amino acids was carried out by a distribution ascending paper chromatography.

Results and discussion

The obtained optical density data by the method of biuret reaction showed that the change in the amount of proteins observed throughout the culture period. Moreover, the accumulation of proteins occurs up to 28 days, for strain 419 (0.243), and for the strain 639 (0.357). Subsequently, the optical density decreased and amounted to 419 strain (0.044), and for strain 639 (0.08). Data obtained from the equation of the calibration curve showed that the accumulation of proteins in the culture filtrate of the strain 639 was more intense. However, the maximum amount of proteins was observed on day 28 in the culture filtrates of both strains. Thus, on day 7, the concentration (C) of the protein in the culture filtrate of the strain 419 was 0,172 mg / 10ml, and strain 639 - C = 0,325 mg / 10ml. On day 14, the concentration (C) of the protein in the culture filtrate of the strain 419 was 0,999 mg / 10ml, strain 639 - C = 1,45 mg / 10ml. After 21 hours the concentration (C) of the protein in the culture filtrate of the strain 419 was 1.14 mg / 10ml, strain 639 - C = 1,61 mg / 10ml. After 28 hours the concentration (C) of the protein in the culture filtrate of the strain 419 was 1.22 mg / 10ml, strain 639 - C = 1,69 mg / 10ml. With further culturing the strains on the culture medium revealed that the amount of protein in the culture filtrates decreased. After 35 hours the concentration (C) of the protein in the culture filtrate of the strain 419 was 0.824 mg / 10ml, strain 639 - C = 1,17 mg / 10ml. After 42 hours the concentration (C) of the protein in the culture filtrate of the strain 419 was 0,312 mg / 10ml, strain 639 - C = 0,392 mg / 10ml.

In determining the optical density of the culture filtrates of strains 419 and 639 by the method of Lowry obtained data similar results varying the amount of protein by the method of biuret reaction. That is, the maximum amount of proteins was observed on day 28 in the culture filtrates of both strains. Subsequently - amount of protein in the culture filtrates decreased. Thus, on day 7, the concentration (C) of the protein in the culture filtrate of strain 2000 was 419 mg / 100ml, strain 639 - C = 4100 g / 100ml. On day 14, the concentration (C) of the protein in the culture filtrate of strain 6900 was 419 mg / 100ml, strain 639 - C = 12500 g / 100ml. After 21 hours the concentration (C) of the protein in the culture filtrate of strain 7680 was 419 mg / 100ml, strain 639 - C = 14000 g / 100ml. At 28 hours the concentration (C) of the protein in the culture filtrate of strain 8200 was 419 mg / 100 ml, strain 639 - C = 15,000 g / 100ml. After 35 hours the concentration (C) of the protein in the culture filtrate of strain 5600 was 419 mg / 100ml, strain 639 - C = 11300 g / 100ml. After 42 hours the concentration (C) of the

protein in the culture filtrate of strain 1400 was 419 mg / 100ml, strain 639 - C = 2600 g / 100ml. The findings suggest that the deadline to 42 daily cultivation of the fungus in a nutrient medium nutrient reserves were exhausted, and the fungus was unable to continue further growth and, as a consequence - to accumulate toxic metabolites.

The diversity and specificity of the proteins existing in nature are determined by amino acid composition and genetically precisely defined sequence of amino acids in the polypeptide chain. Qualitative analysis of free amino acids showed that in the culture filtrate of strains 419 and 639 revealed the following free amino acids: 7 days in culture filtrate of both strains present cysteine Rf = 0,07; for 14 hours in the culture filtrates of both strains defined serine Rf = 0,22; 21 hours - glutamine Rf = 0,17; 28 hours - asparagine Rf = 0,12; on day 35 - Arginine Rf = 0,14; on day 42 - tyrosine Rf = 0,44. It is known that different functional groups of the natural amino acids are widely used in the synthesis of many biologically active substances. As an example, describe the amino acids defined by us in the culture filtrate of strains 419 and 639 by the distribution ascending paper chromatography.

On day 7 the culture filtrate of strains 419 and 639 defined cysteine. Its concentration in the culture filtrate of the strain 419 was C = 2 • 10-3 mol / l, m (cysteine) = C • V • M = 1,25 • 10-4 g; in the culture filtrate of strain 639 - C = 3,2 • 10-2 mol / l; m (cysteine) = 1,9 • 10-3. A characteristic feature of the chemical structure of the cysteine sulfhydryl group presence, are highly reactive. Under certain conditions, cysteine easily gives hydrogen and then two molecules of cysteine via a disulfide bond to form a new amino acid cystine. .Mutual transition cystine and cysteine in the back is a redox process that is important in the regulation of metabolism. Cysteine is also involved in transamination reactions and sulfur metabolism in the body. n day 14 in culture filtrate of strains 419 and strain 639 recorded the presence of serine. Its concentration in the culture filtrate of the strain was 419 C = 1,9 • 10-2 mol / l, m (serine) = 1,2 • 10-3 g in the culture filtrate of strain 639 C = 1,2 • 10-1 mol / l; m (serine) = 7,8 • 10-3 g L-serine is involved in the construction of almost all naturally occurring proteins. Serine is involved in the formation of the active sites of several enzymes (esterases, peptidgidrolaz), providing their function. Phosphorylation of serine residues within proteins is important in the mechanisms of intercellular signaling. In addition, serine is involved in the biosynthesis of other amino acids glycine, cysteine, methionine, tryptophan. Is the initial product of the synthesis of purine and pyrimidine bases, sphingolipids, ethanolamine, and other important products of metabolism. On day 21 in culture filtrate of strains 419 and 639 indicated the presence of glutamine at a concentration of C = 1,1 • 10-1 mol / l, m (glutamine) = 1,12 • 10-2 g in the culture filtrate of strain 419; at a concentration of C = 7,4 • 10-1 mol / l, m (glutamine) = 7,5 • 10-2 g - in the culture filtrate of strain 639. Glutamine plays an important role in the integration of nitrogen metabolism. Also involved in: the synthesis of other amino acids, including histidine, neutralization of ammonia, carbohydrate biosynthesis is involved in the synthesis of nucleic acids, folic acid, the oxidation of brain tissue cells in a yield of energy stored as ATP etc. On day 28 in culture filtrate strains 419 and 639 identified asparagine. Its concentration was C = 1,4 • 10-1 mol / l, m (asparagine) = 1,3 • 10-2 g in the culture filtrate of strain 419; C = 2,0 • 10-1 mol / l, m (asparagine) = 1,8 • 10-2 g - in

the culture filtrate of strain 639. Asparagine - one of the 20 most common naturally occurring amino acids. The functional group of the side chain of asparagine is carboxamide. This amino acid is not essential. Its codons are AAU and AAC. Precursor of asparagine is oxaloacetate. On day 35 in culture filtrate of strains 419 and 639 indicated the presence of arginine at a concentration of C = 1,2 • 10-2 mol / l, m (arginine) = 1,3 • 10-3 g in the culture filtrate of strain 419; C = 2,0 • 10-2 mol / l, m (arginine) = 2,2 • 10-3 g - in the culture filtrate of strain 639. The characteristic feature is the presence of arginine in its molecule along with □-amino and amidino groups (NH2-CNH), located at the □-amino group, which plays an important role in the metabolism of nitrogenous substances. Under the influence of arginase amidine group is cleaved from arginine to form urea. When you transfer the group to glycine synthesized guanidinoacetic acid – is precursor of creatine. In transferring the amidino group and the hydrolytic cleavage of its arginine converted to ornithine. After 42 hours in culture filtrate strains 419 and 639 identified tyrosine. Its concentration was found C = 3,0 • 10-3 mol / l, m (tyrosine) = 3,8 • 10-4 g in the culture filtrate of strain 419; C = 8,2 • 10-2 mol / l, m (tyrosine) = 1,04 • 10-3 g - in the culture filtrate of the strain 639. Tyrosine - aromatic alpha-amino acid. Exists in two optically isomeric forms - L, D and the form of the racemate (DL). By the structure of the compound is characterized by the presence of phenylalanine from a phenolic hydroxyl group in the para-position of the benzene ring. There are equally important from a biological point of view, meta- and ortho-isomers of tyrosine. L-tyrosine is a proteinogenic amino acid and a part of the proteins of all known living organisms. Tyrosine is a part of enzymes, many of which it tyrosine play a key role in the enzymatic activity and its regulation. In the biosynthesis of tyrosine intermediates are shikimate, chorismate, prephenate. Central tyrosine metabolites synthesized by microorganisms in nature, fungi and plants. Tyrosine refer to replaceable to most animals and human amino acids as the amino acid in the body is formed from another (essential) amino acids - phenylalanine.

Conclusion

Thus, the presence in the culture filtrate of the fungus Colletotrichum lini amino acid cysteine (product of synthesis of phenylalanine), from an early period of culturing the fungus suggests the possible presence, at a certain stage of phenylalanine - a potent inhibitor of the growth of plant cells.

Acknowledgment

It is therefore possible that one of the terms of toxicity culture filtrates of the fact that there is a given amino acid. At the same time, the presence of amino acids such as asparagine, glutamine, serine prove the possibility of inducing morphogenetic activity of the cells of flax with a suitable choice of optimal concentrations of culture filtrate in a selective medium for the production of new resistant to anthracnose forms of flax biotechnological methods.

References

Kretovich VL **(**1971) Основы биохимии растений. 5-е изд. исправл. и доп., pp. 464, Moscow. Rassia: Graduate School.

Kudryavtseva LP, Proletova NV, Loshakova NI Pavlova LN, Vinogradoav EG, Sokolova NS (2013) Методы оценки и отбора исходного материала при селекции льна-долгунца на устойчивость к антракнозу – Scientific research breeding centers - flax. The results of research on flax-flax and flax oilseed research institutions breeding centers for the years 2001-2012. Tver, Rassia: Tver University, 40 – 41.

Monastirskiy OA **(**1996) Токсины фитопатогенных грибов. Plant Protection and Quarantine, Rassia Volume № 3: 5-7.

Pleshkov BP (1975) Биохимия сельскохозяйственных растений. 3-е изд., доп. и перераб.. pp. 496, Moscow: Kolos. Rassia

Proletova NV, Kudryavtseva LP, Vinogradova EG. (10.04.2013) Способ получения регенерантов льна-долгунца, устойчивых к антракнозу, методами *in vitro.* The patent for the invention № 2478282 (priority of invention 20 April 2011). Bulletin. № 10.

Shkalikov VA . (2001) Защита растений от болезней pp.199, Moscow: Kolos. Rassia.

Severin SE, Solovyova GA (1989) Практикум по биохимии. pp. 378, Moscow State University.

Dawson R., Elliot W., Elliot Y., Jones K. (1991) Справочник биохимика. pp 250, Moscow: Rassia: World .