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ESTABLISHING THE SYSTEMATIC POSITION OF FUNGI OF THE GENUS TRICHODERMA, ISOLATED FROM SOILS OF THE BUKHARA REGION

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ABSTRACT

Studying Trichoderma species represents a large practical and theoretical interest in connection with their use in human activities. The widespread use of these mushrooms requires the study of their environmental features, species diversi-ty.Trichoderma fungi are widespread in nature. The systematic position of fungi of the genus Trichoderma studied from soil samples of the Bukhara region. Isolated fungus according to the identifier Rifai are classified as Trichoderma koningi, Trichoderma harzianum, Trichoderma aureoviride, Trichoderma longibrachiatum.

Keywords Trichoderma Phialospore Colony Mycelium Gyphus Strain Phialis Conidiaphore Conidia Species

INTRODUCTION

Fungi of the genus Trichoderma belong to ascomycetes, they are widely distributed in nature, especially on cellulose-containing substrates, soil. Various types of fungus of this kind are used in the production of cellulolytic and hemi cellulolytic enzymes, in the biological control of plant diseases, in the bioconversion of cellulose-containing raw materials, etc. To date, the taxonomy of fungi of the genus Trichoderma is discussed; there is no unified classification of species belonging to this genus.

Recently, a number of researchers have been inclined to use molecular genetic me-thods along with conventional classical methods based on morphological features. The fungi of the genus Trichoderma were first described by Pers (1794).

He described three species of fungi belonging to the genus. Until 1969, almost all Trichoderma species were difficult to distinguish morphologically; therefore, they were identified in the literature as T.viride.

The description of this genus in 1969 was taken by (Rifai.Mien Rifai) was the first modern mycologist who classified the genus Trichoderma and established that the genus includes more than several species. According to his classification, he combined species according to the totality of morphological characteristics of cultures. According to the totality of morphological characters, he described 9 different species of fungi of the genus Trichoderma. According to his description, Trichoderma septic fungus (see in the determinant) forms branching conidiophores of a conical or pyramidal shape. They form colonies of white, yellow and green.

In 1991, John Bissett (1984, 1991) revised the classification of the genus Trichoderma. He described in detail the morphological features of the genus Trichoderma. According to studies, fungi of the genus Trichoderma should be classified as Trichoderma, Pachybasium, Longibrachiatum and Hypocreanum. He described more than 40 species including 14, which he described as new.

Samuels in 1996 conducted a preliminary study of fungi of the genus Trichoderma. Based on the study of morphology and molecular methods, he significantly expanded the species belonging to the genus Trichoderma. Lieckfeldtet al., (1998) indicates the use of molecular methods to identify species. They stated that molecular genetic me-thods should be used to identify species of the genus Trichoderma, which provide a more reliable form of identification of the species. Drujininaet al., (2005) also studied fungi of the genus Trichoderma and described species within the genus Trichoderma using molecular genetic methods.

Harman et al., (2004) investigated Trichoderma fungi and identified 75 species of Trichoderma according to morphological and molecular-genetic characteristics Gams and Bissett (1998) conducted a detailed study of the morphological features of fungi of the genus Trichoderma. They described the Trichoderma species, which is characterized by rapid growth, mainly forming bright green conidia and the branched form of conidiophores. Theyn described species of the genus Trichoderma, forming many pigments from bright greenish yellow to reddish in color, although some colorless species were also found. Similarly, conidia pigmentation ranged from colorless to various green shades, and sometimes even gray or brown. In addition to pigmentation, the identification of species within the genus is quite difficult due to small changes in the morphology of the genus Trichoderma.

In a number of cases, as described in the literature, the Trichoderma harzianum fungus is incorrectly defined by many researchers; it has been incorrectly systematized for many different varieties (Kullnig et al. 2001).

The Index Fungorum database (http://www.indexfungorum.org/names/names.asp) currently even lists 471 different names for Hypocrea species and 165 species for Trichoderma. However, many of these species were introduced long before molecular methods were introduced to identify species, and thus are likely to become obsolete.

The purpose of our research was to isolate Trichoderma fungi from soil samples from the Bukhara region of the Bukhara district under crops in order to use them in the future to create a biological product based on them.

The reason for this interest is the great practical and environmental importance of the genus. On the basis of antibiotics, toxins, enzymes formed by fungi of this kind, preparations are obtained for the biological control of diseases and stimulation of plant growth, and the production of transgenic plants. Trichoderma is also used for biological soil treatment and for composting waste (Alimova et al., 2002)

RESEARCH METHODOLOGY

Fungi of the genus Trichoderma were isolated from the soil, rotting plant debris. While determining the generic and species affiliation, the Rifai identifier was used. (1969) The study of morphological and cultural characteristics was carried out on wort agar, potato-sucrose medium and Mandels agar medium by filter paper. Dimension measurements were performed on standard medium. Chapeco macroscopic properties of the studied fungi were studied in the yards of colonies grown on the above media in Petri dishes with a diameter of 14 cm at a temperature of 28-30 C. In the process of work, phase contrast, electron and scanning microscopy were used.

Experiments have shown that fungi of the genus Trichoderma are distinguished by great variability of morphological and cultural features. For example, according to the growth rate of colonies, they can be divided into 3 groups: fast-growing, intermediate and slow-growing (see fig.). The figure shows the growth rate of these species. It can be seen that of the 4 species, mushrooms are the most rapidly growing. T.Congo is intermediate

between representatives of the species T. harzianium, T. koningii, and fungi of the species T. aureoviride grow relatively slowly. It should be noted that fast-growing strains were characterized by the highest cellulolytic activity.

According to the existing classifications of fungi of the genus Trichoderma, isolated fungi were assigned to the following species: Trichoderma longibrichiatium, T. harzianium, T. koningii, T. aureoviride. The following is a description of the isolated fungi.

TRICHODERMA KONINGII

Fungi colonies are fast growing. On wort agar and cellulose agar, a transparent, highly branched, fibrous substrate mycelium develops. On the second day of growth, the average diameter of the colonies is 40-45 mm. Before the formation of air hyphae, the colony surface is smooth, the development of air hyphae makes it fibrous.

Conidia formation begins at the center of the colony. Colony coloration is initially whitish green, and then dark green. On the potato-sucrose medium, the conidial zone is represented by white turfs located in the center, and aerial mycelium, representing a loose interweaving of hyphae, is well defined to the periphery. The reverse side of the colonies on cellulose agar is not stained; on wort agar and potato-sucrose medium it has a pale yellow color. Hyphae septate, smooth-walled, with a diameter of 2-10 Chlamydospores are round, smooth, terminal and intercalary, with a diameter of 10-12 microns. Conidiophores are highly branched, the main branch gives several lateral branches in groups of 2-3, which extend at an obtuse angle to the carrier. Lateral branches have a pyramidal branching system (Fig.) The terminal phialides are elongated, narrowed at the base, have a bottle-shaped shape. The intercalar phialides are ampuloid, already at the base sharply thinning in their conical apex. Phialospores are ovoid-ellipsoidal with a clearly distinguishable truncated base, sizes 3.2-5.0 x 2.0-3.0 microns.

TRICHODERMA LONGIBRICHIATIUM RIFAI

Fungi colonies on all media are rapidly growing. On the 3rd day of growth on pota-to-sucrose medium and cellulose agar (Fig. 1), colonies occupy almost the entire surface of the Petri dish. The sporulation zone is represented by a dense low mycelium, on which are located turfs formed by sporulation organs, located close to each other. The colony color is initially white, on the 5th day of growth it turns into an olive-green color. On wort agar in the center of the colony, a friable, felt colorless mycelium is formed with increasing development of aerial mycelium to the periphery (Fig.). On all media, the fungus produces a bright yellow-green pigment. Hyphae are transparent, septate, smooth-walled, with a diameter of 3-14 microns. Chlamydospores terminal or intercalary are formed more often in submerged mycelium. They are widely elliptical, from 5 to 14 microns in diameter. Conidiophores have a fairly simple branching system (Fig.). On the main long branches, lateral branches with a diameter of 2 to 7 μm are located at right angles to the carrier. Lateral branches are longer at the base and sparser and shorter at the apex of the main trunk. The terminal branches end with one terminal phialid, elongated to 15 microns. Intercalar phialides are more often formed unevenly, singly, bowling in the size of 6.5-15 x 2.5-3.1 microns. Phialospores are elliptic, subcylindrical 5.5-2.5 microns.

TRICHODERMA AUREOVIRIDE RIFAI

Fungi colonies are slow growing. On wort agar and cellulose agar forms creeping substrate mycelium. Aerial mycelium is poorly developed, not dense, represented by a radially diverging loose weave of hyphae, initially dirty white, with the formation of color on 5-6 days, it turns yellow-green.

On potato-sucrose medium after four days, the radius of the colonies reaches 80-85 mm. The colony is initially white, then yellowish brown. Sods with conidiophores in the center of the colony with a diameter of 70-75 mm form a radial conidial zone characteristic of this medium.

The reverse side of the colonies on wort agar and potato-sucrose medium is brownish yellow, and on cellulose agar is not stained. Hyphae are smooth-walled, branched, septate, colorless, up to 2-3 microns in diameter. They form chlamydospores, often intercalated, 7-10 microns in diameter.

Conidiophores are thin, up to 4 microns in diameter, and form in the form of lateral branches, of which small lateral branches depart. The phialides are collected whorled, long, bottle-shaped, sizes 7-15 x 2.0-2.6 microns. The phialospores are smooth-walled, ovoid with a severed base, sizes 3-4.5 x 2.0-3.5 microns.

TRICHODERMA HARZIANIUM

Colonies in all environments grow rapidly. On wort agar in the center, a dense continuous mycelium is formed. The peripheral growth zone is represented by a ring of radially oriented hyphae ending in a flat edge (Fig.).

Sporulation begins in three days from the center of the colony. The color of the colony is initially light green, and then acquires a dark green color.

On cellulose agar, the colony has the shape of ring-shaped concentric circles. The color changes from whitish green to dull green. The reverse side of the colonies is not stained.

Sporulation organs form compact pads in annular conidial zones. Hyphae are branched, smooth-walled, septate 3-12 microns in diameter. Most chlamydospores are intercalary, smooth, rounded 6-12 microns in diameter.

Conidiophores have a pyramidal branching system, the main branches produce 2-3, and sometimes 4 lateral branches, located under the straight to the nose. The lower lateral branches form secondary branches, at the ends of the twigs whorls are located phialids, with which the branches of conidiophores end, which can arise singly and asymmetrically. The terminal phialides are elongated, long up to 16x2.5 microns. Pear-shaped intercalary phialids, short, sharply elongated to the conical apex, their sizes are 5.0-8.0 x 3.5-4.8.

Phialospores are obovate, smooth-walled, pale green 3.0-2.6 μm (2.4-4.0 x 2.4-2.8).

Thus, it can be seen from our studies that the cultural and morphological characters of the isolated fungi on different nutrient media differ sharply, which is well shown in the figures presented. Fungi differ in the appearance of the colony, the method of sporulation, the period of its appearance, color, shape and size of the phialospores. A detailed study of these characteristics in accordance with the Rifai 1 system made it possible to attribute the isolated strains to Trichoderma longibrichiatium, T. harzianium, T. koningii, T. aureoviride.

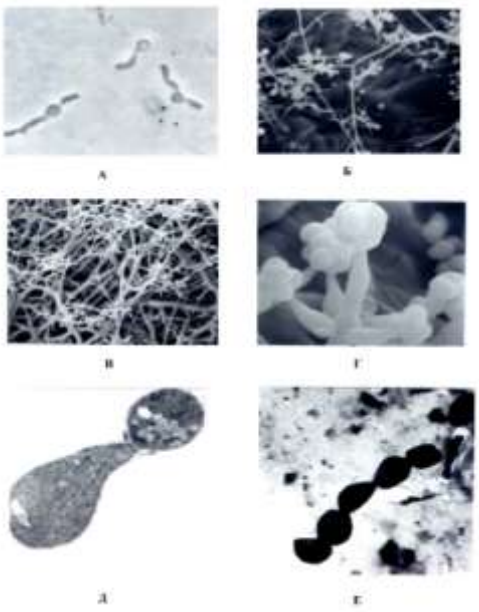

Fig 1. The development of fungi of the genus Trichoderma on cellulose agar with a surface cultivation method

A) germination of spores

B) the formation of first-order hyphae

C) second-order hyphae formation

D) the formation of phialids with spores

E) conidiogenesis

F) chaining

CONCLUSION

Thus, from our researches it can be seen that culturally the morphological characters of the isolated species of fungi of the genus Trichoderma on different nutrient media differ sharply in all the characters of the colony, the method of sporulation, the timing of its appearance, color, shape and size of the phalospores, which is well shown in the figures. A detailed study of these characteristics in accordance with the system Rifai allowed to assign the selected strains to Trichoderma Congibrichiatum, T. Koningi, T. harrianum, T. aureoviride.

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