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EXPERIMENTAL ARTICLES

Autoregulation of Conidium Germination in Micromycetes of the genus *Trichoderma*

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Abstract—The amount of germinated conidia of micromycetes belonging to the genus *Trichoderma* considerably decreased with an increase in the population density. Strains exhibited different ecological strategies. The maximum number of germinated conidia (30–70%) was recorded when the average distance between conidia was 50 μm.

Key words: micromycetes, conidia.

Strains of the genus *Trichoderma* are widely applied in agriculture to control the development of pathogenic fungi in soil, rhizosphere, phyllosphere, and on seeds of various plants [1]; their application is also recommended for the protection of harvested crops [2–5]. The efficient employment of live microbial preparations depends on the ability of the introduced microorganisms to actively colonize the substrate. Conidia of fungi belonging to the genus *Trichoderma* are highly suitable for the production of dry preparations; however, it is necessary to know the conditions for conidium germination in nature. The effect of environmental factors on the behavior of microbial populations has been extensively studied; it was shown that of particular ecological importance are intrapopulational interactions. Data have been published demonstrating the role of autoregulation in the activation of resting forms of microorganisms [6, 7].

The aim of this work was to elucidate the intrapopulational regulation of conidium germination in different strains of the genus *Trichoderma.*

MATERIALS AND METHODS

The micromycete strains were isolated from the rhizospheres of roses (Grand Gala, Royal Velvet, and Dallas varieties). The four strains used in this work were typical representatives of the genus *Trichoderma* (table).

To obtain conidia, micromycetes were cultivated on Czapek agar with sucrose (2%) at 26°C for 7 days; conidia were washed off of the agar, centrifuged, washed thrice, and subjected to ultrasonic treatment on an UZDN-1 disintegrator at 22 kHz and 0.44 A for 30 s to obtain a suspension of single spores. Viability of the

Strains tested

Strain	Species	Author	Isolation source	
			Rose variety	Anatomic part
FW826	*Trichoderma longibrachiatum*	Rifai 1969	Grand Gala	Rhizosphere
FW829	*T. longibrachiatum*	Rifai 1969	Dallas	Rhizoplane
FW827	*T. harzianum*	Rifai 1969	Royal Velvet	Dead root
FW828	*T. harzianum*	Rifai 1969	Dallas	Internal part of root

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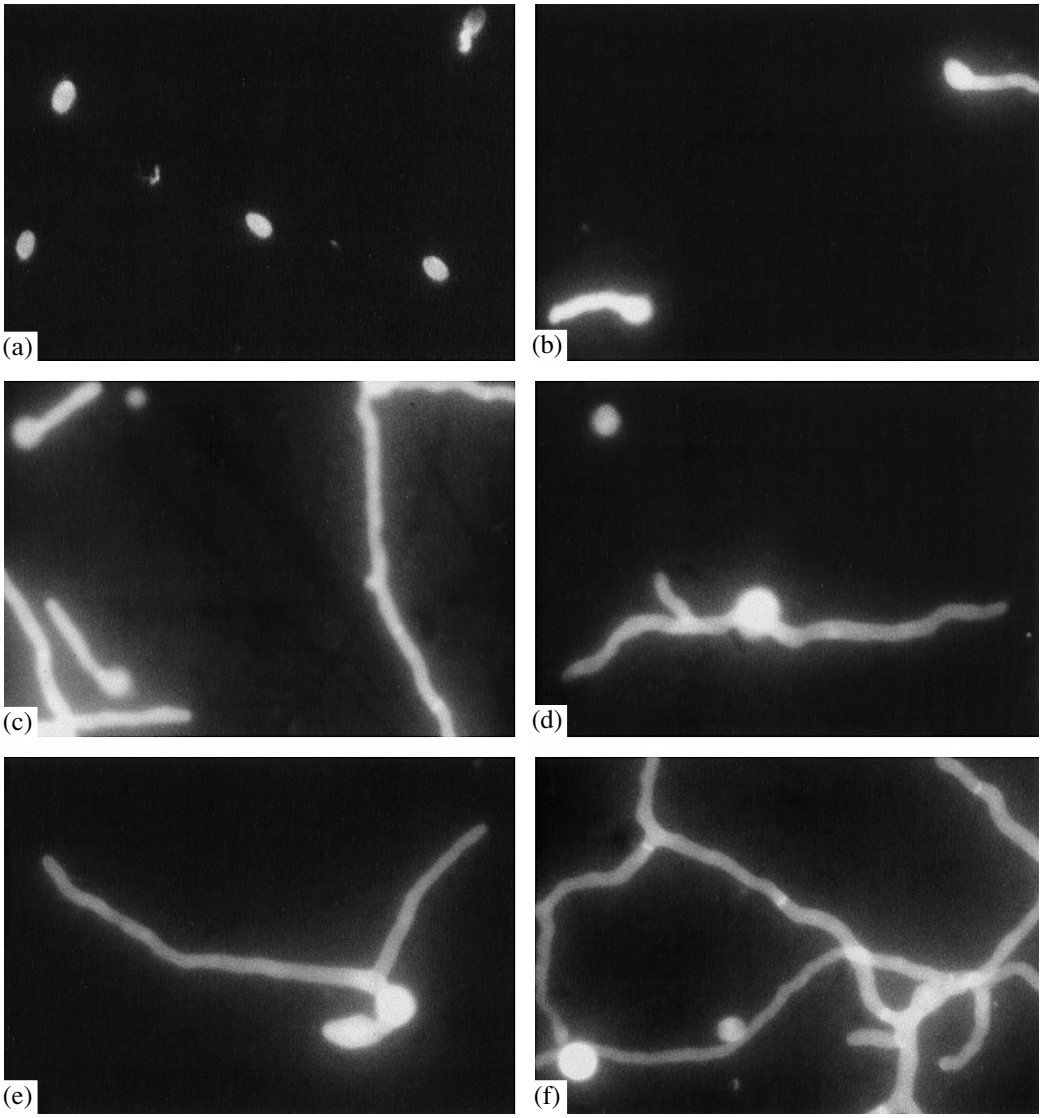

Fig. 1. Conidium germination in micromycetes of the genus *Trichoderma*: (a) the onset of experiment; (b) germination after 15 h of incubation; (c–e) further growth; and (f) formation of microcolonies. Magnification, 1 : 1000.

conidia was determined microscopically by a routine method [8]; 0.02 ml of a conidium suspension was placed on a thoroughly degreased microscope slide and distributed evenly with a loop over an area of 4 cm^2. For each sample, 12 specimens were prepared. The number of conidia per 1 ml of suspension was calculated by the formula:

$$N = S_1 an / vS_2,$$

where N is the number of conidia per 1 ml of suspension; S_1 is the specimen area (μm^2); a is the number of conidia per microscope field (averaged over all specimens); n is the index dilution of the suspension (ml); v is the volume of the drop placed onto the slide (ml); S_2 is the area of the microscope field (μm^2).

Population density was determined by microscopic examination of stained fixed specimens. To estimate the reproductive capacity of conidia, specimens, prior to drying, were incubated in a humid chamber at 26°C and 100% humidity for 6, 15, 18, 24, and 39 h. Then, the specimens were air-dried, fixed, and stained with calcofluor white. Conidia were counted under a LYuMAM I-3 luminescent microscope; the microscope field area was 9800 μm^2. The conidium numbers of 1, 5, 10, and 20 per microscope field corresponded to population densities of 2×10^6, 1×10^7, 2×10^7, and 4×10^7, respectively. The average distance between conidia ($r = \sqrt{1/4m}$, where r is distance and m is the population density calculated per area unit) was used as the abundance index. The course of conidium germination in

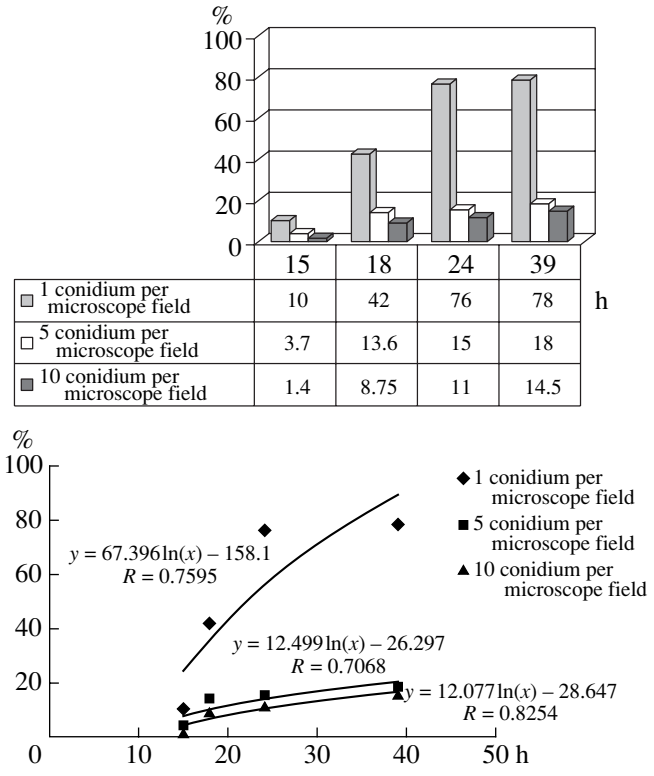

Fig. 2. Germination of *T. longibrachiatum* FW826 conidia in a humid camera as a function of their abundance.

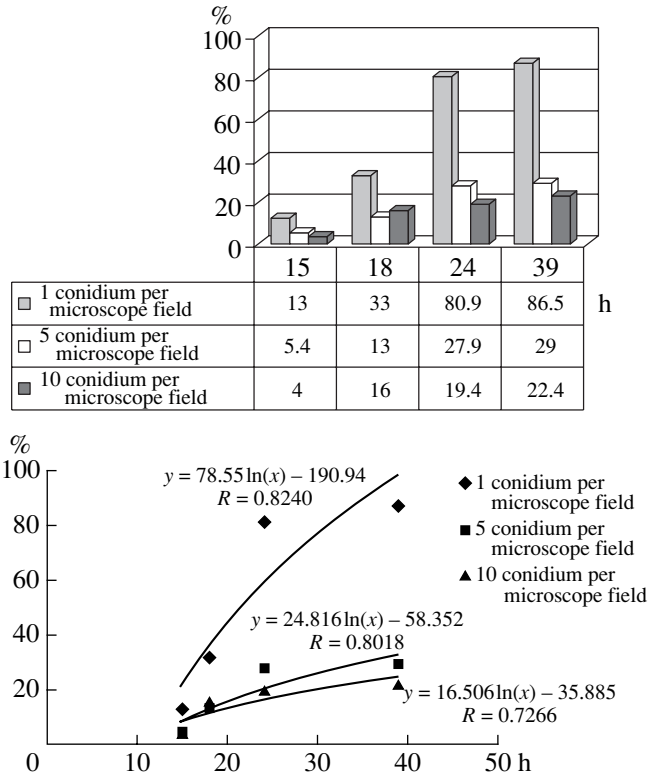

Fig. 3. Germination of *T. harzianum* FW827 conidia in a humid camera as a function of their abundance.

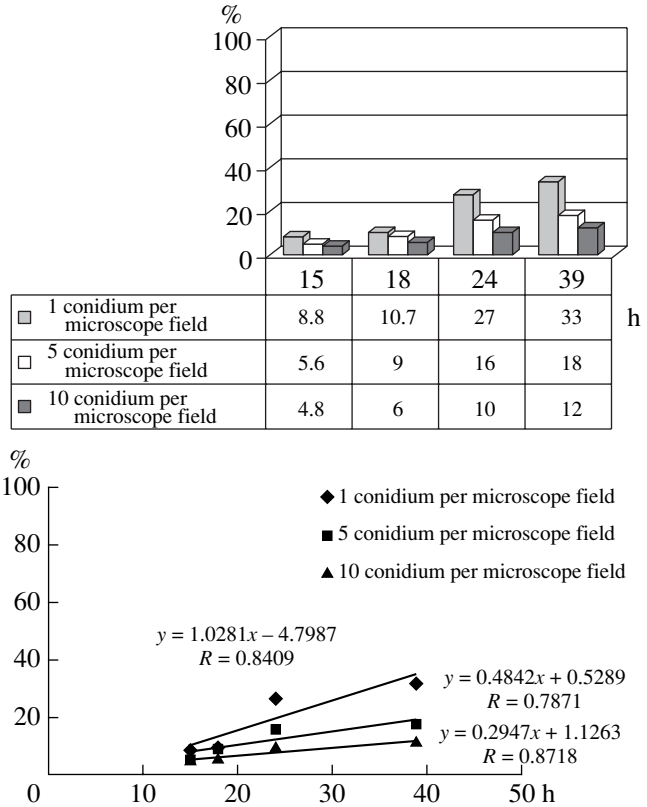

Fig. 4. Germination of the conidia of *T. harzianum* FW828 as a function of their abundance.

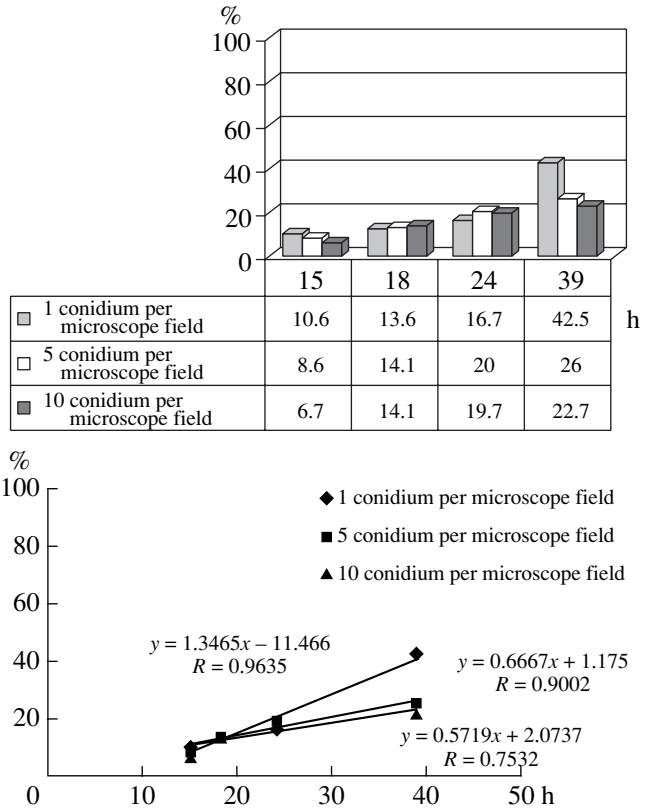

Fig. 5. Germination of the conidia of *T. longibrachiatum* FW829 as a function of their abundance.

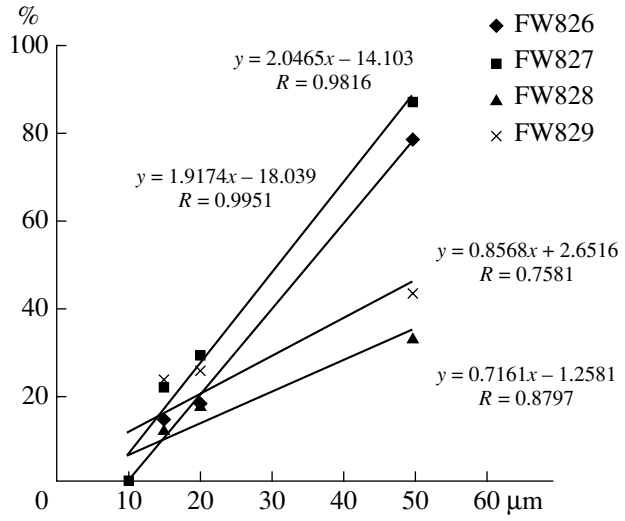

Fig. 6. Correlation between the portion of germinated *Trichoderma* conidia and the average distance between them (μm)

micromycetes of the genus *Trichoderma* is illustrated in Fig. 1.

Standard deviations (δ_{n-1}) for conidium number did not exceed 10%.

RESULTS AND DISCUSSION

The data on the conidium germination in strain *Trichoderma longibrachiatum* FW826 isolated from the rhizosphere of the rose variety Grand Gala are shown in Fig. 2. The germination of conidia in a humid camera was dependent on their abundance and could be described by logarithmic regression equations with correlation coefficients *R*. Conidium germination could be recorded by the 15th hour of incubation; the proportion of germinated conidia comprised 10, 4, and 1.5% in specimens containing 1, 5, and 10 conidia per microscope field, respectively. On subsequent incubation, the amount of germinated conidia increased four- to sixfold in all specimens; conidium germination slowed down in the period of 18–39 h and then ceased. No conidium germination was observed in specimens containing 20 or more conidia per microscope field.

In conidia of strain *T. harzianum* FW827 isolated from the rhizosphere of the rose variety Royal Velvet, germination also started by the 15th hour of incubation; however, the effect of conidium density on the germination process was not so pronounced as in the first experiment. Conidium germination also declined in hours 18–39 and then stopped. No conidium germination was revealed in the specimens containing 20 or more conidia per microscope field.

With strains *T. harzianum* FW828 and *T. longibrachiatum* FW829 isolated from the rhizosphere of the rose variety Dallas, the germination of conidia also started by the 15th hour of incubation. In this case, it could be described by linear regression equations with rather high correlation coefficients *R* ranging from 0.78 to 0.96 (Figs. 4 and 5). Correlation between conidium density and the number of germinated conidia was pronounced in the specimens containing from 1 to 5 conidia per microscope field, although in the former case, the final number of conidia germinated by the 39th hour was lower than in the other strains studied. In the case of strain *T. longibrachiatum* FW829, the amount of germinated conidia did not decrease with an increase in conidium density from 5 to 10 conidia per microscope field, although no conidium germinations was revealed in the specimens containing 20 or more conidia per microscope field.

The data on the effect of the average distance between conidia on the germination process are summarized in Fig. 6. In all of the strains studied, a 50 μm-distance was the most favorable for conidium germination, whereas a distance of 15 μm was unfavorable for three strains of the four. Micromycete populations isolated from different sources were different in their behavior. The correlation between the amount of germinated conidia and the distance between them was more pronounced for strains isolated from the rhizosphere of roses belonging to the Grand Gala and Royal Velvet varieties than for the strain isolated from the rhizosphere of the rose variety Dallas.

Thus, despite certain variations in the strain behavior, a common property of the micromycetes studied was intrapopulational regulation of conidium germination. It can be assumed that the mechanism of this regulation involves cell response to the concentration of signal metabolites (e.g., trimethylamine [6]), which depends on the distance between the cells.

The autoinhibition of conidium germination should be taken into account when formulating recommendations for agricultural practices. The optimal application dose should be experimentally determined for each *Trichoderma* strain.

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