

## SCANNING ELECTRON MICROSCOPY OF CONIDIA OF *TRICHODERMA STROMATICUM*, A BIOCONTROL AGENT OF WITCHES' BROOM DISEASE OF COCOA

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### SHORT COMMUNICATION

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#### ABSTRACT

A field emission electron microscope (SEM) was used to study the conidia surface of *Trichoderma stromaticum*, a biocontrol of witches broom disease of cocoa. Surface features of conidia were difficult to distinguish by light microscope. Conidia appeared to be verrugose and minutely roughened, but the nature of the roughening was not easy to discern. It was common to observe sheath-like structures that completely covered groups of conidia, and also details of wide cells that form the pustules.

**Key words:** Biological control, cocoa, *Crinipellis perniciososa*, morphology of conidia

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Conidia surface ornamentation and conidial matrix, as observed with the scanning electron microscopy, is significant in the taxonomy of *Trichoderma* species (8).

The *Trichoderma* species aggregates have been difficult to subdivide into biological species. Each aggregate represents a number of species (1,12) and morphological characters that can reliably define biological species have not been identified. This type of classification is highly artificial and Domsch *et al.*, (6) and Bissett (1) have revised partly the genus for useful system to differentiate among phenotypically similar species.

Species of the genus *Trichoderma* are ubiquitous active components of the soil microbiota and well known for their production of hydrolytic enzymes (11), antibiotics (5), heterologous proteins (3,9) and as biocontrol agents against phytopathogens (4) and its current commercial use in some countries (10).

*T. stromaticum* sp. nov. (13), isolated from dry brooms of cocoa, has been used in the field to control the basidiomycete *Crinipellis perniciososa*. The antagonist acts preventing the basidioma formation. The new species belongs to *Trichoderma*

sect. *Pachybasium* (1), with *T. harzianum* and *T. virens* representing the closest relations.

This paper presents results of scanning electron microscopy of conidial surface ornamentation of *T. stromaticum*.

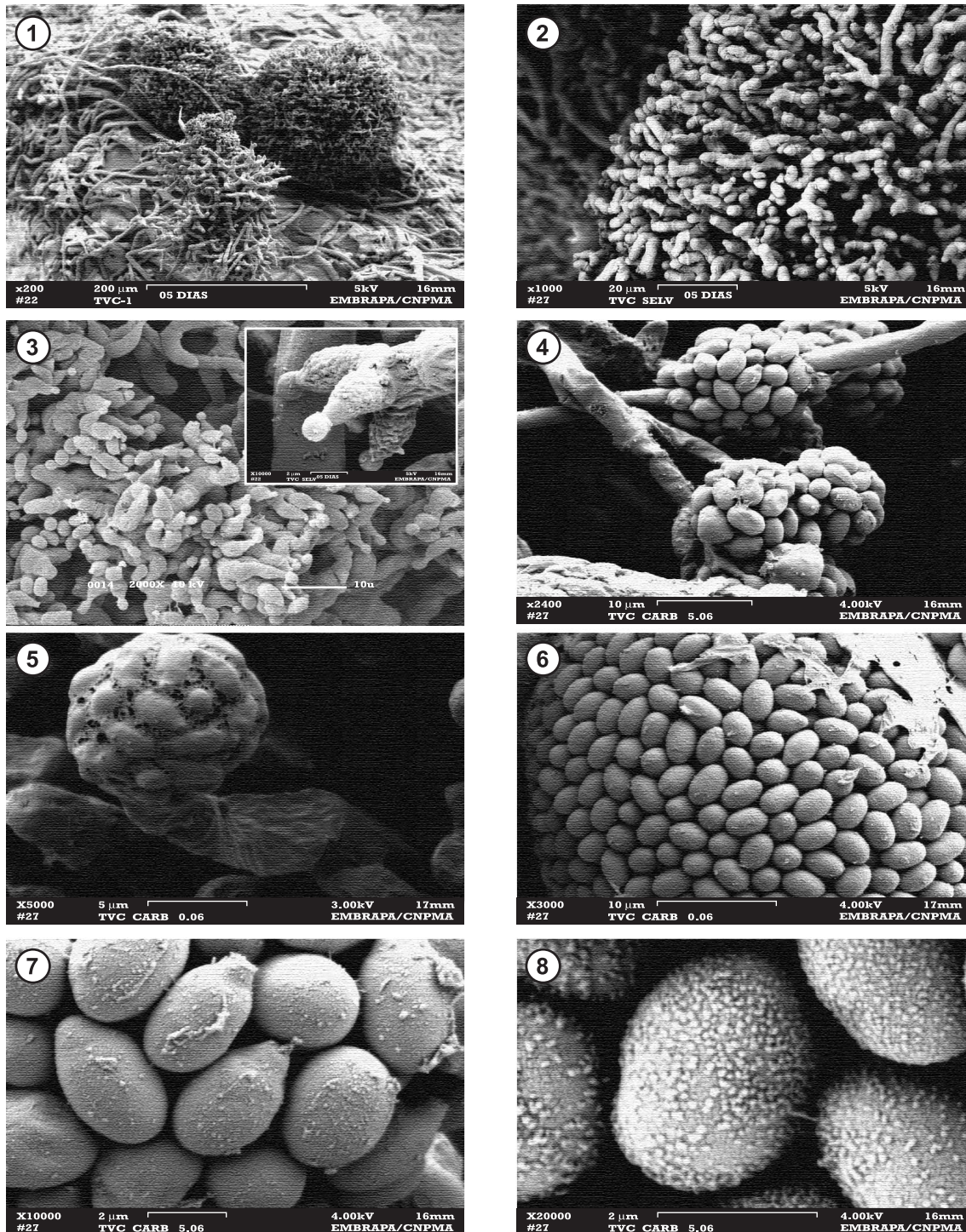
**Strain and culture methods.** One strain of *T. stromaticum*, TVC, isolated from dead cocoa broom was provided by Dr. J. C. B. Costa of CEPEC / CEPLAC, Bahia State, Brazil. Cultures were maintained on 2% malt extract agar.

Material for SEM examination of conidial surfaces was obtained from cultures that were grown on 2% MEA for 12 days at 26°C, and grown under cool white fluorescent lamp with an alternating cycle of 12h of light and 12h of darkness.

Agar blocks were cut into small pieces with conidiating hyphae and fixed in 2% glutaraldehyde in 0.1 M-NaPO<sub>4</sub> buffer and post-fixed in buffered 1% O<sub>5</sub>O<sub>4</sub> for 2h. The material was then dehydrated in an ethanol series (10, 25, 40, 60, 75, 85, 95, 100%) with 15 min per change. The specimens were dried in a critical point drying apparatus and sputter-coated with gold and viewed using a field emission scanning electron microscope, Leo 982 (Zeiss + Leica).

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**Figures 1 - 8.** Scanning microscopy of fixed conidia and conidial masses of *Trichoderma stromaticum*. 1) Compact, velvety-surface conidiogenous pustules on ME after 6 days. 2) Pustules compact formed of vesicular or pseudoparenchymatous thin-walled (4.0 – 4.5  $\mu\text{m}$ ) wide cells arranged in chains that tend to branch dichotomously near the surface of the pustules. 3) Ampulliforme and sharply constricted phialides. 4) Conidial balls developed from terminal phialides. 5) Conidial mass showing tight aggregation of conidia and presence of an extensive sheath surrounding the conidia. 6-7) Large conidia oblongs to ellipsoidal. 8) Phialospores which are verrucose at high magnification.



## RESULTS AND DISCUSSION

Fine structure of conidial surfaces and conidial *en masses* on a phialide was observable by means of a field emission scanning electron microscope.

Conidial surface morphology of *Trichoderma stromaticum*, although appeared to be smooth when viewed in light microscope they are roughened and verrucose (Fig. 8) when viewed in high magnification of 10.000X, and more distinctly with increase of magnification of 20.000X.

This ornamentation type could not be reliably distinguished with light microscopy. Conidia broadly ellipsoidal to ovoid (5.8 µm – 2.0 µm), presenting both ends broadly rounded or end rounded and the base narrowed or apiculate (Figs. 6 and 7). This variation existed even in a single conidial mass. Conidia are formed in compacted pustules (Fig. 1). The pustules are formed of wide cells arranged in chains that tend to branch dichotomously (Fig. 2). Thus, conidial *en masses* on phialides are observed (Fig. 4), with ampulliforme and sharp constricted phialides (Fig. 3). In some cases, it was common to observe conidia forming mucilaginous conidial head, that is, conidial mass showing tight aggregation of conidia and presence of extensive sheath surrounding the conidia, which is characteristic to the section *Pachybasium* (Fig. 5). These sheaths are also present in *Trichoderma virens* (8).

Phialospores of many species of *Trichoderma* are fastened slightly each other by means of epispore scars. *T. viride*, for example, is characterized by the rough-walled conidia in the microscope level (oil immersion). In *T. longibrachiatum*, the roughness of spore surface was pointed out under light microscopic level. At the electronmicroscopic level this roughness was due to intermittent partial thickenings of the outer wall of an epispore (7).

SEM has the potential to reveal previously unknown characters that could be helpful in species definition. This study reports on the features of the conidial surfaces of *T. stromaticum* as useful morphological tools.

## RESUMO

### Microscopia eletrônica de varredura de conídios de *Trichoderma stromaticum*, um agente de biocontrole da vassoura-de-bruxa do cacauero

Estudos morfológicos de conídios de *Trichoderma harzianum*, um agente de biocontrole da vassoura-de-bruxa do cacauero, foram feitos sob microscopia eletrônica de varredura

com emissão de campo. Características da superfície de conídios do fungo mostraram ser rugosas quando observadas em alta magnificação; fato esse impossível de ser visualizado por microscopia ótica. Também foram observados, com frequência, massas de conídios completamente envolvidos por material mucilaginoso e detalhes de células ramificadas dicotomicamente que formam as pústulas.

**Palavras chave:** Controle biológico, cacau, *Crinipellis pernicioso*, morfologia de conídios

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