

ISOLATION OF CELLULOLYTIC CHRYSONILIA SITOPHILA FROM TRIBOLIUM FERRUGINEUM

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SUMMARY

Chrysonilia sitophila was isolated from macerates of the insect *Tribolium ferrugineum*, found in rice hull from the State of Goiás, Brazil.

It was found that *C. sitophila* has cellulolytic activity, determined by the method of Smith, using Petterson's medium, with cellulose-azure as carbon source. In addition the essential aminoacid composition was determined in the fungi.

The possible interrelations between *C. sitophila* and *T. ferrugineum* are discussed.

INTRODUCTION

Cellulosic materials which are 95% lignocellulose (1) represent the most abundant natural resource on earth (2). Generally these materials are recalcitrant and cause ecological problems when they are transformed by different processes. The biodegradation of these constituents, especially cellulose and lignin is extremely slow, and the majority of animals are unable to digest it. However, numerous microorganisms are able to degrade them either directly or in association with higher organisms, providing the necessary degrading enzymes. These microorganisms, providing the necessary degrading enzymes. These microorganisms produce hydrolytic enzymes that act on polysaccharides. Especially interesting is an enzymatic complex (3), due to its importance in the conversion of lignocellulosic materials to single cell protein for animal or to sugars, alcohols, adhesives and solvents (4).

Rice hull and rice straw together with bagasse are the most abundant vegetal crops in Brazil (5). In a previous work an insect (6) was found which was identified as *Tribolium ferrugineum* (Coleoptera)

RESUMEN

[*Aislamiento de Chrysonilia sitophila con capacidad celulolítica de Tribolium ferrugineum*]

El hongo *Chrysonilia sitophila* fue aislado a partir de macerados del insecto *Tribolium ferrugineum*, encontrado en muestras de cáscaras de arroz procedentes del Estado de Goiás, en Brasil.

Se encontró que *C. sitophila* presenta actividad celulolítica, la que fue determinada por el método de Smith, utilizando el medio de Petterson's y celulose-azure como fuente de carbono. Por otra parte se determinó en el hongo la composición de aminoácidos esenciales.

Se discuten además las posibles interrelaciones entre *C. sitophila* y *T. ferrugineum*.

and which feeds on a wide variety of products including all kinds of grains, flour, starchy materials, beans, peas and baking powder (7).

In this work the isolation and characterization of the fungus *Chrysonilia sitophila* is reported. The fungus was found in association with *T. ferrugineum*. Its cellulolytic activity on various substrates was measured and its essential aminoacid composition was determined.

MATERIAL AND METHODS

Insect collection and identification. *T. ferrugineum* specimens were collected from samples of rice hull originally from Goiás State (Brazil). The identification of the insect as *Tribolium ferrugineum*, Fabricius, (Ph. 1) Coleoptera Tenebrionidae was done by Prof. H. Toro, Department of Entomology, Universidad Católica de Valparaíso, Chile.

Culture media. The culture media used were Czapek Agar, Sabouraud Agar and Dextrose Agar for fungi and Nutrient Agar for bacteria. Besides the above

described media a culture medium was used which contained: Sodium nitrate 3 g., dipotassium phosphate 1 g., magnesium sulfate 0.5 g., potassium chloride 0.5 g. ferrous sulfate 0.01 g. per liter of distilled water; rice hull, cellulose and filter paper were used as carbon sources. For determining cellulolytic activity Petterson's medium was used (9).

Sample preparation and microorganism isolation. *T. ferrugineum* specimens were disinfected by using concentrated HCl and then washed three times with sterile distilled water, macerated and inoculated in to Czapeck Agar, Sabouraud Dextrose Agar and Nutrient Agar for the isolation of the different microorganisms. Ten insects were macerated per sample, which was plated in triplicate.

Cellulolytic Activity test. The cellulolytic activity of the fungus was determined by the method of Smith (10), using Petterson's medium, which includes cellulose-azure (SIGMA) as carbon source. When the fungus degrades the cellulose-azure the dye Remazol brilliant blue R is liberated and can be measured qualitatively and thus, the cellulase activity can be determined. For a quantitative measurement of cellulase activity Petterson's medium without Agar and 2% of the cellulose-azure was used; 0.1 ml of fungal inoculum was added. The inoculum turbidity was equivalent to the value of tube N° 3 of the Mac-Farland standard. The absorbance at different times and at 595 nm was measured in a Microanal Spectrophotometer Model 382, after centrifuging the sample at 7.000 rpm in a Exelse 2 centrifuge Model 205 N.

The cellulolytic complex was studied by cultivating the fungus in Petterson's medium without Agar with 1% saccharose and 0.75% crystalline cellulose as inductor. The filtrate was analyzed at different times to measure the total cellulase activity on filter paper, endoglucanase activity on carboxymethylcellulose, cellobiohydrolase activity on microcrystalline cellulose, betaglucosidase activity on p-nitrophenylglucopiranoside and cellobiose, and hemicellulase activity on Xylan (11-13).

Aminoacid composition. Aminoacid analysis of mycelial protein from fungi was performed in a Beckman 199 CI Aminoacid Analyser.

RESULTS AND DISCUSSION

In all microbial cultures obtained from macerated insects we found a fungus with irregular tufts at the margins of the petri dish. Initially colourless, it became pinkish to orange at the second day of incubation at 28°C and showed more or less ascending conidiogenous hyphae, septate with lateral branches which form chains of conidia (Ph. 2). The fungus was identified as *Chrysonilia sitophila* (Mont) Von Arx, with a teleomorphic state that

corresponds to *Neurospora sitophila* (Shear and Dodge) (14). Identification was done by Dr. E. Piontelli from the Department of Mycology, Universidad de Valparaíso, Chile. The habitat of this fungus is related to agricultural products, such as silages and meat, and to transportation and storage rot of fruit. It has been reported in Europe, USA, Japan, Surinam and Indonesia (8).

Occasionally, bacteria were isolated on Nutrient Agar but they were not considered in this work.

The cellulolytic activity of *C. sitophila* was determined in Petterson's medium and is shown in Ph. 4.; C. represents the control and X the fungus. Fig. 1 exhibits the absorption value at 595 nm at different growth times. Results of the activity of the cellulolytic complex on different substrates is shown on Table 1.

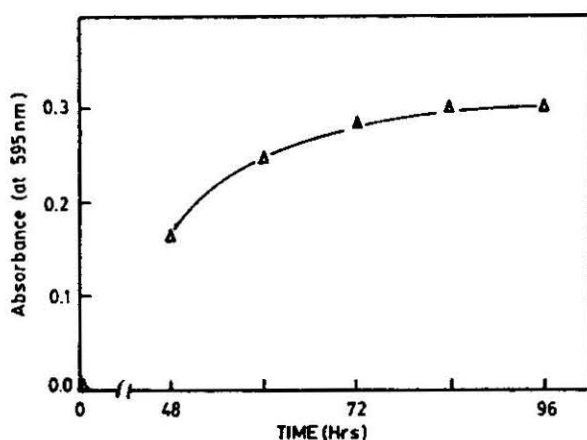


Fig. 1. Cellulase activity.

Table 1: ACTIVITY OF THE CELLULOLYTIC COMPLEX

ENZYMES	ACTIVITY
Endonuclease	+
Cellobiohydrolase	+
B Glucosidase	+
Hemicellulase	+

The aminoacid composition of the mycelial protein is on Table 2.

Table 2: AMINOACID OF THE MYCELIAL PROTEIN

(Gr. Aminoacid/100 Gr. Protein)

AMINOACIDS (A)	C. sitophila
Alanine	6.70
Arginine	2.50
Aspartic Acid	7.50
Cysteine	0.01
Glutamic Acid	8.30
Glycine	7.30
Histidine	1.45
Isoleucine	3.30
Leucine	5.70
Lysine	3.90
Methionine	1.45
Phenylalanine	2.90
Proline	4.30
Serine	4.10
Treonine	4.65
Tryptophane	-
Tyrosine	2.00
Valine	6.90

(A) Calculated as total aminoacid plus amount of liberated ammonia divided by the weight of the sample x 100.

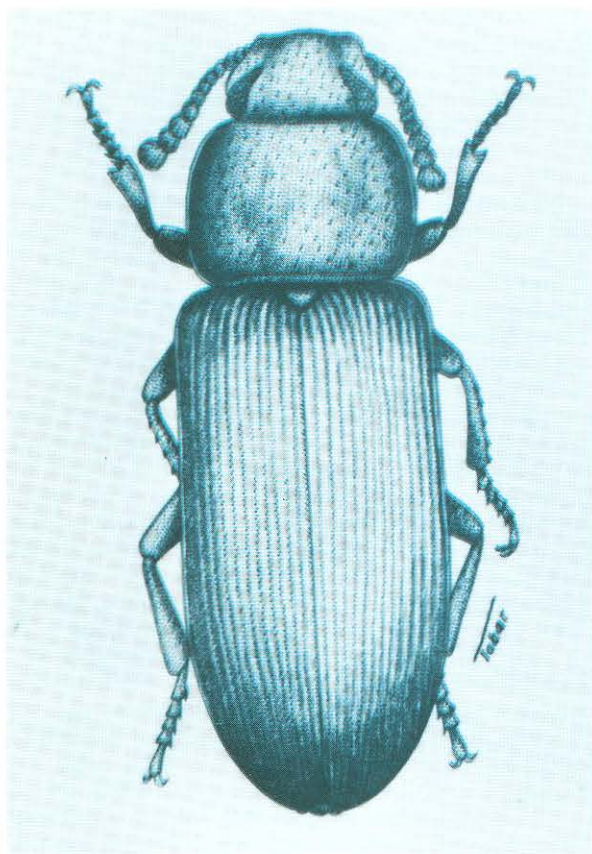
The data indicate that *C. sitophila* has a cellulolytic activity (Ph. 3) which appears after 24 hrs. reaching a maximum at 84 hrs. in the logarithmic growth phase. It should be interesting to evaluate this activity in comparison with known cellulolytic fungi in order to study its possible industrial applications.

C. sitophila was isolated from macerated insects. This indicates a permanent presence of the fungus in the insect. From the experimental data is difficult to say if *C. sitophila* is in symbiosis with *T. ferrugineum*. On other hand, it is possible that in this situation different microorganisms participate in symbiotic relation or in successive chains as previously described in other systems; Total analysis of the intestinal microbiota is actually in progress in our laboratories. However, *C. sitophila* on the bases of its aminoacid and cellulase contents, should play an important role in the feeding of *T. ferrugineum*, by providing essential aminoacids and carbon sources the latter from the biodegradation of lignocellulosic materials. In addition, the insect could play an important role in the dissemination of the fungus.

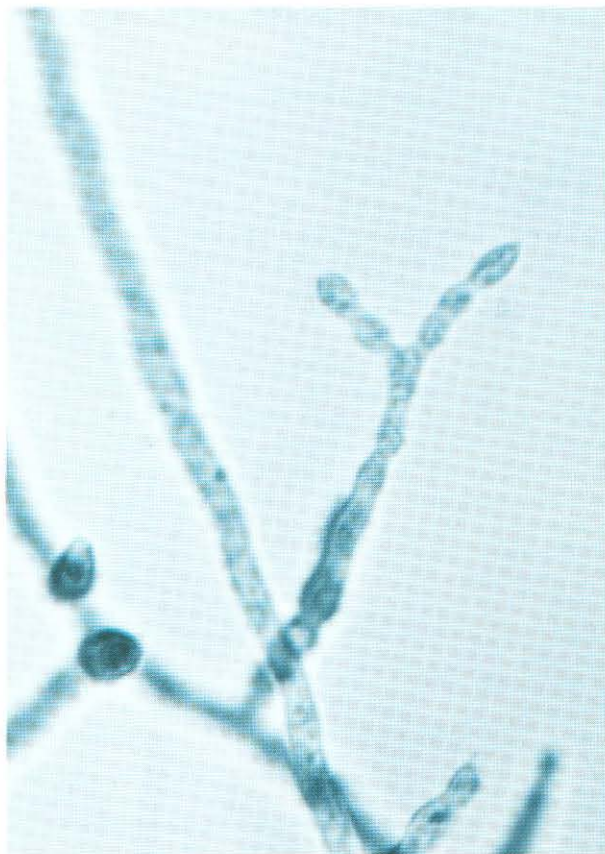
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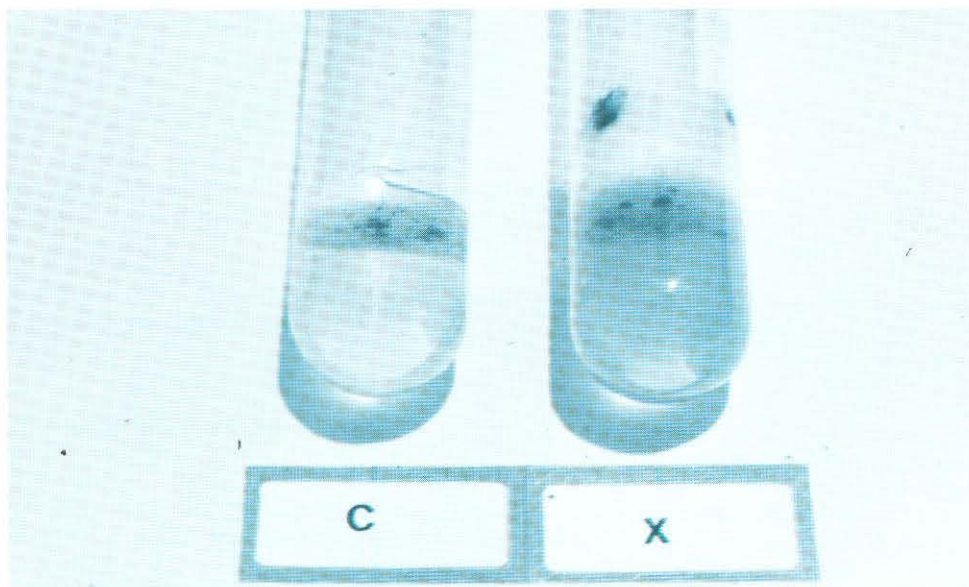
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Ph. N° 1 *Tribolium ferrugineum* (Fabricius)



Ph. N° 2 *Chrysonilia sitophila* x 1000



Ph. N° 3 Cellulase azure test: C = control, X = tube inoculate with *C. sitophila*.

REFERENCES

1. Janshekar, H. and Fiechter, A. (1983) "Lignin Biosynthesis, Application and Biodegradation". *Advan. Biochem. Eng. Biotechnol* 27: 119–178.
2. Reese, E.t., Mandels and Weis, A.H. (1972). Cellulase as novel Energu source. *Advan. Biochem. Eng.* 2: 181–200.
3. Rauau, X. and Odier, E. (1986). Production of extra-cellular enzyme by the white-rot fungus *Dichonitus squalens* in cellulose-containing liquid culture. *Enzyme Microb. Technol.* 8: 22–26.
4. Mandels, M. (1985). Applications of cellulases. *Biochem. Soc. Trans.* 13 : 414–415.
5. Viegas, J. A. and Motta de Barros, P. (Eds.) (1985). *Biotechnología e desenvolvimento nacional. Secr. Ind. Comer. Cienc. Tecnol. Dep. Cienc. Tecnol. Sau Paulo, Brazil.*
6. Durán, N., Mansilla, H. and Reyes, J.L. (1986). Biomass photochemistry-X: Modifications in Lignin under ultraviolet irradiation. *Polymer Phototechm.* submitted.
7. Metcalf, C.L. and Flint, W.P. (1951). "Destructive an useful insects, their habitas and control" Mc. Graw-Hill Book Company, New York.
8. Samson, R.A. y Hoekstra, E.S. y Von Oorschot, C.A.N. (1981) "Introduction to food-horne fungi". Ed. entralalbureau Voor Schimmelcultures. Baarn Holand.
9. Patterson, G., Cowling, E.B. and Porath, J. (1963). Sudies on cellulolytic enzymes. I. Isolation of a low-molecular-weight cellulase from polyporus versicolor *Bioquim. Biophys. Acta* 67 : 1–8.
10. Smith, R.E. (1977). Rapid tube test for detecting fungal cellular production. *Appl. Environ. Microbiol.* 33: 980–981.
11. Mandels, M. Andreotti, R. and Roche, C. (1976). Measurement of Saccharifying cellulose. *Biotechnol. Bioeng. Symp.* 6 : 21–33.
12. Herr, D. (1979). Secretion of Cellulose and B. Glucosidose by *Trichoderma Viride* ITCC-433 in Submerged culture of Different, Substrates. *Biotechnol. Bioeng.* 21 : 1361–1371.
13. Fadda. M.B., Dessi, M.R., Maurici, A. and Satta, G. (1984). Highly efficeint solubilization of natural lignocellulosics materials by a commercial cellulase immobilized on various solid support, *Appl. Microbiol. Biotechnol.* 19 : 306–311.
14. Shear, C.L. and Dodge, B.O. (1927). Life histories and heterothallism of the red bread mold fungi of the *Monilia sitophila* groups. *J. Agr. Res.* 33 : 1019–1042.
15. Krieg, N. and Holt, J.G. (Edit.) (1984). *Bergey's Manual of Systematic Bacteriology*, Vol. 1 9th. Ed. Baltimore, Usa. Williams and Wilkins Co.