Summary. Enzymes from a range of fungi have been obtained from culture filtrates and by squeezing wooden cubes in which fungi had penetrated. They were directly mixed with 2 concentrations of 3 wood preservatives and incubated with either starch or cellulose (CMC) so that any subsequent formation of glucose would indicate the in vitro enzymic tolerance to the toxicants. With every fungus, substrate and method of obtaining the enzymes there was some enzymic activity irrespective of the presence of a preservative. The implications of this tolerance for wood preservation are discussed.

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Sensitivity to U.V. Treatment and Nuclear Size of Mycoplasma-Like Organism Infected *Humicola* sp.

In a previous paper we reported the presence of a mycoplasma-like organism (MLO) in fungi belonging to the genus *Humicola* Traaen[1]. The MLO was successfully transmitted within some related *Humicola* strains by infecting healthy strains with ultrafiltrates of MLO carrying cultures[1]. The morphological feature of the most interesting strain (named 2–1) in which the MLO infection has been transmitted, has been described elsewhere[2]. The MLO infection greatly influenced its morphology and the infected substrain was named Strain Z-1. This anomalous morphology is actually maintained over a 2-year period of subculturing on malt agar.

On malt agar Strain Z-1 colonies, completely lacking aerial mycelium as opposed to 2-1 colonies with aerial mycelium, have a crusty aspect, and are easily distinguishable from any other *Humicola* colony.

Time after time, but at very low frequency, colony sectors very similar to the well known saltations in fungi arose on Z-1 colonies grown on malt agar (Figure 1). Colonies obtained from sectors (R-type colonies) have a white colour with abundant aerial mycelium. Under optical and electron microscope checks, MLO were still found in R-type subcultures although their frequency was noticeably lowered (unpublished data).

According to these findings, we supposed that Strain Z-1 and R-type strains represent different types of host-MLO interrelationships, their expression being dependent on physiological or biochemical characters of the microfungus, and possibly of the MLO too. As a consequence, mutagenic agents such as U.V. treatment of Strain Z-1 would increase the frequency of R-type colonies.

The present work deals with the U.V. treatment and the nuclear sizing of parental Strain 2-1, of MLO -infected Strain Z-1, and of one of R-type substrains.

Materials and methods. Strain 2-1 has been described elsewhere[2]. Strain Z-1 has been obtained by infecting Strain 2-1 with ultrafiltrates of mycelium of a MLO-carrying microfungus[1]. The colony morphology on malt extract agar (BBL, Maryland) is the following: lack of aerial mycelium; colonies rough, first yellow brown; in old cultures the mycelium becomes brown; pigment not diffused into the agar; hyphae hyaline, wide, often broken and empty, always distorted. Intercalary chlamydospores are abundantly produced, sometimes in chains. In old cultures aleuriospores are formed, first light yellow, later yellow brown, 10 µm in diameter, very irregularly shaped. At the cytomorphological level the strain differs from Strain 2-1 both in the lack of regular aleuriospores and typical phialoconidia, and the morphology of the developing mycelium (Figure 2).

Substrains of R-type have been obtained by subculturing the spontaneous sectorial mutation of Strain Z-1. Strain R-1 is characterized as follows: colonies white with aerial compact mycelium; hyphae hyaline, narrow; few intercalary chlamydospores are present. After 4–5 days aleuriospores are produced, later abundant, irregu-

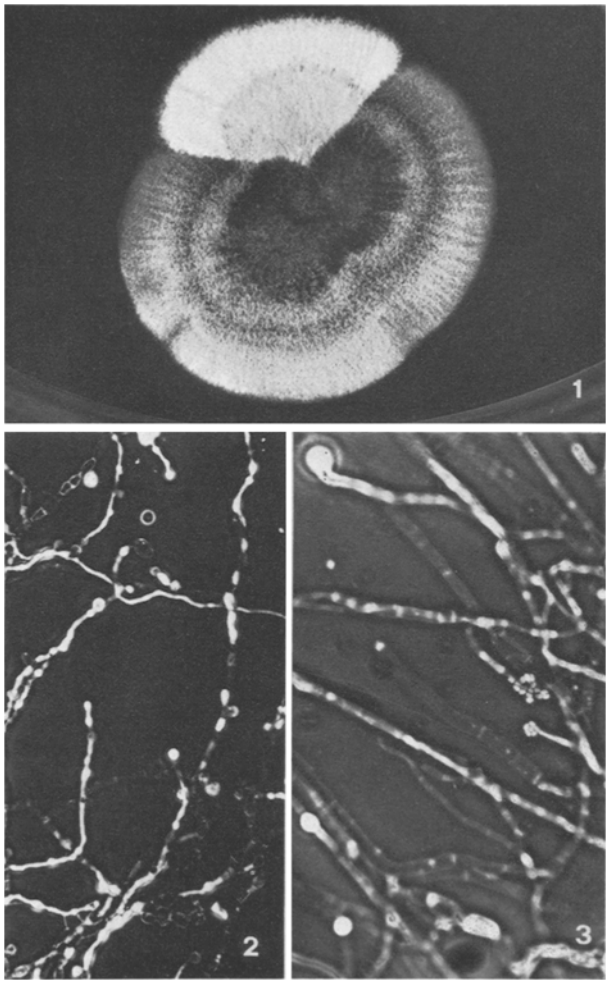

Fig. 1. Sector of R-type arizing on Strain Z-1 colony.
Fig. 2 and 3. Morphological feature of Strain Z-1 and Strain 2-1.

[1] A. A. LEPIDI, M. P. NUTI, C. FILIPPI, G. BAGNOLI, V. GHERARDUCCI and G. PICCI, Ann. Microbiol., Milano *24*, 241 (1974).

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larly shaped, 10 µm in diameter. Phialoconidia are sometimes formed, grouped in persistent chains. At the cytomorphological level it looks like an intermediary organism between Z-1 and 2-1 (Figure 3). All the present strains were routinely maintained on slants of malt extract agar at 26 °C.

As far as the U.V. treatment is concerned, samples of actively growing mycelia in stirred cultures on malt broth (BBL) were collected and treated with a Potter homogenizer until the mycelium was uniformly dispersed.

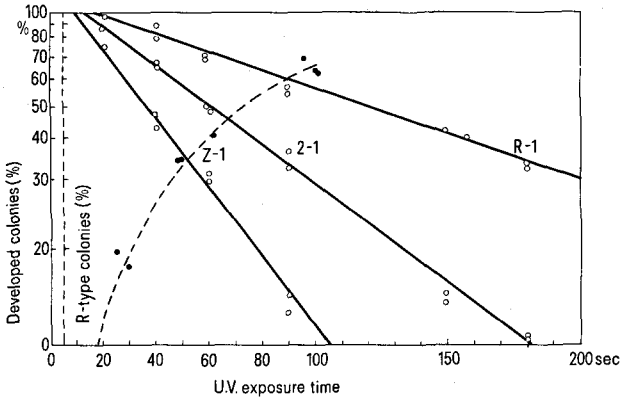

Fig. 4. U.V. sensitivity of Strains 2-1, Z-1 and R-1 (solid line) and U.V. induced R-type colonies from Z-1 (dotted line).

Diameters and volumes of the Giemsa-positive bodies

Strain	Diameter (µm)	Volume (µm³)
2-1 [a]	1.1	0.72
Z-1 [a]	0.9	0.41
[b]	0.3	0.03
R-1 [a]	1.2	1.07

[a] Nucleus of the fungal cell. [b] Giemsa-positive bodies related to the MLO infection.

Plates of malt agar of 11 cm in diameter were inoculated at a density giving 150 growing colonies, on average. Just after the inoculum, the surface of the plates was exposed to a Philips U.V. germicidal lamp (mod. TUV, 15 Watt) at a distance of 25 cm for various times; the cultures were incubated at 26 °C for 1 week. On the plates inoculated with Strain Z-1 both the number of surviving cells and the colonies showing the typical Z-1 morphology were recorded. In order to ascertain the persistence of the colony type, the colonies having a R-type morphology were again treated in a Potter homogenizer, and dilutely plated on malt agar.

As far as the staining of nuclei and electron microscopy is concerned, the following procedures have been employed: a) Slide cultures according to RIDDEL[3] were harvested after 4 days of development and stained with the Gurr-Giemsa solution for 12 min; before staining, the specimens were fixed for 10 min in Carnoy's fluid, and hydrolyzed with 1 *N* HCl for 10 min at 60 °C. b) Specimens were fixed with 3% glutaraldehyde in phosphate buffer 0.05 *M*, pH 6.1, for 1 h at 4 °C, then washed in the same buffer; they were postfixed in 1% OsO_4 in the same buffer for 1 h at room temperature. After the embedding in Epon-Araldite by conventional methods, the specimens were stained according to DE BERTOLDI et al.[4] an observed in a Siemens E. M. Elminskop I.

Results and discussion. The responses of the assayed strains to the U.V. treatment are illustrated in Figure 4. In the same figure, the frequency of the R-type colonies deriving from Z-1 after U.V. treatment is reported. All the strains here considered show a 100% survival after short exposure to U.V., so indicating the presence of more than one nucleus per colony forming unit (a mycelial fragment consisting of cells which are also known from cytological evidence to contain more than 1 nucleus per cell)[5].

[3] R. W. RIDDEL, Mycologia *42*, 265 (1950).
[4] M. DE BERTOLDI, F. MARIOTTI and C. FILIPPI, Can. J. Microbiol. *20*, 237 (1974).
[5] K. C. ATWOOD and A. NORMAN, Proc. natn. Acad. Sci., USA *35*, 696 (1949).

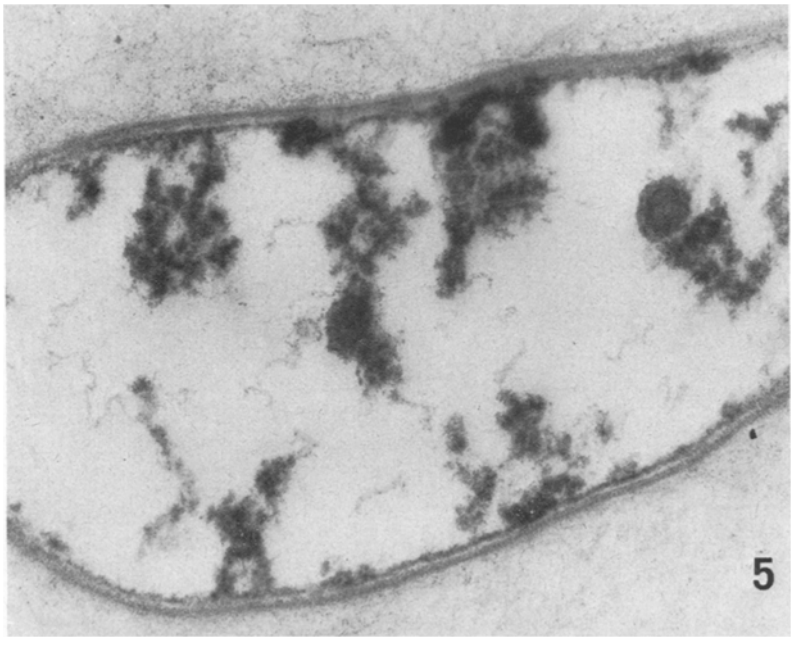

Fig. 5. Electron micrograph of the small bodies present in the Strain Z-1 cells.

Linear inactivation curves are obtained by plotting on a semilog scale the viable cell number against the exposure time. The LD_{50} ranged from 40 to 112 sec. The different shape of the curves could be connected with different factors, such as either a different transparency of the external cell layers to U.V. light, or differences at the nuclear level (e.g. the nuclear ploidy or the number of nuclei per colony forming unit).

The percentage of R-type colonies present on plates of Strain Z-1 after U.V. treatment does constantly increase in proportion to the duration of the treatment, thus indicating that metabolic changes, presumably due to U.V.-induced mutations in the host genome, may interfere with the expression of the host-parasite interrelationship. Further suggestion of a metabolic complementarity between MLO and its host, likely at the protein synthesis level, arose also during attempts to eliminate the presence of MLO from infected strains by treatment with antibiotics effective against mycoplasmas, e.g. erytromycin, kanamycin, lincomycin, spiromycin, and tetracyclines. Evidence is available that tetracycline does inhibit the microfungus strain according to whether MLO is invasive, latent, or absent (work in progess).

As far as the dimensions of Giemsa-positive bodies are concerned, the strains here studied have the diameters and volumes summarized in the Table.

Two different populations of Giemsa-positive bodies have been observed in Strain Z-1, having diameters of 0.9 μm (fungal nuclei, several nuclei per cell as in Strains 2-1 and R-1) and 0.3 μm on the average. More than 1 dozen of the small bodies (an electron microscopic picture of these bodies is reported in Figure 5) may be present in 1 fungal cell, their presence having a probable connection with the MLO infection. In cells of R-type strains their number is significantly reduced, more than 70% (unpublished data).

As a concluding remark, the present data seem to support the evidence that an interplay does take place between the nuclear characters of the host strain and the invasiveness and pathogenicity of the MLO.

Summary. The infection of MLO in a soil microfungus (*Humicola* sp.) originates different types of non-lethal parasitism. Differences have been found in the U.V. sensitivity and nuclear characters according to the type of MLO-microfungus interrelationship.

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Involvement of a Surface Concanavalin A-Binding Glycoprotein in the Adhesion of *Trichomonas vaginalis* to Substrates

Trichomonas vaginalis cells will adhere to various types of solid support[1]. When this occurs on the walls of culture vessels, monolayer cultures not unlike those of multicellular organisms, are formed[2]. Adhesiveness is probably dependent on the properties of the cell membrane and many lines of evidence indicate that, in eucaryotic cells, surface glycoproteins are involved in adhesion phenomena[3].

A study was therefore made on the *T. vaginalis* surface to investigate the role of carbohydrate-containing proteins in cell-to-glass adhesion.

Materials and methods. The *Trichomonas* strain employed (FC) was maintained axially as previously described[2]. Only 12-h cultures containing $>98\%$ viable cells, as determined by trypan blue exclusion test[4], were used.

To determine binding of the jack bean lectin concanavalin A (ConA) to the surface of *T. vaginalis*, an indirect immunofluorescent test was performed[5]. Washed cells (4×10^4/ml in 0.14 *M*, pH 7.0 phosphate buffered saline – PBS) were incubated with excess ConA (Pharmacia Fine Chemicals AB; batch No. 7623) for 15 min at 37 °C and, after repeated washing, with a fluorescein isothiocyanate (FITC)-labelled immunoglobulin fraction of anti-ConA rabbit antiserum. Further washing was followed by resuspension in PBS and observation under a Leit Ortolux microscope with transmitted UV-light.

The ethylene diaminetetraacetic acid (EDTA) treatment of *Trichomonas* cells was performed as previously described[6]. The EDTA soluble fraction obtained (EDTA-SF) was then separated by affinity chromatography with ConA covalently bound to Sepharose-4B. 20 ml of EDTA-SF containing 35 mg of protein was dialyzed against 0.1 *M* sodium acetate buffer, pH 6.0 containing 1 *M* NaCl, 0.001 *M* $CaCl_2$, 0.001 *M* $MgCl_2$ and 0.001 *M* $MnCl_2$. The solution was then applied to a column of ConA-Sepharose (Pharmacia Fine Chemicals AB; batch No. 6745), bed volume 48 ml, and eluted with the above buffer. After the unbound fraction was collected (ConA-UF), elution with α-methyl-D-glucopyranoside gave a fraction (ConA-BF) that proved homogeneous on sodium dodecyl sulphate-(SDS)-polyacrylamide gel electrophoresis and was apparently a glycoprotein since it was stainable with Coomassie blue and PAS-positive[7].

Results and discussion. The distribution of ConA receptors on the surface of normal *T. vaginalis* is shown in the Figure a; fluorescence pattern is uniform with dots of varying intensity distributed over the entire cell surface. The appearance of fluorescence on the membrane is specific, since it is completely inhibited when ConA is pre-incubated with 0.1 *M* α-methyl-D-glucopyranoside as a hapten inhibitor.

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