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EXPERIMENTAL MYCOLOGY 15, 206–214 (1991)

# Infrared Monitoring of the Adhesion of *Catenaria anguillulae* Zoospores to Solid Surfaces

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Accepted for publication April 10, 1991

TUNLID, A., NIVENS, D. E., JANSSON, H.-B., AND WHITE, D. C. 1991. Infrared Monitoring of the adhesion of *Catenaria anguillulae* zoospores to solid surfaces. *Experimental Mycology* 15, 206–214. Electron microscopic studies of nematodes infected with the chytridiomycetous fungus *Catenaria anguillulae* indicated that zoospores of the fungus adhered to the cuticle of nematodes by a layer of extracellular polymers. The chemical composition of the adhesive polymers and their interaction with a solid surface were examined with Fourier transform infrared spectroscopy, using an attenuated total reflectance cell. On-line monitoring of the adhesion of zoospores to a germanium crystal with this technique showed that the adhesive polymers consisted of a protein(s) containing amide I and II bands. The adsorption of these proteins, measured as the increase in the amide II band, had a rapid initial phase of ca. 20 minutes, followed by a slower increase during the course of incubation. Fluorescein isothiocyanate staining of the attached cells at the end of the experiment showed that the adhesion of the zoospores occurred before the formation of the cyst wall. 

INDEX DESCRIPTORS: adhesion, *Catenaria anguillulae*, FTIR, proteins, zoospores.

Adhesion to the host surface is an important step in the infection process of fungal zoospores and ensures that the pathogen is not washed away before subsequent germination and invasion (Bartnicki-Garcia and Sing, 1987). Adhesion in zoospores is considered to be an initial phase of the encystment, swimming zoospores are not adhesive but become so just before a cyst wall is formed (Sing and Bartnicki-Garcia, 1975a,b). For an individual zoospore, the duration of time over which adhesion occurs is extremely short and lasts only about 30–60 s for *Phytophthora palmivora* (Bartnicki-Garcia and Sing, 1987) and between 1 and 4 minutes for *Phytophthora cinnamomi* (Gubler *et al.*, 1989). The adhesive materials of the *Phytophthora* species as well as of *Pythium aphanidermatum* are rapidly secreted from vesicles, located inside the plasma membrane, immediately after induction of encystment (Sing and Bartnicki-Garcia, 1975a,b; Gubler and Hardham, 1988; Estrada-Garcia *et al.*, 1990). Cytochemistry and chemical characterization of the adhesive polymers from these zoospores have shown that they are glycoproteins.

So far, adhesion studies of zoospores have been performed in systems using intact roots, preparations of plant cells or protoplasts, or inert surfaces such as glass and plastics (Sing and Bartnicki-Garcia, 1975a; Bartnicki-Garcia and Sing, 1987; Longman and Callow, 1987; Hohl and Balsiger, 1986; Gubler *et al.*, 1989). The main limitation of these methods is that they are destructive and do not allow for real time monitoring of the molecular interactions

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0147-5975/91 $3.00

between the zoospore and the surface. Fourier transform infrared (FTIR)[2] spectroscopy in combination with an attenuated total reflectance (ATR) flow cell is a sensitive and nondestructive technique that has been used to characterize the adsorption of proteins and other macromolecules to surfaces (Gendreau, 1986). The IR portion of the spectrum is rich in information regarding the vibrational and rotational motions of atoms in molecules (Bellamy, 1958; Parker, 1971). Specific IR absorptions can be assigned to particular types of covalent bonds, and modifications of these bonds by the local electronic environment can be detected in the details of the spectra.

In contrast to conventional IR methods, FTIR spectroscopy has the speed, sensitivity, and precision to monitor the interactions between molecules and surfaces, even in water solutions (Gendreau, 1986). In combination with an ATR cell, FTIR spectroscopy measures the absorption spectra of compounds located within part of a micrometer of a crystal surface (Griffiths and deHaseth, 1986). This technique has been utilized to study the interactions between blood proteins and surfaces (Gendreau and Jacobsen, 1979), growth of mammalian cells (Hutson *et al.*, 1988), development of biofilms on surfaces (Baier, 1980; Nichols *et al.*, 1985), and the adhesion mechanisms of bacteria (Nivens *et al.* 1988).

All work on the adhesion mechanisms of zoospores has so far been performed on Oomycetes such as *Phytophthora* spp. and *Pythium* spp. and virtually nothing is known about these phenomena in other zoosporic fungi, e.g., Chytridiomycetes. In this study, we have used the FTIR–ATR technique to examine the adhesion of zoospores of the chytridiomycetous fungus *Catenaria anguillulae* to a solid surface. *C. anguillulae* is one of the most common endoparasitic fungi attacking nematodes (Barron, 1977). Zoospores of this fungus adhere and encyst at the nematode cuticle and after germ tube formation, the nematode is penetrated and infected.

[2] Abbreviations used: ATR, attenuated total reflectance; FTIR, Fourier transform infrared; DRIFT, diffuse reflectance FTIR; TEM, transmission electron microscopy; SEM, scanning electron microscopy.

## MATERIAL AND METHODS

*Culture of organisms. C. anguillulae* Sorokin (CBS 117.87) was maintained on YPSS medium (20.0 g soluble starch, 1.0 g yeast extract, 1.0 g $K_2HPO_4$, 0.5 g $MgSO_4$, 15.0 g agar, 1000 ml distilled water) at 30°C. To produce zoospores the fungus was transferred to PYG medium (1.25 g peptone, 1.25 g yeast extract, 3.0 g glucose, 15.0 g agar, 1000 ml distilled water, pH 6.8) and grown for 2–4 days at 30°C. The nematode *Panagrellus redivivus* (Linn.) T. Goodey was grown axenically in a soya peptone–liver extract medium (Nordbring-Hertz, 1977).

*Transmission electron microscopy (TEM).* Zoospores, both motile and encysted, were fixed by adding 4% glutaraldehyde in cacodylate buffer drop-wise to a final concentration of 3% and left over night at 4°C. The zoospores were washed three times in the buffer and treated with 2% $OsO_4$ in cacodylate buffer at 4°C and again washed three to four times in the buffer. The specimens were dehydrated in an ethanol series and embedded in Epon, sectioned on a Reichert-Jung Supernova ultramicrotome, and examined in a Jeol Temscan 200CX electron microscope.

*Scanning electron microscopy (SEM).* Nematodes with adhering, and encysted, zoospores were treated with unbuffered 2% (final concentration) glutaraldehyde added drop-wise during 3–5 min and fixed for 20 min at 22°C. The nematodes were washed three to four times in distilled water, dehydrated in an ethanol series, collected on a Nucleopore filter, and critical-point dried. The specimens were mounted on aluminum stubs and coated with Au and observed in a

Philips SEM 515 scanning electron microscope.

*Cell adhesion.* Zoospores of *C. anguillulae* were harvested by flooding 2- to 4-day-old culture plates (PYG medium) with distilled, deionized water ($dH_2O$). The spores were washed twice with filtered (0.22 μm) $dH_2O$ by centrifugation (3000*g*, 10 min). The zoospores were diluted in $dH_2O$ to a concentration of $10^7$ cells/ml and injected with a syringe into an ATR flow cell. The experimental setup is shown in Fig. 1. The ATR cell with zoospores was placed in the FTIR instrument and IR spectra were recorded about every 2.5 min for 30 min, every 6–7 min between 30 and 60 min, every 20 min between 1 and 3 h, and every hour between 3 and 12 h of incubation. At this time, any free or loosely adherent zoospores were removed by extensively rinsing the ATR cell with several volumes of $dH_2O$. Final FTIR spectra were recorded of the attached zoospores. The ATR cell was then dismounted and the adhered spores were examined with epifluorescence microscopy, after staining with fluorescein isothiocyanate (Babiuk and Paul, 1970). The ATR experiments were performed at room temperature (20°C) and repeated twice.

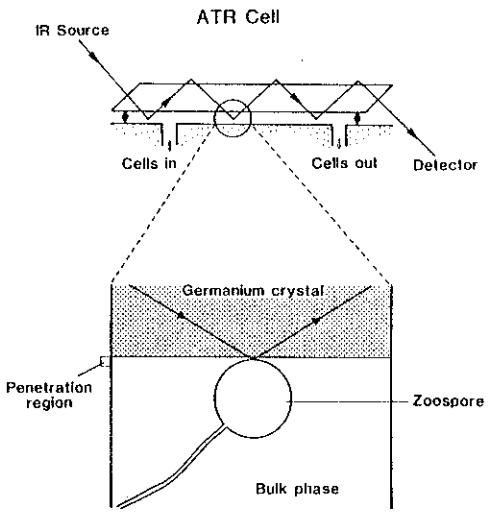


FIG. 1. Schematic diagram of the attenuated total reflectance (ATR) cell used in the adhesion experiments. The IR beam on the internal reflection in the germanium crystal penetrates into the aqueous medium, and IR absorption spectra can be recorded continuously of compounds located in the region of penetration.

*Fourier transform infrared spectroscopy.* The ATR cell was supplied by Harrick Scientific Corp. (Ossining) and consisted of a germanium crystal, O-ring seals, and a crystal holder assembly. The assembly is made of two metal plates which contain an inlet, an outlet, and a groove. The metal plates were clamped around the germanium crystal to form the flow channels and the O-rings provided the seals. Using this cell, the germanium crystal surface is held vertically. The ATR cell was placed in the microbeam chamber of a Nicolet 60SX FTIR instrument, equipped with a liquid nitrogen cooled, high sensitivity, mercury:cadmium: tellurium detector (range 5500–710 $cm^{-1}$), a mid-IR Globar source, and a KBR beamsplitter (Nicolet Instrument Corp., Madison, WI).

The internal reflection element in the ATR cell was germanium metal (50 × 10 × 2 mm, refractive index, 4.01, Harrick Sci. Corp.) cut to provide a 45° angle of incidence (θ). The depth of penetration ($d_p$) of the infrared energy into the water solution of zoospores with respect to the wavelength of light (λ) is approximately $d_p = 0.0638 * \lambda$ (Fink and Gendreau, 1984). This results in a $d_p$ for the amide I band (1650 $cm^{-1}$) of 385 nm. Each sample scan resulted in a simple-sided interferogram of 4096 data points which provided a resolution of 4 $cm^{-1}$. Interferograms were zero-filled and apodized by the Haap–Genzel function prior to the Fourier transformation, utilizing the Nicolet SX software (TMON version 2.0). Signals averaging 256 scans per sample required 72 s of total measurement time. All spectra were ratioed to a background of the dry ATR cell. Transmission spectra were converted to absorption spectra, and water background spectra of

filtered (0.22 μm) $dH_2O$ were subtracted. Spectra of water vapor were also subtracted when necessary. The sample chamber (outside the ATR cell) and optical bench were under a constant nitrogen purge throughout the analysis.

The IR spectra from the ATR experiments were compared with spectra of lyophilized zoospores obtained using the diffuse reflectance FTIR (DRIFT) technique. Samples of zoospores, harvested from the PYG medium, were washed with $dH_2O$ and lyophilized. Weighed amounts of the lyophilized cells were mixed with potassium bromide at a ratio of 1:10. The DRIFT accessory was from Spectra Tech Inc. (Stamford, CT), and the samples were scanned as described above. The spectra were interpreted based on the Kubelka–Munk analysis (Griffiths and Fuller, 1982).

## RESULTS

Examination by SEM indicated that the adhesion of zoospores of *C. anguillulae* to the cuticle of the nematode *P. redivivus* was mediated by a layer of extracellular polymers (Fig. 2A). A polymer layer outside the cell wall was also visualized by TEM on encysted zoospores but was absent on nonadhering zoospores (Fig. 2B).

On-line IR monitoring of the adhesion of *Catenaria* zoospores to the germanium crystal surface in the ATR cell showed the appearance of molecules with amide I (1645 $cm^{-1}$) and amide II (1550 $cm^{-1}$) bands after only 2.5 minutes of incubation (Fig. 3). The intensity of these bands increased rather rapidly during the first 20 minutes of incubation, and then more slowly during the course of the experiment (Fig. 4).

After about 2 h of incubation, at least two new IR bands at frequencies lower than the amide II bands appeared in the IR spectra at 1450 and 1400 $cm^{-1}$ (Fig. 5). These bands, as well as the amide I and II bands, were also present in the IR spectra recorded after 12 h of incubation. No peaks were seen in the carbohydrate region of the IR spectra (1150 $cm^{-1}$). Subtracting of sequential scan sets during the course of the experiment did not reveal any qualitative changes in the IR spectra during the incubation. Washing the ATR flow cell with water did not change the IR spectra, as shown by subtracting the IR spectra before and after the wash (Fig. 6).

Examination of the crystal surface by epifluorescens microscopy at the end of the experiment showed the presence of attached zoospores. The number of attached zoospores was estimated to about $10^5$ cells/$cm^2$. Notably, the attached zoospores were not encysted.

The FTIR spectra of lyophilized zoospores recorded using the DRIFT technique were considerably different from the FTIR–ATR spectra (Fig. 7). Except from the amide bands, the DRIFT spectrum contained prominent IR bands at 1748, 1240, 1100, and 1032 $cm^{-1}$.

## DISCUSSION

The results presented here constitute the first report on the mechanisms involved in the adhesion of zoospores of the chytridiomycete *C. anguillulae*. On-line monitoring of the FTIR spectra of zoospores from this fungus adsorbing onto a germanium crystal surface in the ATR flow cell showed that they adhered to solid surfaces using polymers containing amide I and II bands. These bands are in the region of C=O stretching (amide I), and N–H in-plane bending and C–N stretching (amide II), which is characteristic for proteins (Gendreau, 1986). The frequencies observed at 1450 and 1400 $cm^{-1}$ are probably related to the structure of the side chains ($CH_2$ and carboxyl) of the proteins (Gendreau, 1986). That these protein bands were due to the IR absorption of an adhesive from the zoospores was indicated by the fact that the spectra did not change after washing away free cells and extracellular material from the ATR cell, and by demonstrating that the remaining cells were attached zoospores.

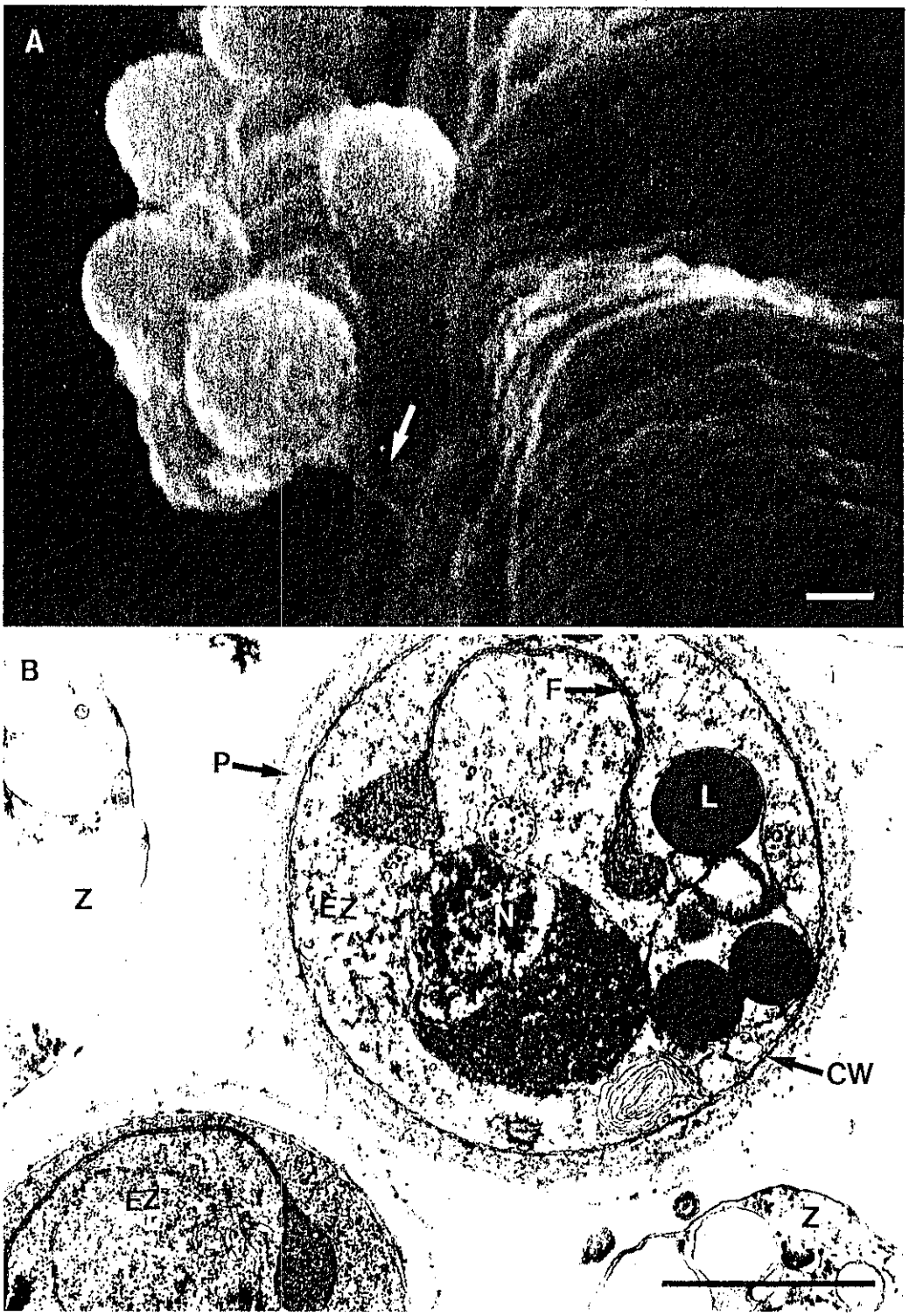


FIG. 2. (A) Scanning electron micrograph of encysted zoospores of *C. anguillulae* adhering to the cuticle of the nematode *P. redivivus*. Germ tube penetrating in the cuticle (arrow). Micrograph recorded after about 1 h of interaction. Bar: 1 μm. (B) Transmission electron micrograph of *C. anguillulae* zoospores (Z; no extracellular polymers visible) and encysted zoospores (EZ; with a polymer layer). N, nucleus; L, lipid body; P, polymer layer; F, withdrawn flagellum; CW, cell wall. Bar: 1 μm.

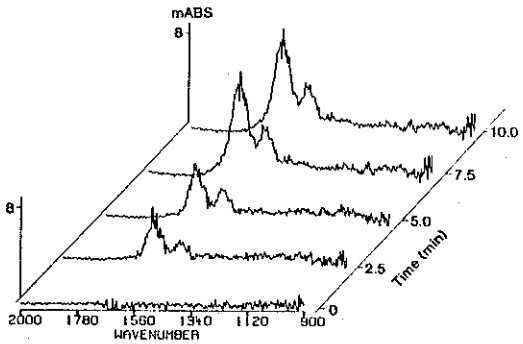


FIG. 3. Three-dimensional plot of FTIR spectra collected every 2.5 min plotted as a function of time of adhesion of *C. anguillulae* onto a germanium crystal surface in an ATR cell. The major IR bands are at 1645 $cm^{-1}$ (amide I) and 1550 $cm^{-1}$ (amide II).

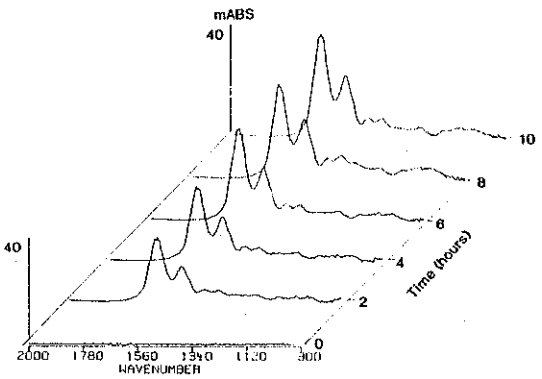


FIG. 5. Three-dimensional plot of FTIR spectra collected every 2 h plotted as a function of time of adhesion of *C. anguillulae* onto a germanium crystal surface in an ATR cell. The major IR bands are at 1645 $cm^{-1}$ (amide I) and 1550 $cm^{-1}$ (amide II).

The depth of penetration of the IR beam with the used ATR cell was about 0.4 μm, which can be compared with the thickness of 0.2 μm of the extracellular polymer layer present on the encysted zoopores, as visualized by TEM. The FTIR spectra recorded using the ATR cell might therefore include both extracellular and intracellular components. However, the major differences between the IR spectra from the ATR cell and the DRIFT accessory indicate that the ATR technique mainly record IR absorption of extracellular components. The depth of penetration of the DRIFT technique is several millimeters (Griffiths and Fuller, 1982). The prominent band at 1748 $cm^{-1}$ in the DRIFT spectrum can be assigned to C=O stretching in esters of glycerides (Kates, 1986). Encysted zoospores of *C. anguillulae* contain big lipid bodies (Fig. 2B) and analysis of lipids in both zoospores and encysted cells has shown that they have a high content of acylated neutral lipids (5–10%, Tunlid unpublished data). The correspond-

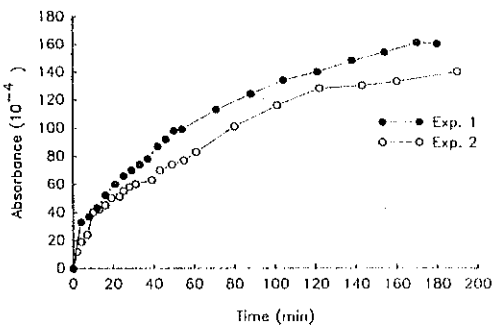


FIG. 4. The intensity of the amide II bands as a function of incubation time of *C. anguillulae* in the ATR cell. Data from two different experiments are presented.

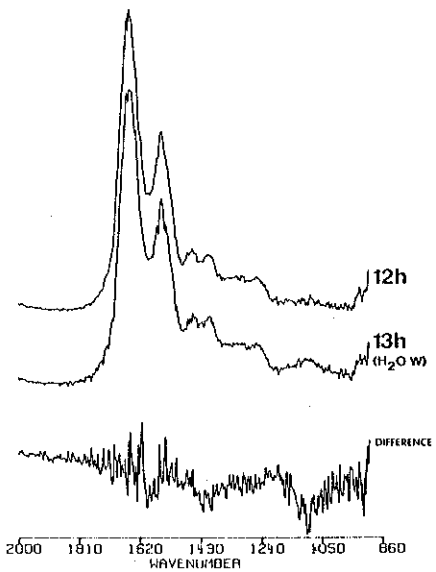


FIG. 6. The FTIR spectra of *C. anguillulae* attached to a germanium crystal surface for 12 h (top), for 13 h, including 1 h of extensive washing of the ATR cell with water (middle), and these two spectra subtracted (bottom).

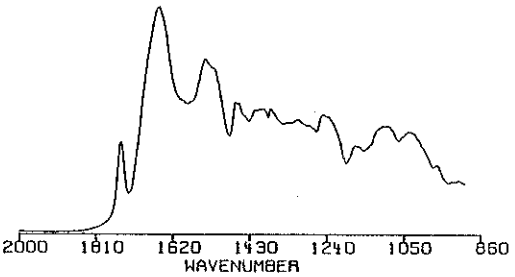


FIG. 7. Reflectance IR spectra (DRIFT) of lyophilized zoospores of *C. anguillulae*.

ing IR band from intracellular fatty acid esters was absent in the FTIR ATR spectra.

Previous studies have shown that the kinetics of protein adsorption to surfaces in ATR experiments can be determined by plotting the intensity of the amide bands, which are not sensitive to conformation or structural changes, against the time of incubation (Gendreau, 1986). The amide II band is such a band and the corresponding plot from our experiments showed that the adsorption of proteins from the zoospores had an initial rapid phase of about 20 minutes, followed by a slower increase during the course of incubation. This suggests that the adhesion phase of individual zoospores of *C. anguillulae* is very short, as has also been reported for *Phytophthora* zoospores (Bartnicki-Garcia and Sing, 1986; Gubler *et al.*, 1989). The following slower increase in the intensity of the amide II band indicates that the zoospores were adhering to the crystal during the whole incubation period, which can be expected since the encystment was not synchronized in these experiments. Microscopy of the attached cells showed that they, contrary to the zoospores adhered to the nematode cuticle, had flagella and remained as zoospores. Thus, the adhesion of zoospores of *C. anguillulae* to the germanium crystal seems to occur before the cyst wall is formed. Similarily, in *P. palmivora* adhesion occurs before or during the initiation of cyst wall microfibril synthesis (Sing and Bartnicki-Garcia, 1975a,b).

The reasons why the zoospores adhering to the germanium crystal were not encysted are not known. Experiments have shown that the encystment of *C. anguillulae* can be induced by the lectin concanavalin A in a mechanism similar to that which has been reported for *P. cinnamomi* (Jansson, unpublished data) (Hardham and Suzaki, 1986). This observation indicates that the encystment process of *C. anguillulae* is triggered by the interactions between proteins on the surface of the zoospores and the cuticle of the nematode. The question, therefore, arises as to whether the adhesion mechanism of the *Catenaria* zoospores observed in the ATR cell is similar to that of those adhering to the nematode cuticle. Results from studies using zoospores of *P. palmivora* and *P. cinnamomi* indicate that the adhesion mechanisms expressed during the early stages are nonspecific and similar on both inert and living surfaces (Sing and Bartnicki-Garcia, 1975a; Gubler *et al.*, 1989). However, experiments of the adhesion mechanisms of *P. aphanidermatum* to plant roots (Longman and Callow, 1987), as well as of several nematophagous fungi to nematode surfaces (Nordbring-Hertz, 1988; Jansson and Nordbring-Hertz, 1988), have indicated that the adhesion of these fungi to their host cells is a complex process involving both specific lectin–receptor interactions and other mechanisms.

FTIR spectra of living cells are enormously complex and it is very difficult to assign the absorption bands to specific molecules (Nichols *et al.*, 1985; Hutson *et al.*, 1988). Subtractions of spectra obtained from isolated polymers and the Fourier self-deconvolution technique can be very valuable tools in such studies (Gendreau, 1986). However, rather than trying to assign the bands to specific molecules, FTIR spectroscopy of whole cells should be focused on detecting reproducible spectral changes occurring during specific cellular or physiological processes. We think that the FTIR–ATR technique as demonstrated

in this paper can be a very valuable tool for monitoring the molecular interactions between microorganisms and surfaces and for studying how these processes are affected by the surface structure and the surrounding environment.

## ACKNOWLEDGMENTS

This study was supported by grants from the Swedish Natural Science Research Council to A.T. and H.-B. J. D.E.N. and D.C.W. were supported in part by Grant No. N00014-87-K-0012 from the Office of Naval Research U.S. Department of Defense and Environmental Science Division, Oak Ridge National Laboratory.

## REFERENCES

BABIUK, L. A., AND PAUL, E. A. 1970. The use of fluorescein isothiocyanate in the determination of the bacterial biomass of grassland soil. *Can. J. Microbiol.* 16: 57–62.

BARRON, G. L. 1977. *Topics in mycology No. 1. The Nematode-Destroying Fungi.* Canadian Biological Publications Ltd., Guelph.

BAIER, R. E. 1980. Substrata influences on adhesion of microorganisms and their resultant new surface properties. In *Adsorption of Microorganisms to Surfaces* (G. Bitton and K. C. Marshall, Eds.), pp. 59–104. Wiley, New York.

BARTNICKI-GARCIA, S., AND SING, V. O. 1987. Adhesion of zoospores of *Phytophthora* to solid surfaces. In *Zoosporic Fungi in Teaching and Research* (M. S. Fuller and A. Jaworski, Eds.), pp. 279–283. S.E. Pub. Corp., Athens, GA.

BELLAMY, L. T. 1958. *The Infrared Spectra of Complex Molecules.* Wiley, New York.

ESTRADA-GARCIA, M. T., CALLOW, J. A., AND GREEN, J. R. 1990. Monoclonal antibodies to the cell adhesive coat secreted by *Pythium aphanidermatum* zoospores recognise 200 × $10^3$ M, glycoproteins stored within large peripheral vesicles. *J. Cell Sci.* 95: 199–206.

FINK, D. J., AND GENDREAU, R. M. 1984. Quantitative surface studies of protein adsorption by infrared spectroscopy. I. Corrections for bulk concentrations. *Anal. Biochem.* 139: 140–148.

GENDREAU, R. M., AND JAKOBSEN, R. J. 1979. Blood–surface interactions: Fourier transform infrared studies of protein surface adsorption from flowing blood plasma and serum. *J. Biomed. Mater. Res.* 13: 893–906.

GENDREAU, R. M. 1986. Biomedical Fourier transform infrared spectroscopy: Applications to proteins. In *Spectroscopy in the Biomedical Sciences* (R. M. Gendreau, Ed.), pp. 21–52. CRC Press, Boca Raton, FL.

GRIFFITHS, P. R., AND DEHASETH, J. A. 1986. *Fourier Transform Infrared Spectrometry.* Wiley, New York.

GRIFFITHS, P. R., AND FULLER, R. E. 1982. Mid-infrared spectrometry of powdered samples. *Adv. Infrared Raman Spectrosc.* 9: 63–129.

GUBLER, F., AND HARDHAM, A. R. 1988. Secretion of adhesive material during encystment of *Phytophthora cinnamomi* zoospores, characterized by immunogold labelling with monoclonal antibodies to components of peripheral vesicles. *J. Cell Sci.* 90: 225–235.

GUBLER, F., HARDHAM, A. R., AND DUNIEC, J. 1989. Characterising adhesiveness of *Phytophthora cinnamomi* zoospores during encystment. *Protoplasma* 149: 24–30.

HARDHAM, A. R., AND SUZAKI, E. 1986. Encystment of zoospores of the fungus *Phytophthora cinnamomi* is induced by specific lectin and monoclonal antibody binding to the cell surface. *Protoplasma* 133: 165–173.

HOHL, H. R., AND BALSIGER, S. 1986. A model system for the study of fungus–host surface interactions: Adhesion of *Phytophthora megasperma* to protoplasts and mesophyll cells of soybean. In *Recognition in Microbe–Plant Symbiotic and Pathogenic Interactions* (B. Lugtenberg, Ed.), pp. 259–272. Springer, Berlin.

HUTSON, T. B., MITCHELL, M. L., KELLER, J. T., LONG, D. J., AND CHANG, M. J. W. 1988. A technique for monitoring mammalian cell growth and inhibition *in situ* via Fourier transform infrared spectroscopy. *Anal. Biochem.* 174: 415–422.

JANSSON, H.-B., and NORDBRING-HERTZ, B. 1988. Infection events in the fungus–nematode system. In *Diseases of Nematodes* (G. O. Poinar Jr, and H.-B. Jansson, Eds.), Vol. II. pp. 59–72. CRC Press, Boca Raton, FL.

KATES, M. 1986. *Techniques of Lipidology, Isolation, Analysis and Identification of Lipids,* 2nd ed., pp. 172–175. Elsevier, Amsterdam.

LONGMAN, D., AND CALLOW, J. A. 1987. Specific saccharide residues are involved in the recognition of plant root surfaces by zoospores of *Pythium aphanidermatum. Physiol. Mol. Plant Pathol.* 30: 139–150.

NICHOLS, P. D., HENSON, J. M., GUCKERT, J. B., NIVENS, D. E., AND WHITE, D. C. 1985. Fourier transform–infrared spectroscopic methods for microbial ecology: Analysis of bacteria, bacteria–polymer mixtures and biofilms. *J. Microbiol. Methods* 4: 79–94.

NIVENS, D. E., TUNLID, A., FRANKLIN, M., SMIT, J., AND WHITE, D. C. 1988. *Infrared Monitoring of In-*

*teractions between Caulobacter Species and Solid Surfaces*. Abstract Am. Soc. Microbiol., Annual Meeting, Miami. .

NORDBRING-HERTZ, B. 1977. Nematode induced morphogenesis in the predacious fungus *Arthrobotrys oligospora*. *Nematologica* 23: 443–451.

NORDBRING-HERTZ, B. 1988. Ecology and recognition in the nematode–nematophagous fungus system. In *Advances in Microbial Ecology* (K. C. Marshall, Ed.), Vol. 10, pp. 81–114. Plenum, New York.

PARKER, F. S. 1971. *Applications of Infrared Spectroscopy in Biochemistry, Biology and Medicine*. Plenum, New York.

SING, V. O., AND BARTNICKI-GARCIA, S. 1975a. Adhesion of *Phytophthora palmivora* zoospores: Electron microscopy of cell attachment and cyst wall formation. *J. Cell Sci.* 18: 123–132.

SING, V. O., AND BARTNICKI-GARCIA, S. 1975b. Adhesion of *Phytophthora palmivora* zoospores: Detection and ultrastructural visualization of concanavalin a-receptor sites appearing during encystment. *J. Cell Sci.* 19: 11–20.