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FTIR spectroscopic evaluation of changes in the cellular biochemical composition of the phytopathogenic fungus *Alternaria alternata* induced by extracts of some Greek medicinal and aromatic plants

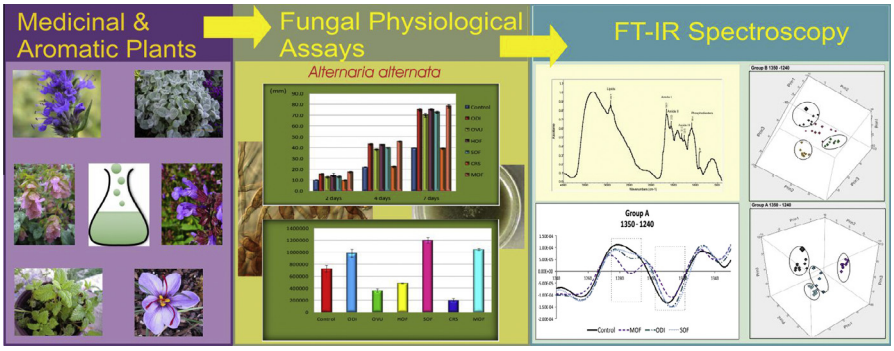
Efstathia Skotti [a], Sophia Kountouri [b], Pavlos Bouchagier [c], Dimitrios I. Tsitsigiannis [b], Moschos Polissiou [a], Petros A. Tarantilis [a,*]

[a] Laboratory of Chemistry, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
[b] Laboratory of Phytopathology, Department of Crop Science, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece
[c] Department of Food Technology, Technological Educational Institute of Ionian Islands, GR 28100 Kefallonia, Greece

HIGHLIGHTS

- Evaluation of medicinal and aromatic plants in the physiology of the fungus *Alternaria alternata*.
- Detection of biochemical changes in the phytopathogenic fungus *Alternaria alternata* by spectroscopy.
- Correlation between FTIR band area ratios and mycelium growth development of *Alternaria alternata*.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, the biological activity of aquatic extracts of selected Greek medicinal and aromatic plants to the phytopathogenic fungus *Alternaria alternata* was investigated. *Lamiaceae* species (*Hyssopus officinalis* L., *Melissa officinalis* L., *Origanum dictamnus* L., *Origanum vulgare* L. *and Salvia officinalis* L.) were found to enhance significantly the mycelium growth whereas *Crocus sativus* appears to inhibit it slightly. *M. officinalis* and *S. officinalis* caused the highest stimulation in mycelium growth (+97%) and conidia production (+65%) respectively. In order to further investigate the bioactivity of plant extracts to *A. alternata*, we employed Fourier Transform Infrared Spectroscopy (FTIR). Differences of original spectra were assigned mainly to amides of proteins. The second derivative transformation of spectra revealed changes in spectral regions corresponding to absorptions of the major cellular constituents such as cell membrane and proteins. Principal component analysis of the second derivative transformed spectra confirmed that fatty acids of the cell membranes, amides of proteins and polysaccharides of the cell wall had the major contribution to data variation. FTIR band area ratios were found to correlate with fungal mycelium growth.

* Corresponding author. Tel.: +30 210 529 4262; fax: +30 210 529 4265.
E-mail address: ptara@aua.gr (P.A. Tarantilis).

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Introduction

Fungal species have diverse impacts on plants, animals, ecosystems and the environment [1–3] ranging from nutrient recycling, to biotechnology [4,5] or emerging animal and plant pathogens. Many fungal pathogens cause serious damage to a large number of crops with significant impacts to agricultural economy [6,7]. Fungal cells contain genetic material (DNA and RNA), gene products (proteins) and products of proteins (carbohydrates and lipids) [8]. Fungal species interact with their surroundings through their cell walls and by means of the compounds they secrete or store and cell function is related to cell composition [9]. *Alternaria alternata*, commonly grown on vegetation, is one of the most important species among the allergenic fungi [10] and a devastating plant pathogen of several crops worldwide. Bioactive compounds or mixtures have been used for the effective control of fungal pathogens such as *A. alternata* [11]. The evaluation of bioactive compounds or mixtures on specific cells is usually conducted through bioassays and molecular tests [12–15]. Biopesticides represent a new trend in the European Union; they can control pathogen and pests effectively with the minimum environmental impact [16]. Biopesticides are a form of pesticide based on microorganisms or natural products that may act directly controlling pathogen and pest epidemics or indirectly by enhancing the development of natural enemies [17,18].

Plant extracts, either aqueous or in organic solvents, have shown antimicrobial activity when examined by different screening models [19] mainly towards finding successful drug candidates. Biological activity of plants extracts is being investigated through alterations in photosynthetic mechanism or stress status of plants [20,21], bioassays [22,23], tissue or cell culture [19], receptor enzyme [24] and biochromatography [25–27]. The evaluation of bioactive compound and mixtures except the common microbiological methods lately conducted through novel techniques in metabolomic procedures such as Fourier Transform Infrared Spectroscopy (FTIR) [28–31].

The use of (FTIR) spectroscopy has been extensively used as a tool for understanding the main cellular composition [32,33,8,34] as infrared spectra provide detailed information on several cellular components such as proteins, polysaccharides and lipids [35]. While spectra are complex and comprise contributions from all the cellular components, FTIR spectroscopy has been successfully used as a valuable tool in identification, discrimination and classification of specific microorganisms [36–39] and fungal species [40–42]. FTIR spectroscopy was successfully used for identification of cells infected with viruses [39], cancer cells [43,44] as well as for detections and identification of infections caused by pathogens [37]. Detections of changes on cellular components under several stress conditions have also been approached using FTIR technique [45–48], as well as fungal species growth under optimal or stress conditions [8,49]. According to previous studies fungal FTIR spectra reflect the biochemical cellular composition of hyphae, mycelium and spores, and can distinguish fungal species and biochemical changes under stressed conditions [33,8,46]. Past research has shown that lipid accumulations have been detected in fungal cells as a response to environmental stress [46]. Evaluation of antimicrobial agents activity and efficiency was also performed through the use of FTIR spectroscopy [30,31].

Sample preparation and the method of spectra recording are among the most critical factors in the application of FTIR spectroscopy to biological samples. The diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) has been proved as a rapid method that can be applied to various solid samples without any other chemical pretreatment [50] and has been applied to freeze-dried biological specimens [51–53]. In combination with multivariate statistical methods such as principle component analysis, this technique seems to have a potential to be applied as a screening tool to identify and characterize biochemical changes related to fungal mycelium growth.

The aim of this study was to apply FTIR spectroscopy technique for evaluating changes in cellular composition of the phytopathogenic fungus *A. alternata* induced by aquatic extracts of some medicinal and aromatic plants.

Materials and methods

Plant material

Air dried samples (harvest 2010) of the *Lamiaceae* family were offered by Aetoloakarnania's Rural Cooperative of Aromatic, Pharmaceutical and Energy Plant Cultivators (Agrotikos Syneterismos Kalliergiton Aromatikon, Farmakeftikon, Energiakon Fyton Aetoloakarnanias, ASKAFEFA), Greece. These samples were, namely, lemon balm (*Melissa officinalis* L.), sage (*Salvia officinalis* L.), oregano (*Origanum vulgare* L.; chemotype carvacrol) and hyssop (*Hyssopus officinalis* L.). Dittany (*Origanum dictamnus* L.; chemotype carvacrol) was supplied from the local market in Crete, Greece and saffron (*Crocus sativus*) was offered by Agricultural Cooperative of Kozani. Only leaves of the aforementioned samples were used, with the exception of oregano and hyssop, for which leaves and flowers were used together, and saffron for which only stems were used. The samples were dried at room temperature. Dry plant material was stored at −20 °C until used.

Preparation of plant extracts

Extracts of five *Lamiaceae* species (*H. officinalis, M. officinalis, O. dictamnus, O. vulgare*, and *S. officinalis*) at a concentration of 10 g/100 ml, and *C. sativus* at 0.1 g/100 ml were prepared by soaking dry plant material in boiling double distilled water (ddH_2O), mixing thoroughly and then allowing to stand for 15 min. The herbal extracts were first filtered though a Whatman filter No. 1. Prior to inoculation, an aliquot was further filtered though a sterile and endotoxin free 0.2 μm PES filter media (Whatman Puradisk 25 mm) to reduce the risk of interference by micro-organisms.

Reagents and test organisms

Potato dextrose agar (PDA) was supplied by Sigma Aldrich. Potato dextrose broth medium was prepared in the lab using the filtered broth of 200 g of boiled potato small pieces (approx. 1 cm × 1 cm) in 500 ml of ddH_2O, adding 20 g of dextrose and the correct amount of ddH_2O to bring the volume to 1 l; the broth was autoclaved for 20 min at 120 °C. *A. alternata* was provided by the laboratory of Phytopathology of the Agricultural University of Athens.

Bioassays

Fungal physiological assays

A. alternata was grown in PDA plates under light conditions in order to produce enough conidia. Fungal mycelium development and spore production studies were carried out on plates containing 19 ml solid PDA medium plus 1 ml of each of the aromatic plants extracts that were added in PDA after autoclaving and cooling down and before medium solidification. The experiments were conducted in 90 mm sterile petri dishes. Conidia of *A. alternata* were collected in 1 ml of ddH_20 (supplemented with 0.01% Tween 80