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Selective and rapid detection of soil fungi using surface modified long period fiber gratings

Shilpi Gupta

ECED, SVNIT, Surat, India

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ABSTRACT

We present fast and reliable bio-engineered long period gratings for detection of fungal species in soil. Qualitative and quantitative measurements of airborne microorganisms is a challenge for microbiologists. The optical sensors based on long period gratings have been used for the identification of a very common *Trichoderma Viride* species which is a genus of *Trichoderma* fungi present in soil. *T.Viride* is among the first and most used bio-fungicide. The proposed procedure of sensing enables selective binding of *T.Viride* fungal spores in presence of other species. Binding of the spores is made confirmed by capturing SEM micrographs of the surface of fiber gratings. Significant shift of resonance peak from 1553 nm to 1548.78 nm was recorded. We demonstrate instant detection and effective selectivity of sensor for desired spores, suppressing the chances of false detection.

1. Introduction

Greenhouse vegetables and floriculture crops suffer from diseases which can be managed effectively with bio-fungicides. Bio-fungicides are composed of beneficial microorganisms. These organisms protect the plants against pathogens that are responsible for diseases [1]. Being an alternative to chemical fungicides, bio-fungicides can be used as part of an integrated disease management program as they attack and control pathogens from developing resistance to traditional chemical based fungicides.

Among these biofungicides, *Trichoderma* species are widely being used wordwide to protect plant roots against pathogen. *Trichoderma* is a genus of soilborne fungi, that can be found all over the world. They are known as successful colonizers of their habitats in comparison to their counterparts and have been studied with respect to various characteristics and applications. Besides benefits provided by *Trichoderma* species, these have been reported to cause respiratory problems to human beings due to volatile organic compounds they produce. These are also reported to cause diseases in plants. Moreover, *Trichoderma* species have also been reported as a source of causing green mould disease to plants [2–5].

Analysis of characteristics of *Trichoderma* species in an ongoing research [6]. Traditional methods of detection can no longer meet the requirements of the existing era because of the long procedures, limited sensitivity and no possibility of remote sensing [7]. Optical fiber based sensors can fulfil all these requirements at the same time at very low cost. Long period fiber gratings have been used for detection of E.coli [8,9], fungi [10], bacteriophages [11]. Such sensors for detection of *T.Viride* is reported in this article. Surface of the LPFGs are functionalized in a way to detect the species very selectively and with ultra high sensitivity.

E-mail address: sgupta@eced.svnit.ac.in

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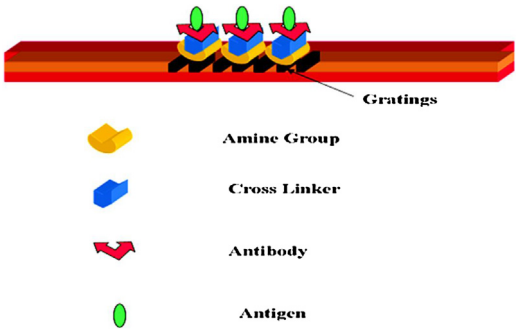

Fig. 1. Systematic Representation of Antibody immobilization strategy employed for surface modification of long period gratings.

The paper is organized as follows. Section 1 introduces the state of art in the field of bio-fungicides. Theoretical background of the sensor is given in Section 2. Section 3 describes chemicals and procedures used in the process of fungi immobilization. Results and discussions have been provided in Section 4. Finally work is concluded in Section 5.

2. Theory

Coupling of co-propagating fundamental guided mode LP_{01} and cladding modes represented by LP_{0m} takes place in long period gratings due to dielectric perturbation, which results in series of attenuated resonance peaks in transmission spectrum [12]. Assuming $A_{co}(z)$ and $A_{cl}(z)$ as complex amplitudes of forward propagating and backward propagating modes. Variation of these modes in presence of other modes of azimuthal order ν near dielectric perturbation is given by coupled mode equations -

$$\frac{dA_{co}(z)}{dz} = ik_{co-co}A_{co}(z) + i\frac{s}{2}k_{co-co}A_{cl}(z)e^{-2\delta(z)} \tag{1}$$

$$\frac{dA_{cl}(z)}{dz} = ik_{cl-co}A_{co}(z)e^{2\delta(z)} + i\frac{s}{2}k_{cl-cl}A_{cl}(z) \tag{2}$$

where $k_{co-co}$and $k_{cl-co}$are the coupling coefficients, s is the modulation depth or peak induced-index change and δ is the detuning parameter. The coupling of these modes is determined by area of overlap of the transverse fields of the resonant modes E_i and average index of the grating Δn_i [13].

$$K_{ij} = \frac{\omega\varepsilon_0 n}{4}\int \Delta n_i E_i(r)E_j^*(r)dr \tag{3}$$

According to coupled mode theory grating transmission is a function of coupling coefficient K. Grating transmission is given by-

$$T_{dB} = \cos^2(L\sqrt{K^2+\delta^2}) + \delta^2\left[\frac{\sin^2(L\sqrt{K^2+\delta^2})}{K^2+\delta^2}\right] \tag{4}$$

where L is the length of grating. Strength of modulation improves with the increase in peak induced-index change and results in large transmission losses in attenuation bands at resonance wavelengths. Resonance bands have different values of peak loss and bandwidth due to dissimilar coupling coefficients that are the function of modal overlap [14,15]. Position of resonance peak wavelengths and transmission loss are dependent on the fiber core and cladding parameters, grating pitch, length of the grating and grating induced-index modulation.

LPFGs are known to have high refractive index sensitivity, as the evanescent field of coupled mode extends more into the surroundings and enables it to be used as a significant surrounding Refractometric sensor [16,17].

3. Materials and methods

The surface of the fiber gratings were modified layer by layer for final antigen binding as depicted in Fig. 1. Chemicals used in the process of immobilization were APTES- 3-Aminopropyltriethoxylane from aminosilane family, Glutraldehyde 25% in H_2O, Alamethicin from Trichoderma Viride (>98% HPLC), Phosphate buffer solution (pH 7).

The step by step biofunctionalization process is described below. The culture of the fungi *T. Viride* were freshly prepared by taking one gram of *Trichoderma* sample taken in to sterile distilled water for the preparation of 10^{-6} dilution. 1 ml of 10^{-6} was transferred to sterile petriplates and 15 ml of molten cooled potato dextrose agar was added to same petriplates. Plates were incubated at room temperature for 5 days. The average no. of colonies were counted. Minimum colony forming unit of 2×10^7 cfu/gm had been obtained.