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Rapid detection of ciprofloxacin effects on *Fusarium graminearum* and *F. avenaceum* cells in modulating environmental pH using a reactive, non-toxic food-dye indicator

*Xiakun Yuan, Yit Kheng Goh, Nicholas Low, Vladimir Vujanovic**

Department of Food & Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, S7N 5A8 Saskatoon, SK, Canada

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ABSTRACT

The objective of the study was to assess the effect of ciprofloxacin antibiotic on the physiological or phenotypic characteristics of food-borne toxigenic *Fusarium graminearum* and *F. avenaceum* molds under *in vitro* conditions.

In the presence of ciprofloxacin, *Fusarium* mycelia growth and morphology were altered based on the antibiotic concentration range used. Results showed that ciprofloxacin in concentrations $\geq$40 μg/mL induced chlamydospore formation in *Fusaria* and as such, this antibiotic should be considered as an important abiotic stress factor and growth inhibitor.

A novel method was investigated to correlate chlamydospore formation with the colour changes observed in FD&C Green Number 3, a common water soluble food dye. The antibiotic-treated *F. graminearum* and *F. avenaceum* isolates produced chamydospores, which in turn altered environmental pH with concomitant changes in the colour and intensity of the dye. The colour changes observed as a function of environmental pH were supported by instrumental methods (pH meter and spectroscopy), and a commercial pH indicator (thymol blue) results.

In conclusion, we propose that FD&C Green Number 3 can be used as an accurate indicator for the rapid assessment of *Fusarium* molds when grown on ciprofloxacin antibiotic-containing substrate. Special emphasis should be given to an indirect risk assessment of antibiotic effects on toxic molds.

1. Introduction

Demands for safe, reliable and cost effective food colourants, have increased significantly in the past few decades due to huge socio-economic and technological pressures on the food industry to create more visually appealing food products (Downham and Collins, 2000; Spears, 1988). Consumers rely on the appearance and colour of foods as indicators of quality. Artificial dyes or colourants applied to foods or drinks may also serve to stimulate the appetite of consumers. Consequently, industries involved in food colourant production have experienced an increased market demand for these products (Downham and Collins, 2000).

Because of the public need to have visually appealing and safe foods, which are free from harmful microorganisms, the application or incorporation of antimicrobial agents in

* *Corresponding author*. Tel.: +1 306 966 5048; fax: +1 306 966 8898.
E-mail address: vladimir.vujanovic@usask.ca (V. Vujanovic).
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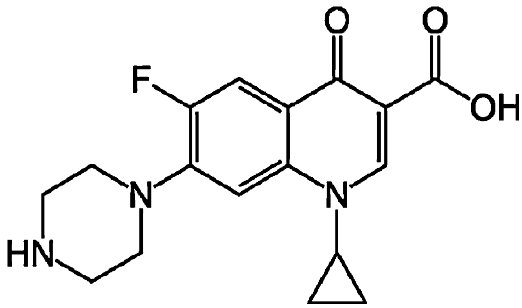

Fig. 1 – Antibiotic: ciprofloxacin.

food products are relatively common. The overarching goal of antimicrobial addition is to provide a safer food supply by constraining the spread of human and animal diseases, reducing the impact of food spoilage, and controlling toxin producing micro-organisms (Bhunia et al., 1988; Biedenbach and Jones, 1996; Collignon, 2005; Collignon et al., 2009; Di Corcia and Nazzari, 2002; Jack et al., 1996; Prescott, 2008). Antimicrobial products recently developed for medical use, such as those based on ciprofloxacin, are considered more environmentally-friendly or safer (Smid and Gorris, 1999) than traditional drug treatments based on trimethoprim-sulfamethoxazole antibiotics. Ciprofloxacin is a first-line, broad-spectrum and highly efficient systemic antibiotic used in human medicine, pharmaceuticals and veterinary medicine (Goffin et al., 2004). It has many potential interactions including those with food (apple, cereals, garlic), beverages (fruit juices), dairy products (milk, yoghurt), vitamins and minerals (Ca, Fe, K, Zn), and caffeine-fortified products. Co-administration with these foods or compounds results in a complexation reaction altering its bioavailability (Akinleye et al., 2007; Conly and Stein, 1994; Neuhofel et al., 2002; Pápai et al., 2010).

Ciprofloxacin (Fig. 1) is a second generation of fluoroquinolone [1-cyclopropyl-6-fluoro-4-oxo-7-piperazin-1-yl-quinoline-3-carboxylic acid] with efficient antibacterial properties. It works by interfering with DNA and protein synthesis. However, data related to effects of ciprofloxacin antibiotic on fungal food-borne *Fusarium* Link. molds are limited. *F. graminearum* Schwabe (teleomorph: *Gibberella zeae* Petch) and *F. avenaceum* (Fr.) Sacc. (teleomorph: *Gibberella avenacea* Cooke) are important toxigenic food-borne taxa, producers of vomitoxin/deoxynivalenol and zearalenone (Desjardins, 2006) and enniatins (Ivanova et al., 2006), respectively. Human infection by fusaria usually occurs in immunocompromised patients as a result of inoculation of the body surface, thus causing skin infection, onychomycosis, keratitis (following an ocular trauma), endophthalmitis, and arthritis (Martino et al., 1994). These microbes are associated with pre- and post-harvest cereal grain diseases resulting in multi-billion dollar annual economic losses in North America alone (Bai and Shaner, 2004). In Europe, these molds were often identified with, and isolated from, food products, including fruit (Thrane, 1996). Mycotoxin contaminated foods/feeds are placed under severe restrictions due to the high risk factors associated with their consumption by humans and/or animals (Desjardins, 2006; Anderson and Thrane, 2006).

In this study, we examined the effects of ciprofloxacin on *F. graminearum* and *F. avenaceum* mycelia by monitoring changes in morphological and physiological traits such as chlamydospore formation, colour and growth. In addition, visible spectroscopy shifts for FD&C Green Number 3 associated with environmental pH changes induced by ciprofloxacin treated *Fusarium* were examined. The aim of this study was to investigate the use of FD&C Green Number 3 as a rapid assessment non-toxic reagent to detect the presence of *Fusarium* molds in ciprofloxacin treated substrates, and to evaluate the potential impact of ciprofloxacin on pH changes in the surrounding environment of *Fusarium*-cells.

2. Materials and methods

2.1. *Fungal growth and antibiotic treatment*

Fusarium graminearum 3-acetyldeoxynivalenol (3-ADON) chemotype SMCD 2243 and *F. avenaceum* enniantins A and B chemotype SMCD 2241 isolates were obtained from the Saskatchewan Microbial Collection and Database (SMCD) and maintained on 15% potato dextrose agar (PDA) at 4 °C in darkness prior to the antibiotic study.

Mycelial plugs (approximately 0.5 cm^2) from the actively growing zones of both *Fusarium* species were placed, mycelial side down, on PDA (Beckon Dickinson, Cockeysville, MD) amended with ciprofloxacin (Sigma–Aldrich Canada Ltd., Oakville, ON), at four different concentrations: 40 μg/mL, 80 μg/mL, 160 μg/mL and 320 μg/mL (Partida-Martinez and Hertweck, 2005). Inoculated plates were incubated at 23 °C in darkness for two weeks. The experiment was performed in five replicates and repeated twice. Morphology and growth of both *Fusarium* isolates was examined and recorded daily (Leslie and Summerell, 2006).

FD&C Green Number 3 was kindly donated by Dyeco Ltd. (Kingston, ON).

2.2. *Microscopic observation*

Mycelia from both *Fusarium* isolates inoculated on PDA with and without antibiotic were studied under Carl Zeiss Axioskop2 microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY) with 20× and 100× objectives. Images were captured with the attached Carl Zeiss AxioCam ICc1 camera (Carl Zeiss MicroImaging, Inc., Thornwood, NY).

2.3. *Food dye, pH measurement and absorbance*

FD&C Green Number 3 (C.I. no. 42053; molecular weight: 808.86) is food colourant (Fig. 2), which can be safely used at concentrations of up to 100 mg/kg (FAO, 2010). It is also used as a protein stain in electrophoresis and as a quantitative stain for histones (alkaline proteins found in eukaryotic cell nuclei) at alkaline pH.

In this study, colour changes in FD&C Green Number 3 (0.1%.) at pH 1.0, 3.0, 5.0, 7.0, 9.0, 11.0, and 14.0 were investigated. Absorbance (at 680 nm) readings for this colourant at the aforementioned pH values were determined using a DU 800 Spectrophotometer (Beckman Coulter, Mississauga, ON).