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Influence of nitrogen sources on growth and mycotoxin production by isolates of *Pyrenophora tritici-repentis* from wheat

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ABSTRACT

The fungus *Pyrenophora tritici-repentis* (Died.) Drechs. infects the leaves and kernels of wheat, causing tan spot and red smudge, respectively. Isolates of *P. tritici-repentis* have been reported to be both phytopathogenic and mycotoxigenic. This research investigates the influence of nitrogen sources on growth and production of mycotoxins by eight different isolates of *P. tritici-repentis*. A synthetic agar medium (SAM) was used with different nitrogen sources, both inorganic [(NH_4Cl, NH_4NO_3 and $(NH_4)_2SO_4$)] and organic (L-alanine, L-histidine, and L-lysine), at a concentration of 37.5 mmol L^{-1}. Individual isolates exhibited different growth rates that varied according to the nitrogen source added to the medium. The choice of nitrogen source also had a major effect on production of the mycotoxins emodin, catenarin and islandicin. The highest concentrations of emodin, 54.40 ± 4.46 μg g^{-1}, 43.07 ± 23.39 μg g^{-1} and 28.91 ± 4.64 μg g^{-1} of growth medium, were produced on the complex medium (V8-potato dextrose agar) by the isolates Alg-H2, 331-2 and TS93-71B, respectively. A relatively high concentration of emodin also was produced by isolates Az35-5 (28.29 ± 4.71 μg g^{-1} of medium) and TS93-71B (27.03 ± 4.09 μg g^{-1} of medium) on synthetic medium supplemented with L-alanine. The highest concentrations of catenarin (174.54 ± 14.46 μg g^{-1} and 104.87 ± 6.13 μg g^{-1} of medium) were recorded for isolates TS93-71B and Alg-H2 on synthetic medium supplemented with L-alanine and NH_4Cl, respectively. The highest concentration of islandicin (4.64 ± 0.36 μg g^{-1} medium) was observed for isolate 331-2 in the presence of L-lysine. There was not a close relationship between mycelial growth and mycotoxin production by the fungal isolates. This is the first report on the influence of nitrogen sources on the production of mycotoxins by *P. tritici-repentis*.

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1. Introduction

The ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.), causal agent of tan spot, is an important foliar pathogen of wheat: *Triticum aestivum* L. and *T. turgidum* subsp. *durum* (Desf.) Husn. This fungus occurs worldwide in all major wheat growing regions [1] and can cause yield losses that range from 3% to 50%, depending on weather conditions and the susceptibility of the host cultivar [2,3]. Moreover, *P. tritici-repentis* also infects the kernels, resulting in blackening of the germ end of affected seeds known as black point, and/or a reddish discoloration termed red smudge [4]. For Canadian Western Amber Durum (CWAD), 0.25% of red smudge or a 10% combination of black point and smudge will lower the grade from #1 to #2, representing an average loss of $12 CAD per ton [5]. Isolates of *P. tritici-repentis* can produce several mycotoxins of the anthraquinone class (polyketides). The most important mycotoxins produced by this fungus are emodin, islandicin and catenarin [6,7]. These mycotoxins have been isolated previously as mold metabolites [8,9]. Emodin is a mutagenic, genotoxic and diarrheagenic mycotoxin [10–12]. A crude extract of emodin administered orally caused severe diarrhea in 1-day-old cockerels and the mean lethal dose of emodin was 3.7 mg kg^{-1} [10]. Clinical symptoms included loss of appetite, accumulation of fecal material with acute epidermal irritation around the cloaca, general debilitation, anorexia, diarrhea, and death within 5 days of ingestion [10,13]. Islandicin is a mutagenic mycotoxin [11] and catenarin also is classified as an undesirable mycotoxin [14,15]. Therefore, *P. tritici-repentis* is not only a phytopathogen, but also a mycotoxigenic fungus [7].

Fungi are important contaminants of food and feed, where they are responsible for spoilage and more importantly the production of dangerous toxins. While the most common mycotoxigenic fungi are *Aspergillus*, *Penicillium* and *Fusarium* [16], several other fungal genera are also able to produce mycotoxins, including *Acremonium*, *Alternaria*, *Bipolaris*, *Byssochlamys* [syn. *Paecilomyces*], *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Claviceps*, *Cochliobolus*, *Cylindrocarpon*, *Gliocladium*, *Helminthosporium*, *Monascus*, *Mortierella*, *Mucor*, *Myrothecium*, *Neotyphodium*, *Phomopsis*, *Pithomyces*, *Stachybotrys*, *Stagonospora*, *Trichoderma*, *Trichothecium* and *Verticimonosporium* [6,16–23]. Little attention has been paid to these fungi, although they could be of concern to public health.

Bouras and Strelkov [7] reported that wheat kernels harvested at maturity contained approximately 0.05 mg catenarin and 0.06 mg emodin per gram of tissue following inoculation with *P. tritici-repentis* at the mid-to-late milk stage. No mycotoxins were detected in non-inoculated tissues. Given the production of several anthraquinone mycotoxins by *P. tritici-repentis*, infection of wheat kernels by this fungus may represent a potential risk for human and animal health [7]. Wheat is an important food commodity worldwide, and is an important ingredient in the production of baked goods. Unfortunately, wheat also is an excellent substrate for growth of several saprophytic, pathogenic and mycotoxigenic fungi.

Fungal contamination is one of the main causes of deterioration of stored grains. Thus, early detection of fungal toxins on food and feedstuffs is crucial for safety purposes and to eliminate mycotoxins from the food chain. The development and application of methods to achieve this detection must be based on knowledge of the genetic features of mycotoxin producers [24], as well as knowledge of their needs for growth and mycotoxin production. Moreover, to further explore the biosynthesis of these mycotoxins, it is necessary to determine the environmental factors that regulate their production. Generally, the biosynthesis of secondary metabolites such as mycotoxins in microfungi is controlled by nitrogen and carbon sources [24]. Several studies found that nitrogen source can have an important influence on mycotoxin production, including kojic acid [25], aflatoxins [26], gibberellin [27], citrinin [28] and ochratoxin A [29].

The objective of this study was to examine the influence of different nitrogen sources on the production of emodin, catenarin and islandicin by *P. tritici-repentis*. Improved knowledge of the factors affecting the production of these mycotoxins is the first step in understanding mycotoxin biosynthesis by this wheat pathogen. This is the first study in which the influence of nitrogen sources on mycotoxin production by *P. tritici-repentis* has been assessed.

2. Material and methods

2.1. Isolates of *P. tritici-repentis*

Eight single-spore isolates of *P. tritici-repentis* were included in this study (Table 1). These isolates were originally collected from infected wheat leaves sampled from four different regions worldwide (Canada, Algeria, Azerbaijan, and the Turkish–Syrian border [30,31]). The isolates (ASC1, 86-124, 331-2, 90-2, Alg 3-24, Alg-H2, Az35-5 and TS93-71B) were selected because they represent all of the known races of *P. tritici-repentis*. All isolates were provided by the late Dr. L. Lamari (University of Manitoba, Winnipeg, Canada). The fungal cultures were maintained on potato dextrose agar (PDA) at 4 °C (Difco Laboratories, Detroit, Michigan, USA) until use.

Table 1 – Isolates of *Pyrenophora tritici-repentis* included in this study.

Isolate	Host	Origin	Reference
ASC1	Winter (bread) wheat *T. aestivum* (Norstar)	Canadian prairies, Manitoba	[32]
86-124	Winter (bread) wheat *T. aestivum* (BH1146)	Canadian prairies, Manitoba	[32]
331-2	Durum wheat *T. durum* (Newton)	Canadian prairies, Manitoba	[32]
90-2	Winter (bread) wheat *T. aestivum*	Canadian prairies, Manitoba	[32]
Alg 3-24	Durum wheat *T. durum*	Guelma (Heliopolis), eastern Algeria	[32]
Alg-H2	Durum wheat *T. durum*	Guelma (Heliopolis), eastern Algeria	[30]
Az35-5	Durum wheat *T. durum*	Azerbaijan	[31]
TS93-71B	Durum wheat *T. durum*	Turkish–Syrian border	[31]