International Journal of Food Microbiology 266 (2018) 158–166

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Influence of water activity and temperature on growth and fumonisin production by *Fusarium proliferatum* strains on irradiated wheat grains

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ARTICLE INFO

Keywords:
Fumonisins
Wheat grains
Fusarium proliferatum
Abiotic stress

ABSTRACT

Wheat is the most important cereal consumed by the Argentine population. In previous studies performed in durum and common wheat grains in this country it has been observed fumonisin contamination as well as high incidence of *Fusarium proliferatum*. Fumonisins are toxic fungal metabolites, and consumption of fumonisin-contaminated maize has been epidemiologically associated with oesophageal cancer and neural tube defects in some human populations. Using irradiated wheat-grains, the effects of abiotic factors, temperature (15, 25, and 30 °C) and water activity (a_W; 0.995, 0.98, 0.96, 0.94, 0.92, and 0.88), on mycelial growth and fumonisin biosynthesis were compared for three *F. proliferatum* strains isolated from wheat grains in Argentina. Although all isolates showed similar profiles of growth, the fumonisin production profiles were slightly different. Maximum growth rates were obtained at the highest a_W (0.995) and 25 °C, with growth decreasing as the a_W of the medium was reduced. Maximum amounts of total fumonisins (FB_1, FB_2 and FB_3) were produced at 0.995 a_W and 15 °C for 2 strains, and at 25 °C and 0.995 a_W for the third one. Fumonisins concentrations varied considerably depending on the a_W and temperature interactions assayed. Studied strains showed different fumonisin production profiles. Two-dimensional profiles of a_W by temperature interactions were developed from these data to identify areas where conditions indicate a significant risk of fumonisins accumulation on wheat. As a result, temperature and a_W conditions that resulted in fumonisins production are those found during wheat grain development (especially milk and dough stages) in the field. This is the first study made using irradiated wheat grains and provides useful baseline data on conditions representing a low or a high risk for fumonisins contamination of wheat grains which is of concern because this cereal is destined mainly for human consumption.

1. Introduction

In Argentina human consumption of wheat products is much greater than for products made from other cereals (Pacin et al., 2012). Wheat grains production in this country during 2016 reached 18,3 million - tons, and human wheat flour consumption was estimated at 3.8 million tons (89 kg/person/year) (FAIM, 2015). In the last few years extensively work has been published reporting wheat contamination with fungal species and also with mycotoxins which are molecules that can cause damage in human and animal health (Desjardins, 2006). Among all fungal species, *Fusarium* ones can be isolated from wheat grains and produce a range of mycotoxins that can persist in the grain. Some mycological surveys isolated many *Fusarium* species from wheat grains and wheat-based products, being *Fusarium proliferatum* isolated in a high frequency, and in some cases the predominant (Amato et al., 2015; Chehri et al., 2010; Conner et al., 1996; Mohammadi et al., 2016; Moretti et al., 1999; Palacios et al., 2011; Ramirez et al., 2006; Stankovic et al., 2007; Tančinová and Labuda, 2009). *F. proliferatum* is a member of the *Fusarium fujikuroi* species complex (FFSC). This species has a broad host range: it can be isolated from several agriculturally important plants as the main pathogenic agent, but the highest concern related to it is the ability together with *Fusarium verticillioides,* to be a main pathogen of maize worldwide (Ghiasian et al., 2006; Logrieco et al., 2002). Both species can produce several mycotoxins being

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https://doi.org/10.1016/j.ijfoodmicro.2017.12.001
Received 12 July 2017; Received in revised form 22 November 2017; Accepted 1 December 2017

fumonisins the most dangerous. Fumonisins cause a number of severe mycotoxicoses in animals such as equine leukoencephalomalacia in horses and porcine pulmonary edema in swine (Desjardins, 2006). In humans, they have been associated with oesophageal cancer, neural tube defects and recent studies suggested that human exposure to them could be related to impaired growth in children (Kimanya et al., 2010; Marasas et al., 2004; Missner et al., 2006; Shirima et al., 2015). Moreover, fumonisin B_1 has been evaluated as a possible carcinogen to humans (class 2B) by the International Agency for Research on Cancer (IARC, 2002). Although Shephard et al. (2005) reviewed several reports about fumonisins occurrence in wheat and concluded that careful evaluation of the analytical method and source of contamination is required in order to prevent the report of false positive results, natural occurrence of fumonisins in wheat and wheat-based products have been observed wordwide (Alkadri et al., 2014; Amato et al., 2015; Busman et al., 2012; Castellá et al., 1999; Castoria et al., 2005; Chehri et al., 2010; Cirillo et al., 2003a, 2003b; Jakšić et al., 2012; Kushiro et al., 2009; Li et al., 2015; Liu et al., 2012; Mashinini and Dutton, 2006; Mendes et al., 2015; Rodrigues and Naehrer, 2012; Roohi et al., 2012; Roscoe et al., 2008; Rubert et al., 2013; Serrano et al., 2012; Sirot et al., 2013; Stanković et al., 2011, 2012; Sun et al., 2011). Moreover, natural fumonisin contamination (mainly fumonisin B_1) has been reported on durum (Palacios et al., 2011) and common wheat (Cendoya et al., 2014b) in Argentina. In both kinds of wheat, fumonisins were found in high frequency, and also some samples in both studies exceeded the limits established for human consumption by the European Union (1000 ng/g) for maize and sub-products. Amato et al. (2015) hypothesized that low levels of fumonisins commonly occur in wheat grains, but that they may remain undetected as long as mycotoxin monitoring programs for wheat not included fumonisins. Due to the increased number of reports about the occurrence of fumonisin and also the presence of *F. proliferatum* on wheat around the world, field pathologist and mycologist showed interest in understanding this pathosystem. Moreover, Guo et al. (2016) reported that *F. proliferatum* strains originating from different host were able to infect wheat via seeds (systemic colonization), causing accumulation of fumonisin and beauvericin in kernels. Furthermore, Amato et al. (2015) stated that the relevant source of fumonisins in wheat grains appears to be *F. proliferatum.* For those reasons, it is relevant to understand the ecology of *F. proliferatum* on wheat.

Since fungal growth and mycotoxin production result from the complex interaction of several factors, an understanding of each factor is essential to understand the overall process and to predict and prevent mycotoxin development (Chamley et al., 1994). Among environmental factors that influence growth and mycotoxin production by fungi, temperature and water activity (a_W) are the primary ones (Marín et al., 2004). Nowadays there is few information about *F. proliferatum* development on this substrate. As a result, investigating *F. proliferatum* behaviour in wheat would provide useful information in order to predict its growth with the consequent fumonisin contamination of the kernels.

The aim of this work was to determine the impact of a_W, and temperature on growth and fumonisin production on irradiated wheat grains by three strains of *F. proliferatum* isolated from wheat in Argentina.

2. Material and methods

2.1. Strains

Three *F. proliferatum* strains (ITEM: 15654, 15661, 15664) isolated from Argentinean wheat grains during 2007–2008 harvest season were used. These isolates have been characterized by a molecular, biological and morphological way (Nelson et al., 1983; Leslie and Summerell, 2006). For the molecular characterization *1-α elongation factor* (*EF-1α*), *calmodulin,* and *FUM8* genes were sequenced indicating that these isolates belong to the FFSC and characterized as *F. proliferatum*. In order to determinate their *Fusarium fujikuroi* mating population (MP) crossing experiments were done (Klittich and Leslie, 1988) with standard testers as female parents and the uncharacterized field isolates as male parents. The three strains belong to the *Fusarium fujikuroi* mating population D. The ability of the strains to produce fumonisins was also tested, and the three of them were able to produce fumonisins (Palacios et al., 2011). These strains were recorded in the Istituto de Tossine e Micotossine, Bari, Italia. Now they are deposited at the Department of Microbiology and Immunology, Universidad Nacional de Rio Cuarto culture collection (RC). Cultures are maintained in 15% glycerol at − 80 °C.

2.2. Grain

Argentinean wheat grains were gamma irradiated (10–12 kGy) using a Cobalt radiation source and stored aseptically at 4 °C until their utilization. The irradiated grain contained no microbial infection or mycotoxin contamination and retained germinative capacity of about 75% (Hamer, 1994). Sterilized controls were performed in order to verify the efficiency of the irradiation treatment. The initial a_w of the grain was 0.654. 500 g of irradiated wheat grains were weighed into sterile beakers and rehydrated to the required a_w (0.995; 0.98; 0.96; 0.945; 0.92 and 0.88) by addition of sterile distilled water using a moisture absorption curve (Table 1). Flasks were subsequently refrigerated at 4 °C for 72 h with periodic shaking to allow absorption and equilibration. Finally, the a_w levels were confirmed by using an Aqualab Series 3 water activity metre (Decágon Devices, Inc., WA, USA). Rehydrated wheat grains were pouring into 9-cm sterile Petri dishes to form a monolayer of grains (20 g).

2.3. Inoculation, incubation and growth assessment

Petri plates containing wheat grains at different a_W levels were inoculated with a 4-mm-diameter agar disc that was taken from the margin of a 7-day-old colony of each *F. proliferatum* strain grown on synthetic nutrient agar (Gerlach and Nirenberg, 1982) at 25 °C. Agar discs were transferred face down to the centre of each plate. To maintain the correct equilibrium of relative humidity inside the boxes, Petri plates containing grains of the same a_w were enclosed in plastic containers together with two beakers of NaCl-water solution of the same a_w as the treatments. Containers were incubated at 15, 25 and 30 °C for 28 days. A full factorial design was used where the factors were: a_W, temperature and strain and the response was growth (total number of plates: 6 a_W values × 3 temperatures × 3 strains × 3 replicates).

Assessment of growth was made daily during the incubation period, with wheat grains cultures being examined using a binocular magnifier (× 10). Two diameters of the growing colonies were measured at right angles to each other until the colony reached the edge of the plate. The radii of the colonies were plotted against time, and a linear regression was applied to obtain the growth rate as the slope of the line. The time at which the line intercepted the x-axis was used to calculate the lag phase in relation to strain, a_W and temperature. Three complete Petri plate cultures per treatment were destructively sampled after 28 days of incubation, dried at 50 °C for 24 h and stored at − 20 °C until toxin

Table 1
Amount of water necessary to reach the different a_W levels in irradiated wheat grains.

a_W	Water (mL/100 g)
0.65	0.0
0.88	8.4
0.92	13.9
0.945	16.4
0.96	22.8
0.98	31.7
0.995	40.7