International Journal of Food Microbiology 289 (2019) 182–188

Contents lists available at ScienceDirect

International Journal of Food Microbiology

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

Quantitative antifungal activity of reuterin against food isolates of yeasts and moulds and its potential application in yogurt

Allison Vimont[a], Benoît Fernandez[a], Gomaa Ahmed[a,b], Helene-Pilote Fortin[a], Ismail Fliss[a,*]

[a] Institute of Nutrition and Functional Foods, Food Science Department, Food and Agriculture Faculty, Laval University, 2425 Agriculture Street, Quebec City, QC G1V 0A6, Canada

[b] Department of Nutrition and Food Science, National Research Center, Cairo, Egypt

ARTICLE INFO

Keywords:
Reuterin
3-HPA
Antifungal
Bio-preservative
Yogurt

ABSTRACT

Reuterin is an antimicrobial agent produced by conversion of glycerol and excreted by several bacterial species including the food grade lactic acid bacterium *Lactobacillus reuteri*. Several inhibitory activities have been reported to reuterin against a broad range of Gram-positive and Gram-negative bacteria, bacterial spores, moulds, yeasts and protozoa. However, the antifungal and anti-yeast activity of reuterin is poorly documented.

The aim of the current work was:1) To quantify the minimum inhibitory activity (MIC) and the minimum fungicidal activity (MFC) of reuterin against a representative panel of the most abundant fungi and yeast species associated with food contamination; 2) To investigate the application of reuterin as antifungal agent for bio-preservation of yogurt.

Reuterin was produced by *L. reuteri* ATCC 53608 in MRS and glycerol solution then purified before using. Our data showed that purified reuterin inhibited the growth of tested microorganisms at a concentration of 11 mM or less. Moreover, reuterin showed a fungicidal activity (killed 99.9% of all tested microorganisms) at concentrations equal or below 15.6 mM as indicated by MFC. Values of MFC were comprised between 1.0 and 4.8 of the MIC values, suggesting a potent fungicidal mechanism on both yeasts and filamentous moulds with one exception only. In yogurt, reuterin showed a fungistatic effect at a concentration of 1.38 mM while a fungicidal effect was obtained at 6.9 mM. Therefore, reuterin has a high potential as a food preservative, particularly owing to its biochemical properties and antibacterial and antifungal activities.

1. Introduction

Yeasts and filamentous moulds are commonly identified as spoilage microorganisms of food products, stored crops and feed (Pitt and Hocking, 2009). Yeasts such as *Candida*, *Kluyveromyces* and *Rhodotorula* are common spoilage of dairy and meat products while *Saccharomyces*, *Schyzosaccharomyces*, *Torulospora* and *Zygosaccharomyces* are frequently found in beverages.

Fungi are recognized as food spoilage microorganisms. Therefore, they render the human consumption of food which represents huge economic losses for the food industry.

Aspergillus, *Aureobasidium*, *Eurotium* and *Penicillium* are reported as spoilage filamentous moulds for a wide range of food products including meat, marine, dairy products and cereals (Ledenbach and Marshall, 2009; Pitt and Hocking, 2009). Fungal spoilage threatens both food quality and public health due to the possible production of mycotoxins. (Pitt and Hocking, 2009). Furthermore, mould growth on food products such as bakery products is a serious economic concern as it is the major factor limiting shelf life of these products (Saranraj, 2012).

To control these microorganisms, preservatives are commonly added. The most commonly used antifungal compounds for the preservation of foods are the weak acids or natamycin (also called pimaricin) (Chen et al., 2008; Pitt and Hocking, 2009). Weak acids, such as benzoic or sorbic acids, cause the microbial death by inducing a stress response that restores homeostasis, resulting in the reduction of available energy for growth and other essential metabolic functions (Brul and Coote, 1999). Natamycin is a member of the polyene antibiotic family which binds specifically to ergosterol, preventing it from performing its functional effects (Welscher et al., 2008).

Food spoilage yeasts and moulds are becoming resistant to antibiotics and also to preservatives (Brul and Coote, 1999). In this regard, some fungal species possess mechanisms of resistance to the preservatives. For instance, a number of *Penicillium* species have acquired

* Corresponding author.
E-mail address: ismail.fliss@fsaa.ulaval.ca (I. Fliss).

https://doi.org/10.1016/j.ijfoodmicro.2018.09.005
Received 11 May 2018; Received in revised form 2 September 2018; Accepted 5 September 2018
Available online 06 September 2018
0168-1605/

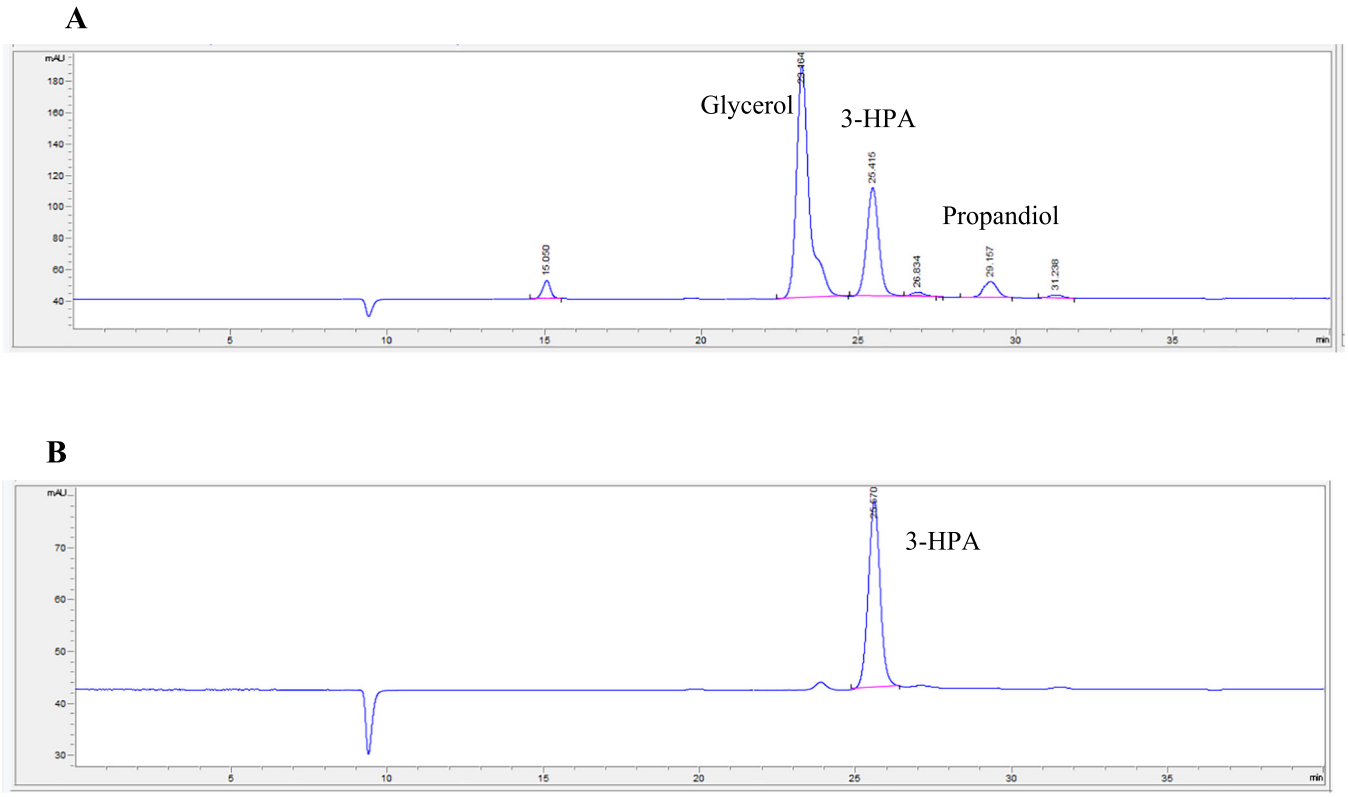

Fig. 1. HPLC chromatograms of reuterin: A) non-purified supernatant; B) purified reuterin.
Ion exclusion HPLC was performed using Coregel ION-300 column (7.8 × 300 mm, sulfonated polystyrene/divinylbenzene copolymers) at 40 °C. Elution was made with isocratic 10 mM H_2SO_4 with a flow rate of 0.4 mL/min and a refractive index detector use polystyrene/divinylbenzene copolymers. The detected peaks are: Glycerol, RT: 23.5; Reuterin (3-HPA), RT: 25.5; and Propandiol, RT:29.2.

the ability to degrade sorbate by its decarboxylation into trans-1,3-pentadiene, causing an off-odor and a "kerosene-like" flavor (Stopforth et al., 2005). The growing consumer concerns about food safety issues have raised the interest in producing bio-preservatives such as protective culture and their metabolites, including reuterin.

Reuterin is an antimicrobial agent produced by *Lactobacillus reuteri*, a hetero-fermentative lactic acid bacterium found in a variety of ecological niches like food fermentations or intestinal gut. Reurerin could also be produced by other genera of bacteria including *Bacillus*, *Citrobacter*, *Clostridium*, *Enterobacter* and *Klebsiella.* Reuterin is produced by converting the glycerol into 3-hydroxypropionaldehyde (3-HPA) through a coenzyme B12-dependent enzymatic reaction catalysed by the glycerol dehydratase (Vollenweider and Lacroix, 2004). Until 2016, reuterin was defined as a dynamic system, containing 3-HPA, its hydrate 1,1,3-propanetriol and the dimer 2-(2-hydroxyethyl) – 4-hydroxy-1,3-dioxane (Vollenweider and Lacroix, 2004). Recently, Engels et al. (2016) demonstrated that 3-HPA spontaneously dehydrates in aqueous solution to form acrolein. They therefore proposed to include acrolein in the definition of reuterin. By the means of 3-HPA and acrolein, reuterin has been proposed to induce oxidative stress in cells, most likely by modifying thiol groups in proteins and small molecules, causing the depletion of glutathione and modification of functional enzymes in particular (Engels et al., 2016).

Regarding its antimicrobial activities, reuterin has been reported to inhibit a broad range of Gram-positive and Gram-negative bacteria, bacterial spores, fungi and protozoa, including various food spoilers and pathogens (Ávila et al., 2014). Moreover, reuterin has been reported as a high potential food preservative (Vollenweider and Lacroix, 2004). However, its antifungal properties are poorly documented, in particular because the inhibiting molecules inside reuterin were not yet chemically characterized. To our knowledge, the only available minimal inhibitory concentrations (MIC) for reuterin against moulds were established from a supernatant of *L. reuteri* 1063 grown in the presence of glycerol (Chung et al., 1989). However, the authors reported the MIC as activity units rather than providing exact effective concentrations. The results showed that MIC comprised between 2 and 14 unit/mL for four yeast strains, and between 8 and 36 unit/mL for two mould strains. These concentration were later on recalculated based on the concentration of the aldehydic monomer and reported as molar concentrations (Stevens et al., 2011). Nakanishi et al., (Nakanishi et al., 2002) also reported a complete growth inhibition of four yeast strains and 11 moulds after 24 h of contact with the supernatant containing reuterin.

Therefore, this study aimed to characterize the antifungal properties of pure reuterin by providing exact effective MIC and minimal fungicidal concentrations (MFC) against 10 moulds and 14 yeasts that represent the most abundant fungi and yeast species associated with food contamination.

2. Materials and methods

2.1. Fungal strains and media

Fungal strains used in this study were isolated from the environment, food or dairy products and were referenced in four different strain collections (Table 1). They represent some of the most abundant fungal species found as food contaminant (Pitt and Hocking, 2009). Moulds were grown at 25 °C for at least 7 days on Potato Dextrose Agar (PDA, BD-Difco, Sparks, MD, USA) while yeasts were grown at 30 °C for 48 h in YPD broth composed of 10 g/L of yeast extract (BD-Difco), 10 g/L of peptone (BD-Difco) and 20 g/L of dextrose (Thermo Fisher Scientific Inc., Ontario, Canada). All fungal strains were reactivated from a