Food Chemistry: X 13 (2022) 100193

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

The synthesis of soluble and volatile bioactive compounds by selected brewer's yeasts: Antagonistic effect against enteropathogenic bacteria and food spoiler – toxigenic *Aspergillus* sp.

Sofía Sampaolesi [a,*], Laura E. Briand [a], Graciela De Antoni [b], Angela León Peláez [b]

[a] Centro de Investigación y Desarrollo en Ciencias Aplicadas – Dr. Jorge J. Ronco CINDECA, CCT La Plata-CONICET, Universidad Nacional de La Plata, Calle 47 No 257, B1900AJK La Plata, Buenos Aires, Argentina

[b] Cátedra Libre en Salud y Derechos Humanos, Cátedra de Microbiología. Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calles 50 y 115, s/No, La Plata, Buenos Aires, Argentina

ARTICLE INFO

Keywords:
Bioactive compounds
Brewery waste
Brewer's yeasts
Antimicrobial properties

ABSTRACT

Contamination by *Aspergillus* sp. and the accumulation of its mycotoxins in food and beverages have a high impact on human health and food safety. This investigation inquires the ability of brewer's yeasts discarded after fermentation (brewing fermentation residue, BFR) to synthesize bioactive compounds and to biocontrol *Aspergillus* sp. BFRs of *Saccharomyces cerevisiae* MBELGA62 and *Pichia kudriavzevii* MBELGA61 proved to have bacteriostatic properties and to be efficient in fungal growth reduction, decreasing the growth rate of *Aspergillus flavus* and *Aspergillus parasiticus* up to 37.8% and 42.5%, respectively. Fungal mycelium degradation along with absentia of conidia was detected near the yeast inoculum. Moreover, the yeasts synthesize volatile bioactive compounds that extend *Aspergillus* sp. lag phase above 100% and decrease fungal growth rates from 20% towards 44%, along with the complete inhibition of conidia synthesis. These results indicate the potential of this residue to be used in biocontrol applications in the food industry.

1. Introduction

Beer is the most consumed alcoholic beverage around the world (Colen & Swinnen, 2016). Its production through malt wort fermentation involves the generation of large amounts of solid by-products, such as the malt bagasse and the yeast sludge composed by live flocculated yeasts and immature beer supernatant (Hellborg & Piskur, 2009). In fact, 1.5–3 kg of yeast sludge (along with 85–90% of moisture) per 100 L of beer is obtained, being the second major by-product of brewing (Mathias et al., 2015). This beer fermentation residue (from now on, BFR) is an abundant and inexpensive source of protein, minerals, vitamins, especially vitamin B complex, as well as nutraceuticals such as β-glucans or mono- and oligosaccharides (Puligundla et al., 2020). In this context, the recycling and reuse of the BFR is interesting from both, an economical and environmental side (Mussatto, 2009).

The BFR has been proposed mainly as a nutritional additive for livestock, including piglets, laying hens, broilers, sheep, ruminants and in aquaculture. Alternative applications to animal feed, such as functional food ingredients and fermentation substrate, had been studied. BFR is the cheapest source of nucleic acids; an inexpensive nitrogen source in L-(+)-lactic acid fermentation and for succinic acid production; a suitable source for methane (biogas) production through anaerobic digestion; a viable nutrient adjunct in ethanol fermentation; a nutrient-rich and cost-effective starting material for yeast extract production; and an excellent biosorbent for the removal of lead from polluted waters (Puligundla et al., 2020).

The cell walls of the yeasts from BFRs are rich in β-D-glucan, which can be recovered by treatment of the residue (alkaline treatment, sonication, dried spray, etc.). β-glucans can improve the functional properties of food, acting as thickening, emulsifying, oil-binding or water-holding agents (Thammakiti et al., 2004). Moreover, β-glucans obtained from BFR have been used in yoghurts as nutraceutical ingredient (Piotrowska et al., 2009) and as functional ingredient in bread (Martins et al., 2018). β-glucans isolated from BFR are effective at lowering the

Abbreviations: BFR, Beer fermentation residue; VOCs, Volatile organic compounds; CFSs, Cell free supernatants; MIC, Minimal inhibitory concentration; MBC, Minimal bactericidal concentration.

* Corresponding author.
E-mail address: sampaolesi@quimica.unlp.edu.ar (S. Sampaolesi).

https://doi.org/10.1016/j.fochx.2021.100193
Received 29 October 2021; Received in revised form 18 December 2021; Accepted 20 December 2021
Available online 22 December 2021
2590-1575/© 2021 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

levels of LDL cholesterol and triacylglycerols in experimental rats (Waszkiewicz-Robak & Bartnikowska, 2009) and is suitable for the adsorption of mycotoxins (Yiannikouris et al., 2004). Mannoproteins can also be recovered from the yeast's cell wall and are used as food additives, especially as stabilizers in wine. In addition, BFR was reported as a suitable biomaterial for the extraction of mannoproteins (Costa et al., 2012; da Silva Araújo et al., 2014).

Angiotensin converting enzyme (ACE)-inhibitory peptides, well-known compounds for their antihypertensive activity, can be recovered by disruption and degradation of the cell wall of the BFR as well as other protein and bioactive peptides (Vieira et al., 2019). Viera and coworkers reported an optimized methodology for the autolysis and enzymatic degradation of BFR in order to obtain enhanced ACE-inhibitory and antioxidant properties (Vieira et al., 2017).

In addition to the nutritional properties either as an additive or as a source of bioactive substances, some research points to the antagonic effect of BRF against bacteria and fungi. The production of ethanol, the synthesis of toxins and mycocins, the secretion of organic acids and the pH modification of the media are among the mechanisms that yeasts developed to counteract other microorganisms (Muccilli & Restuccia, 2015). The metabolites secreted during fermentation by *Saccharomyces* sp., such as SO_2, CO_2 and ethanol, are antagonic effectors against enteropathogens. The ethanol is a toxic compound for cells, with a bactericidal impact widely reported. The SO_2 generates sulfuric acid by dissolving in the aqueous media, reducing the pH with the concomitant bactericidal and bacteriostatic effect. Moreover, certain research proposed that this compound inhibits the microbial enzymatic activity by reducing disulfide bonds (Chichester & Tanner, 1972). In the same manner, the dissolution of CO_2 acidifies the media which in turn has a bacteriostatic effect (White & Zainasheff, 2010).

Most of the bioactive compounds with inhibitory effect produced by yeasts are secreted to the extracellular space. In fact, the investigations reported by McDonnell and Russell suggest that the main product of alcoholic fermentation, that is ethanol, antagonizes bacteria through the denaturalization of its proteins and the dissolution of the lipids of the cellular membrane, inducing the bacterial lysis in *in vitro* studies (McDonnell & Russell, 1999).

In this context, it is not surprising that yeasts are the most studied bio-controlling microorganisms, and that are used not only for the production of fermented food but to the bio-preservation of fruit and vegetables (Muccilli & Restuccia, 2015; Bleve et al., 2006). Its antagonic mechanisms include the secretion of antimicrobial compounds, known as mycocins or killer toxins (Golubev, 2006; Suzuki et al., 2001), initially defined as extracellular proteins, glycoproteins and glycolipids capable of affecting sensitive yeasts. Even though mycocins are defined as toxins secreted by yeasts to compete with other yeasts taxonomically related, previous investigations reported by Bajaj, Raina, & Singh (2013) demonstrated that *Pichia kudriavzevii* RY55 secretes mycocins that inhibit the growth of bacterial pathogens, such as *Escherichia coli, Enterococcus faecalis, Klebsiella* spp., *Staphylococcus aureus, Pseudomonas aeruginosa* and *Pseudomonas alcaligenes.* In this sense, Waema, Maneesri & Masniyom (2009) reported that a killer toxin produced by a *Candida krusei* strain, isolated from fermented vegetables, inhibits the pathogens *E. coli, Salmonella Typhimurium, S. aureus* and *Bacillus cereus* associated with human disease outbreaks.

Moreover, some authors reported killer activity against fungi. Muccilli et al. (2013) described *exo*-β-1,3 glucanase activity against *Botrytis cinerea, Penicillium expansum, Penicillium digitatum* and *Colletotrichum gloeosporioides* in cell free supernatants of a *Wickerhamomyces anomalus* strain. Pretscher et al. (2018) detected an antagonic non-enzymatic activity against *B. cinerea* in the filtrated supernatants of yeasts isolated from termite guts. Bleve et al. (2006) isolated *Issatchenkia orientalis, Metschnikowia pulcherrima, Issatchenkia terricola* and *Candida incommunis* strains with killer properties against *Aspergillus carbonarius* and *Aspergillus niger*.

Additionally, the production of killer toxins has been demonstrated in *Saccharomyces, Candida, Cryptococcus, Debaryomyces, Kluyveromyces, Pichia, Torulopsis, Williopsis* and *Zygosaccharomyces*. The first mycocins were identified in *Saccharomyces cerevisiae* strains isolated from the brewing industry (Bevan & Somers, 1969). Therefore, it is expected that the BFR containing physiologically active brewer's yeasts could produce bioactive compounds with antifungal and antibacterial impact.

The present investigation focuses on the reutilization of the BFR as it comes directly from brewery without any treatment. In particular, the antagonic properties against pathogens of the non-yeast fraction of this residue is thoroughly investigated.

2. Materials and methods

2.1. Fungal and bacterial strains

S. cerevisiae MBELGA62 and *P. kudriavzevii* MBELGA61 were isolated from the yeasts sludge named starter M6, a brewery subproduct composed by live flocculated yeasts kindly donated by a homebrewer from La Plata (Buenos Aires, Argentina). The yeasts were cultured in YPD broth (yeasts extract 1% w/v; bacteriological peptone 2% w/v; dextrose 2% w/v) or in wort, prepared from barley by a local brewer, with adjustment of the pH and the concentration of sugars when necessary, previous to its sterilization by autoclaving (15 min at 121 °C).

Enteropathogens *Salmonella enterica* var. *Enteritidis* CIDCA 101 and *Escherichia coli* EHEC 60169, from the collection of the Centro de Investigación y Desarrollo en Criotecnología de Alimentos CIDCA (Buenos Aires, Argentina), were kept in nutritive agar slants (Biokar, France) at 4 °C. The strains were activated in nutritive broth (Biokar, France) through incubation overnight (ON) at 37 °C.

Aspergillus parasiticus CMUNLP7 (Gamba et al., 2015) and *Aspergillus flavus* CMUNLPI5 strains (typical aflatoxin B_1 (AFB_1) producers) belong to the collection of the microbiology department at the School of Chemistry (Universidad Nacional de La Plata, Argentina). *A. flavus* CMUNLPI5 (previously PJA) belongs to the collection of the Universidad de la República (Uruguay). Fungi were cultivated in potato-dextrose-agar slants (PDA. Britania, Argentina) for 7 days at 30 °C in order to induce its sporulation prior to the experiments.

2.2. Brewing micro-fermentations and cell free supernatants

The micro-fermentations were performed in brewing wort composed by 90.0 g of powder malt extract for blonde beer styles (Brewferm, Belgium) in distilled water to a final volume of 500 mL, 0.25 mg/mL of $ZnSO_4$ (Sigma Aldrich, Merck) and a pH of 4.5 ± 0.1 (adjusted with lactic acid) (Holt et al., 2018). The formulation was autoclaved at 110 °C for 15 min and the sedimented protein fraction was separated by filtration. The supernatant was adjusted to a final sugar concentration of 17.0 °Bx. Finally, 417 μL/L of sterile isomerized hop extract was added.

The pre-cultures of selected yeasts were carried out overnight in malt extract broth (3% w/v microbiological malt extract, 0.3% w/v peptone, pH 5.4) at 28 °C under stirring. Cells in the pre-cultures were quantified in Muse® Cell Analyzer (Merck Millipore, Alemania).

Fermentations were performed with 70 mL of brewing wort inoculated with an initial yeast population of 2×10^6 CFU/mL, capped with sterilized Müller valves in 100 mL bottles at 20 °C under stirring. The weight loss was monitored until a constant value was obtained (Pérez-Través et al., 2015).

After the end of fermentation, yeast cells were harvested by centrifugation in sterile conditions and stored at 4 °C and the supernatants analyzed immediately or stored at −20 °C. These experiments were carried out by triplicate. Sterile wort controls and positive fermentation controls (inoculated with pre-cultures of the commercial starter SafaleT-58, Fermentis, France) were also included.

Cell free supernatants (CFS) from yeasts cultures in YPD broth and from the brewing micro-fermentations were obtained by ultracentrifugation and filtration with sterile nylon filters of 0.22 μm of pore size

(Sartorius™, Goettingen, Germany). The fact that no yeasts colonies were observed in YPD agar plates seeded with the CFS demonstrated the absence of cells in the systems obtained as described before.

2.3. Investigation of the bactericidal and bacteriostatic effect of the CFSs

ON cultures of the enteropathogens in nutritive broth were harvested by centrifugation, washed twice with sterile physiological solution (PS. Sodium chloride 0.9% w/v) and re-suspended to obtain a suspension equivalent to McFarland 0.5 standard, meaning a 10^8 bacteria/mL initial inoculum.

A volume of 1 mL of dilutions of the CFSs in sterile nutritive broth (100, 90, 80 70, 60 and 50% v/v) were disposed in sterile Eppendorf-like tubes of 1.5 mL. All the tubes, except for the negative control, were inoculated with 10 μL of the bacterial suspension, achieving a 10^5 bacteria/mL final inoculum, and incubated ON at 37 °C. A positive control (1 mL of sterile nutritive broth) and a negative control (not inoculated 100% v/v CFSs) were included.

The minimal inhibitory concentration (MIC), associated with bacteriostatic effect, was determined as the absence of bacterial growth in the tube by direct observation of visible bacterial growth after incubation. Those suspensions with no visible growth were inoculated in nutritive agar plates for the determination of the minimal bactericidal concentration (MBC), associated with bactericidal effect (detailed methods in Supplementary Material).

2.4. Co-incubation of brewer's yeasts and Aspergillus sp. In solid media

The experiment followed the protocols published by Vero et al. (2013) with modifications. *Aspergillus* sp. suspensions of 10^4 conidia/mL were obtained in "spore solution" (sodium lauryl sulfate 0.1 g/L; glucose 10.0 g/L) from sporulated fungal cultures and counted using Neubauer's chamber. The yeast cultures were made by inoculation of the BFRs in YPD broth, incubating at 30 °C for 48 h.

The microorganisms were co-cultured in PDA. A streak of the yeast culture was seeded at 20 mm of one plate edge. *Aspergillus* sp. suspension was inoculated as a drop of 10 μL at 20 mm of the opposite edge. The inoculation scheme is shown in the Fig. S1 of the Supplementary Material section.

The plates were incubated at 30 °C, with the lid up, preventing conidia dispersion. The measurements of the fungal radius were performed with a gauge from the center of the inoculum towards the edge of the mycelium in the direction of the yeast streak (Fig. S1). In addition, positive fungal growth controls were included. The measurements were performed upon time until the corresponding control assay reached the farthest edge of the plate from the inoculum (70 mm). Three replicas of each treatment and control were performed.

The fungal parameters of growth rate and lag phase were calculated from the curves of fungal radius (expressed in cm) versus time (expressed in hours) according to Armando et al. (2013).

2.5. Incubation of Aspergillus sp. In micro-atmosphere of volatile organic compounds

Again, the experiments were made according to Vero et al. (2013) with modifications. *Aspergillus* sp. suspensions of 10^4 conidia/mL were obtained as described previously. A PDA plate was inoculated in the center with 10 μL of the fungal suspension. Another PDA plate was seeded with BFR, in order to obtain a yeast grass. In a sterile environment, the plates were face to each other and sealed with Parafilm®M (Marienfeld Superior, Alemania), in order to avoid the loss of volatile organic compounds (VOCs) during incubation at 30 °C. The VOCs chamber is illustrated in the Fig. S2 of the Supplementary Material section.

The incubation was carried out placing the VOCs chamber with the plate inoculated with *Aspergillus* sp. facing up, avoiding conidia dispersion and guaranteeing that yeasts and fungi have no direct contact. Positive fungal growth controls in chambers without yeast inoculum were included. Treatments and controls were made in triplicate.

Fungal mycelium diameter was measured upon time of incubation until the positive controls reached the edge of the plates. The fungal parameters of growth rate and lag phase were calculated from the curves of fungal diameter (expressed in cm) versus time (in hours).

2.6. Analysis through high performance liquid chromatography

The supernatants recovered from micro-fermentations were filtered by 0.22 μm nylon pore (Symta, Madrid, Spain) and poured in vials suitable for high performance liquid chromatography HPLC analysis. The chromatograph is equipped with a refraction index detector (Thermo Fisher Scientific, Waltham, MA, USA) and a HyperREZTM XP Carbohydrate H + 8 μm column protected with a HyperREZTM XP Carbohydrate Guard (Thermo Fisher Scientific). The analysis was performed with a mobile phase composed of 1.5 mmol/L H_2SO_4 aqueous solution, pH 2.5 ± 0.1, at 0.6 mL/min and 50 °C. The analysis was performed by duplicate.

2.7. Statistical analysis of the data

Kinetic parameters and chromatographic data were analyzed with the Infostat free software package (Universidad Nacional de Córdoba, Argentina) by one-way ANOVA and Tukey test for means comparison.

3. Results

3.1. Evidences of the antagonic potential of brewery residues against bacterial pathogens: Cell free supernatants (CFSs)

Various cell free supernatants (CFSs from now on) obtained from wort micro-fermentations were tested in order to assess their effect on bacterial growth, owing to their well-known capacity of producing ethanol. The Table 1 compares the bacteriostatic effect of the CFSs obtained from YPD broth cultures and wort micro-fermentations of the starter M6, *P. kudriavzevii* MBELGA61 and *S. cerevisiae* MBELGA62 over *S. Enteritidis* CIDCA 101 and *E. coli* EHEC 60169. The effectiveness of the CFSs was investigated as obtained (100% v/v) and under further dilution (from 90% v/v towards 50% v/v) through direct observation of the visible bacterial growth after overnight incubation. The absence of visible bacterial development indicated a positive bacteriostatic effect of the CFS, that is its ability to prevent bacterial growth.

Those observations demonstrate the bacteriostatic capacity of undiluted CFSs (100% v/v) obtained from wort micro-fermentation (Table 1). The CFSs diluted with sterile nutritive broth showed visible growth associated with bacterial development after 24 h of incubation, except for the CFS obtained from the wort fermentation carried out by *S. cerevisiae* MBELGA62 diluted to 90% v/v and inoculated with *E. coli* EHEC 60169 (Table 1).

Further experiments were performed in order to assay the bactericidal property of the CFSs, that is the ability of killing bacteria. In this context, those treatments which did not show visible growth after incubation were further seeded in nutritive agar plates (a culture media that allows bacteria growth) and incubated at 37 °C overnight. All the seeded plates evidenced bacterial growth after incubation (in contrast to the negative controls), indicating that the CFSs have no bactericidal effect.

3.2. Evidences of the antagonic potential of brewery residues against fungi: Soluble metabolites produced by yeasts cells

Aspergillus sp. is a genus of filamentous fungi that affects global cereal production and its derivatives. *A. parasiticus* and *A. flavus* are the major species that produce a subtype of mycotoxins, the potent

Table 1
Bacteriostatic effect of the CFSs of brewer 's yeasts over *S. Enteritidis* CIDCA 101 and *E. coli* EHEC 60169.

	Visible bacterial growth					
	CFSs obtained from YPD broth cultures			CFSs obtained from wort micro-fermentations		
	Starter M6	*Pk* MBELGA61	*Sc* MBELGA62	Starter M6	*Pk* MBELGA61	*Sc* MBELGA62
CFS [% v/v]	Inoculated with *Salmonella Enteritidis* CIDCA 101					
100%	+	+	+	–	–	–
90%	+	+	+	+	+	+
80%	+	+	+	+	+	+
70%	+	+	+	+	+	+
60%	+	+	+	+	+	+
50%	+	+	+	+	+	+
Positive control	+	+	+	+	+	+
Negative control	–	–	–	–	–	–
	CFSs obtained from YPD broth cultures			CFSs obtained from wort micro-fermentations		
	Starter M6	*Pk* MBELGA61	*Sc* MBELGA62	Starter M6	*Pk* MBELGA61	*Sc* MBELGA62
CFS [% v/v]	Inoculated with *E. coli* EHEC 60169					
100%	+	+	+	–	–	–
90%	+	+	+	+	+	–
80%	+	+	+	+	+	+
70%	+	+	+	+	+	+
60%	+	+	+	+	+	+
50%	+	+	+	+	+	+
Positive control	+	+	+	+	+	+
Negative control	–	–	–	–	–	–

Pk MBELGA61: *P. kudriavzevii* MBELGA61. *Sc* MBELGA62: *S. cerevisiae* MBELGA62. Positive control: the pathogen inoculated in nutritive broth. Negative control: CFSs without the pathogen inoculum. The presence of visible bacterial growth was determined by direct observation of the tubes of CFSs inoculated with the pathogens after incubation overnight at 37 °C. (+): visible growth was observed in the tube. (-): no visible growth was observed in the tube.

carcinogen aflatoxins, being aflatoxin B_1 (AFB_1), aflatoxin B_2, aflatoxin G_1 and aflatoxin G_2 the most important in regard to its impact on public health (IARC, International Agency for Research on Cancer- World Health Organization, 2002). Previous investigations reported by some of us, demonstrated that the germination of conidia of *Aspergillus* sp. was greatly inhibited in the presence of brewery cell free supernatants.

This section expands those findings through the investigation of the effect of the brewer's yeast in co-culture with *Aspergillus* sp. in potato-dextrose agar (PDA). This experimental arrangement allows the observation of the effect of both live yeasts and its secreted metabolites on fungal growth. The Fig. 1 shows the photographs of the plates of co-cultures of *Aspergillus flavus* and *Aspergillus parasiticus* and brewing yeasts in solid media, taken at 216 h and 288 h of incubation. The yeasts are the same that gave rise to the CFSs presented in the previous section that is, *S. cerevisiae* MBELGA62, *P. kudriavzevii* MBELGA61 and the starter M6. Additionally, the Fig. 1(a, b, c, d, e, f) shows a closer look at the plates after incubation for 288 h, pointing out the area near to the yeast where the fungal mycelium is less perceptible or not visible at all. It comes clear from the observation of the plates that *S. cerevisiae* MBELGA62 exerts a greater antagonistic effect than *P. kudriavzevii* MBELGA61 (compare plates A and F with B and D in the Fig. 1). This behavior might be ascribed to the ability of *S. cerevisiae* to rapidly deplete the sugars present in the surrounding media, competing with *Aspergillus* sp. for the nutrients. In addition, the fermentation of the sugars results in the secretion of the toxic compound ethanol. The fast fermentation of sugars into ethanol and carbon dioxide, even under aerobic conditions, is a well-studied mechanism of *S. cerevisiae*, known as the Crabtree effect (Dashko et al., 2014).

Moreover, the acidification of the media due to CO_2 solubilization and the presence of ethanol antagonize other microorganisms of the brewing niche during the fermentation (White & Zainasheff, 2010).

Previous investigations reported a similar behavior when co-culturing *Botrytis cinerea* and the antagonic yeast *Pichia membranifaciens* (Masih & Paul, 2002). The authors reported a region of fungal inhibition near to the yeast inoculum in the agar and the absence of conidia.

The Fig. 2 show the growth curves obtained through the measurement of the fungal mycelium radius upon time. It comes clear from the results that the biological impact of the brewer's yeasts depends on the fungal species. *A. flavus* CMUNLPI5 was the most sensitive to the yeast's presence, showing a significant decrease of the growing rate, even reaching the 288 h of co-incubation (Fig. 2A). Furthermore, the fungus was not capable of synthesizing conidia in the area nearest to the yeast streak in the plates, as can be observed in the photographs a, b and c within Fig. 1.

A significant decrease of the growing rate of *A. parasiticus* CMUNLP7 was observed until 120 h of co-culture. Nevertheless, the fungus retrieved its growing capability and reduced the difference with the control at longer incubation times, as can be seen in the Fig. 2B at 264 h of co-incubation. In contrast with *A. flavus* CMUNLPI5, *A. parasiticus* was able to grow over yeast inoculum and to produce conidia at 288 h of incubation (Fig. 1, plates d, e and f).

The kinetic parameters in the range from 96 h towards 192 h for *A. parasiticus* CMUNLP7 and from 96 h to 216 h for *A. flavus* CMUNLPI5 were calculated (Table S1 within the Supplementary material). Those periods of time correspond to the lowest slope of fungal growth rate registered (and therefore, to the maximum reduction of the growth rate by the presence of the brewer's yeasts).

No antagonic effect against *Aspergillus* sp. was detected until 96 h of co-incubation. However, as the fungi grew and advanced in the direction of the yeast streak, a significant decrease in its growing rate was observed ($P < 0.05$), probably due to the presence of soluble metabolites secreted by yeasts and a nutritionally depleted media.

Interestingly, all the assayed brewer's yeasts possess the ability to diminish the growing rate of the fungi even up to 63%. *A. flavus* CMUNLPI5 showed a significantly greater reduction of its rate in co-cultures with the starter M6, in regard to *S. cerevisiae* MBELGA62 ($P < 0.05$, Table S1). *A. parasiticus* CMUNLP7 showed a deeper decrease of its growing rate in co-cultures with the starter M6, in regard to *S. cerevisiae* MBELGA62 and *P. kudriavzevii* MBELGA61 ($P < 0.05$, Table S1). These data reveal a more powerful effect of the starter M6 than the isolated brewer's yeasts.

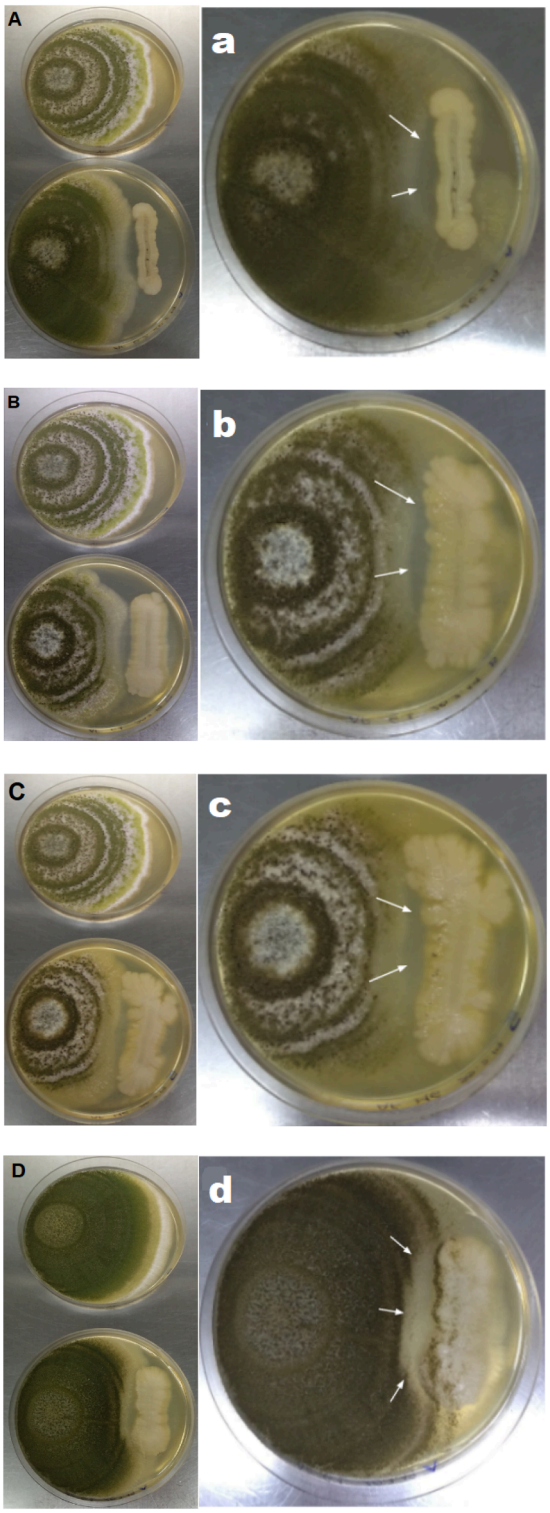

Fig. 1. *Aspergillus* sp. and brewer's yeasts co-cultures in PDA. *Aspergillus* sp. and brewer's yeasts co-cultures in solid media (PDA). The photographs on the left show the treatment (bottom) and its respective control without yeast inocula (top). The pictures of the plates and controls (A, B, C, D, E y F) were taken at 216 h of incubation. The closer picks of the plates (on the right; a,b,c,d,e,f) were taken at the end of the experiment (288 h). White arrows are pointing out the area near to the yeast streak where the fungal mycelium is less visible or not visible at all. **A, a**: *S. cerevisiae* MBELGA62 + *A. flavus* CMUNLPI5. **B, b**: *P. kudriavzevii* MBELGA61 + *A. flavus* CMUNLPI5. **C, c**: starter M6 + *A. flavus* CMUNLPI5. **D, d**: *P. kudriavzevii* MBELGA61 + *A. parasiticus* CMUNLP7. **E, e**: starter M6 + *A. parasiticus* CMUNLP7. **F, f**: *S. cerevisiae* MBELGA62 + *A. parasiticus* CMUNLP7.

In the last stage of incubation, the magnitude of the antagonic effect was related to fungal species. *A. flavus* CMUNLPI5 showed a significant growth reduction in co-cultures with *P. kudriavzevii* MBELGA61 and *S. cerevisiae* MBELGA62. Moreover, in the presence of *S. cerevisiae* MBELGA62, the fungus diminished its growth rate to 90% of the respective control, which evidences the inhibition of fungal growth (Fig. 2A).

On the contrary, *A. parasiticus* CMUNLP7 was not affected by the presence of any of the brewer's yeasts at the last stage of incubation (Fig. 2B). Likely, the aged yeasts begin to die and to autolyze, releasing nutrients that *A. parasiticus* CMUNLP7 could use as nutrients to continue growing.

3.3. Evidences of the antagonic potential of brewery residue against fungi: Volatile metabolites produced by yeasts

The impact of a micro atmosphere of volatile organic compounds (VOCs) produced by brewer's yeasts on the germination and development of filamentous fungi was also investigated. The diameter of the fungal mycelium versus time of incubation is presented in Fig. 4 and the kinetic parameters such as growth rate, percentage of reduction on the growing rate and the lag phase, are summarized in the Table S2 within the Supplementary Material. Additionally, the photographs showed in the Fig. 3 compare the diameter of the mycelium of *Aspergillus* sp. incubated with and without the presence of VOCs for 240 h (bottom and top images, respectively).

The images evidence a smaller diameter of the fungal mycelium and the inhibition of the synthesis of conidia due to the effect of the volatile compounds. A significant decrease of the growing rate of *Aspergillus* sp. is observed in the Fig. 4 from 48 h towards the end of incubation. Moreover, the inhibitory properties of the VOCs are somehow related to the nature of the brewer's yeasts, as concluded from the kinetic parameters presented in the Table S2. In fact, the highest percentage of growing rate reduction (above 40%) of the assayed fungi is registered under the exposition to the VOCs generated by *P. kudriavzevii* MBELGA61. Further evidence of the inhibitory effect is demonstrated in the extended lag phase observed in all the treatments. Nevertheless, the growth rate reduction registered in the presence of the VOCs produced by the starter M6 showed no significant differences with those produced by the isolated brewing strains.

It is quite remarkably that all the treatments showed a total inhibition of the synthesis of fungal conidia after 240 h of incubation under VOCs micro-atmosphere (Fig. 3).

3.4. Insights in the nature of the fermented products of brewer's yeasts with antagonistic properties

The biological impact of the soluble and volatile metabolites within the BFRs over the growth of *Aspergillus* sp. and the propagation of enteropathogenic bacteria led to the hypothesis that the presence of ethanol in the fermented beverage and the micro atmosphere of VOCs is a key factor in the inhibitory effect. In order to prove this hypothesis, the composition of the fermented wort obtained under controlled laboratory conditions was assayed. This investigation focused on certain analytes that are interesting from a technological point of view, such as: glycerol; 2,3-butanediol, erythritol, ethanol, glucose and fructose. Moreover, the 2,3-butanediol has been described as an antimicrobial compound along with ethanol (Madasu et al., 2017). In this context, the Table 2 shows the concentration of those compounds found in the wort fermented by *S. cerevisiae* MBELGA62, *P. kudriavzevii* MBELGA61, the starter M6 and the commercial lyophilized starter Safbrew T-58 (Fermentis, France), used as positive control of the fermentation.

All the brewing strains fully deplete the glucose and fructose present in the brewing wort and produce ethanol, as can be concluded from comparing the ethanol content in the fermentation products with unfermented brewing wort (Table 2). However, it is noticeable that

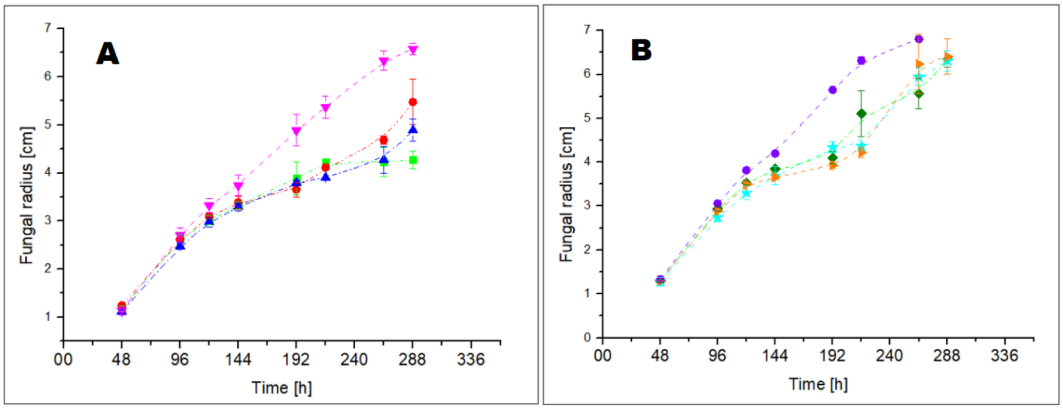

Fig. 2. Evolution of the fungal radius of *Aspergillus* sp. during the co-incubation with brewer 's yeasts in PDA Fungal radius [cm] versus Time [h] for *A. flavus* CMUNLPI5 (**A**) and *A. parasiticus* CMUNLP7 (**B**) co-incubated with brewer 's yeasts. The average of three biological replicas for each point are illustrated. Bars represent standard deviation. **A:** ■ *S. cerevisiae* MBELGA62 + *A. flavus* CMUNLPI5. ▲ *P. kudriavzevii* MBELGA61 + *A. flavus* CMUNLPI5. ● Starter M6 + *A. flavus* CMUNLPI5. ▼ Positive control *A. flavus* CMUNLPI5. **B:** ★ *S. cerevisiae* MBELGA62 + *A. parasiticus* CMUNLP7. ♦ *P. kudriavzevii* MBELGA61 + *A. parasiticus* CMUNLP7. ▶ Starter M6 + *A. parasiticus* CMUNLP7. ⬢ Positive control *A. parasiticus* CMUNLP7.

P. kudriavzevii MBELGA61 and the starter M6 produce significantly lower quantities of ethanol than *S. cerevisiae* MBELGA62 and the commercial strain used as positive control.

S. cerevisiae MBELGA62, a typical strain for brewing *ale* styles, produces quite high amounts of ethanol (6.48% v/v) close to the level of ethanol produced by the control (7.05% v/v). This observation somehow proves the initial hypothesis pointing to ethanol as a key metabolite in the antagonic effect against fungi.

P. kudriavzevii MBELGA61 is considered a non-conventional yeast for brewing. Interestingly, this strain fully depletes the glucose and fructose of brewing wort with a low yield of ethanol equals to 1.19% v/v (Table 2).

Previous investigations of the authors, demonstrated that the *P. kudriavzevii* MBELGA61 catalyzes the carbon flux towards biomass synthesis and therefore, the fermentation is negligible (data not shown). Additionally, the starter M6, composed by *P. kudriavzevii* MBELGA61, produced a fermented beverage with similar composition than that strain (Table 2). Therefore, in these cases, the antimicrobial effect of the BFRs cannot be explained by a high concentration of ethanol.

4. Discussion

It is worth noticing that the brewer's yeasts selected to perform this study were partially characterized in previous investigations. Cell free supernatants from brewing micro-fermentations carried out with *S. cerevisiae* MBELGA62, *P. kudriavzevii* MBELGA61 and the starter M6 were obtained by harvesting and filtration of its fermentation products. These CFSs decrease the germination of *A. flavus* and *A. parasiticus* conidia in *in vitro* studies, proving the biocontrolling potential of the supernatants (in the absence of live yeasts) and its suitability for the preservation of food and feed (Sampaolesi et al., 2019). Moreover, the BFRs obtained from fermentations carried out with these strains revealed the capacity to bind aflatoxin B_1 (AFB_1), a carcinogenic mycotoxin synthesized by most members of the genus *Aspergillus* (Sampaolesi et al., 2019).

The results obtained in the present investigation extends those previous findings. In fact, the supernatants remaining as a waste of wort fermentation carried out with *S. cerevisiae* MBELGA62, *P. kudriavzevii* MBELGA61 and the starter M6 (composed by both, *P. kudriavzevii* MBELGA61 and *S. cerevisiae* MBELGA62) demonstrated bacteriostatic properties rather than bactericidal properties. Conversely, the supernatants obtained from YPD broth cultures of the yeasts showed no antibacterial effect in the same experimental conditions. In fact, the fermentation supernatants without the presence of living cells or even further treatments, were able to inhibit the growth of pathogenic bacteria such as *S. Enteritidis* and *E. coli*, which are usually found as dangerous contaminants in food. This bacteriostatic capacity of CFSs obtained from wort fermentations enlightens the probiotic and prebiotic potential of the BFRs, composed by a large fraction of beer supernatant with bioactive compounds against bacteria. Among those compounds, ethanol has been extensively reported as an antimicrobial agent, and its presence in beer is considered a protective trait against spoilage non-pathogenic bacteria, such as *Lactobacillus* sp. and acetic acid bacteria (White & Zainasheff, 2010). Previous investigations describe the antibacterial properties of secreted compounds produced by non-*Saccharomyces* strains isolated from soil, fruit and vegetables (Bajaj et al., 2013; Waema et al., 2009). To our knowledge, this is the first report of antibacterial properties against enteropathogens reported in the supernatants of wort fermentation.

Moreover, the brewer's yeasts co-cultured with fungi had a profound impact in their growing rate. In fact, the compounds produced by the yeasts diminished the growth rate of *Aspergillus flavus* CMUNLPI5 between 49.0 and 60.9% and of *Aspergillus parasiticus* CMUNLP7 between 54.1 and 63.8% up to 216 h of co-incubation. Further co-incubation demonstrated that *A. flavus* CMUNLPI5 is fully inhibited by *S. cerevisiae* MBELGA62, about 60.0% by *P. kudriavzevii* MBELGA61 and to 37.2% by the starter M6.

These results point out the antifungal properties against *Aspergillus* sp. of brewer's yeasts and its bioactive compounds, in accordance with similar results reported previously. The killer activity is related to different compounds, many of them of peptidic nature, produced and secreted by yeasts. Recently, Vero et al. (2013) isolated a *Leucosporidium scottii* strain capable of producing soluble and volatile compounds that biocontrol gray moho and blue moho disease in apples. The antifungal properties of the live brewer's yeasts present in the BFRs could deliver interesting direct applications of this residue, with no need of pretreatments.

Another reported mechanism of yeasts to antagonize microorganisms is the production of volatile organic compounds (VOCs) (Muccilli & Restuccia, 2015). The production of VOCs by means of the catabolism of amino acids in yeasts, begins with the transamination of alpha-keto acids, followed by decarboxylation to produce the superior aldehyde, which in turn is reduced or oxidized to alcohols or superior acids, respectively, that in turn would generate aromatic esters. The antifungal activity of the ethyl acetate produced by *W. anomalus* was confirmed in hermetic stored grains (Fredlund et al., 2004; Druvefors et al., 2005). Hua et al. (2014) proved the antifungal activity of 2-phenylethanol, produced by a *Pichia anomala* strain, that affected the spore

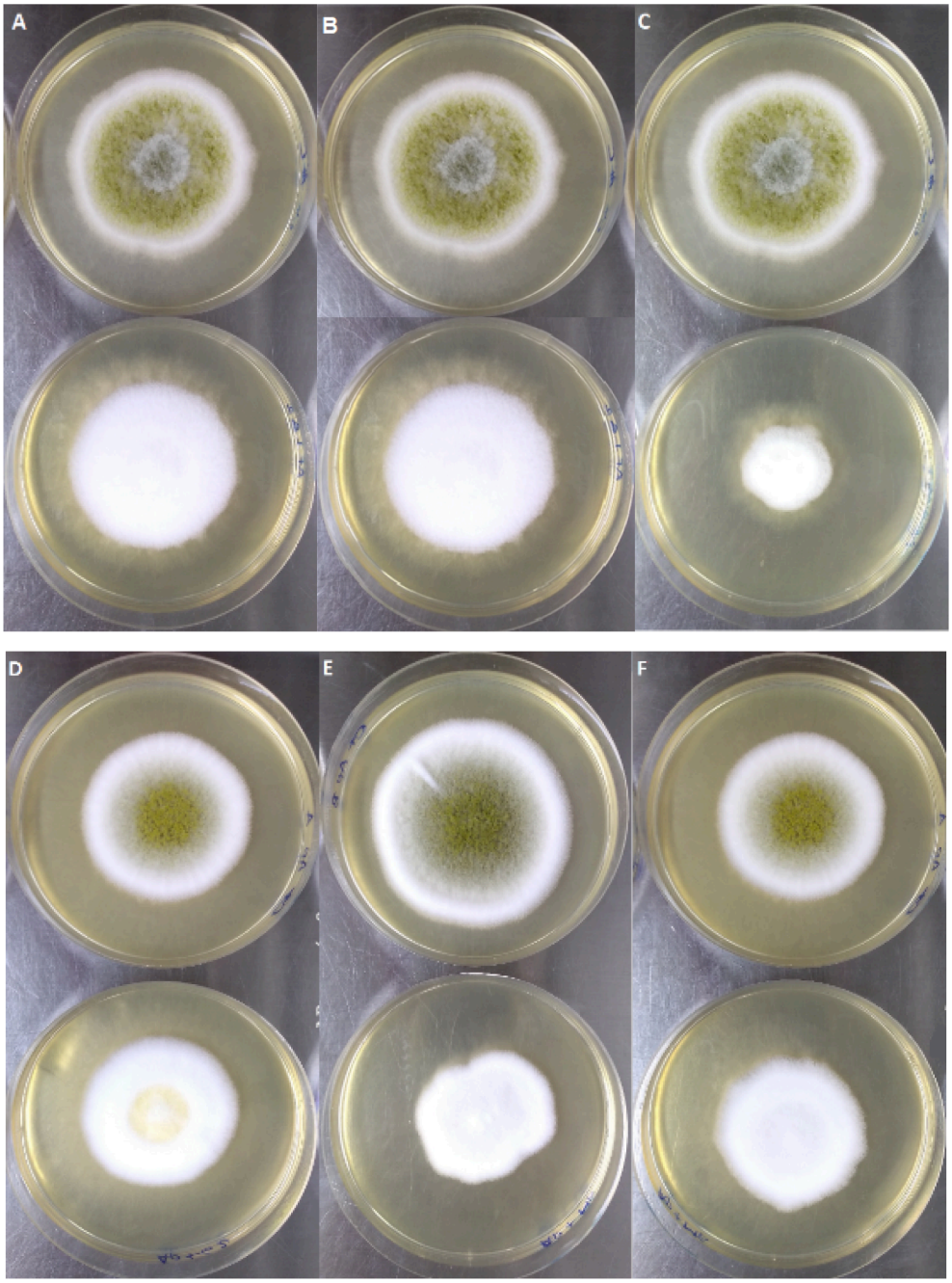

Fig. 3. *Aspergillus* sp. cultured in the micro atmosphere of VOCs produced by brewer's yeasts. The pictures were taken at 240 h of incubation; the bottom plates are the treatments and the upper plates are the respective positive controls (in absence of VOCs). **A**: *S. cerevisiae* MBELGA62 + *A. flavus* CMUNLPI5. **B**: starter M6 + *A. flavus* CMUNLPI5. **C**: *P. kudriavzevii* MBELGA61 + *A. flavus* CMUNLPI5. **D**: *S. cerevisiae* MBELGA62 + *A. parasiticus* CMUNLP7. **E**: starter M6 + *A. parasiticus* CMUNLP7. **F**: *P. kudriavzevii* MBELGA61 + *A. parasiticus* CMUNLP7.

germination, the growth rate and the synthesis of mycotoxins by *Aspergillus flavus*.

This contribution evidences that VOCs significantly reduced the growth rate of *A. flavus* between 30.5 and 57.5%. In addition, VOCs produced by *P. kudriavzevii* MBELGA61 achieve a significant reduction on the growth rate of *A. parasiticus* CMUNLP7. The main impact of the VOCs was observed in the fungal lag phase, which was significantly extended in all treatments, reaching the longest lag phase in the micro atmosphere of VOCs produced by *P. kudriavzevii* MBELGA61.

The fungal growth rate registered in the presence of the VOCs produced by the isolated brewer's strains and the starter M6 is negligible as discussed before. Somehow, this result suggests that there is not a synergic effect of the bioactive compounds produced by each strain in the micro atmosphere of the starter M6. The observation could be interpreted as the result of the accumulation of partial pressures of each VOC until a total limiting pressure is reached in the micro atmosphere of the starter M6, which is lower than the pressure of the same VOC accumulated when cultures of isolated yeast strains are used. This observation allows to conclude that the fungal growth reduction depends both on the nature and the concentration (partial pressure) of the bioactive VOCs accumulated in the micro atmosphere of incubation.

The inhibition exerted by the VOCs over *A. parasiticus* CMUNLP7 and *A. flavus* CMUNLPI5 was observed from the very beginning of the incubation according to the kinetic parameters. In this sense, Hua et al. (2014) reported that the aromatic compound 2-phenylethanol, produced by *Pichia anomala*, inhibits the germination of conidia and the biosynthesis of AFB_1 by *A. flavus*. In fact, the present results indicate that after 24 h of incubation in a micro atmosphere of yeast's VOCs, *Aspergillus* sp. was unable to develop a detectable mycelium, meaning a total fungal inhibition at this point. This phenomenon represents a significant elongation in the fungal lag phase.

Particularly, *P. kudriavzevii* MBELGA61 produced the VOCs with the

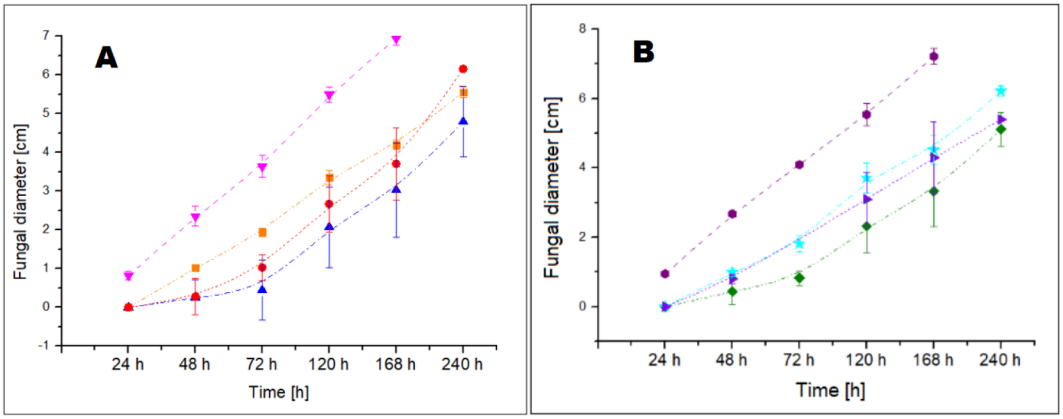

Fig. 4. Evolution of the fungal diameter of *Aspergillus* sp. growing in a micro atmosphere of VOCs produced by brewer's yeasts. Fungal diameter [cm] vs time [h] for *A. flavus* CMUNLPI5 (A) and *A. parasiticus* CMUNLP7 (B) growing in a micro atmosphere of VOCs produced by brewer's yeasts. Each point is the average of three biological replicas; bars represent the standard deviation. **A**: ■ *S. cerevisiae* MBELGA62 + *A. flavus* CMUNLPI5. ▲ *P. kudriavzevii* MBELGA61 + *A. flavus* CMUNLPI5. ● Starter M6 + *A. flavus* CMUNLPI5. ▼ Control positivo *A. flavus* CMUNLPI5. **B**: ★ *S. cerevisiae* MBELGA62 + *A. parasiticus* CMUNLP7. ◆ *P. kudriavzevii* MBELGA61 + *A. parasiticus* CMUNLP7. ▶ Starter M6 + *A. parasiticus* CMUNLP7. ⬢ Control positivo *A. parasiticus* CMUNLP7.

Table 2
Nature and concentration of the products of wort fermentations with brewer's yeasts.

Strain/starter	Glycerol (g/L)	2,3- butanediol (g/L)	Erythritol (g/L)	Ethanol (% v/v)	Glucose (g/L)	Fructose (g/L)
Sc MBELGA62	2.54 ± 0.02^{B}	0.30 ± 0.01^{AB}	0.06 ± 0.00^{B}	6.48 ± 0.04^{B}	0.00 ± 0.71^{B}	0.00 ± 0.00^{B}
Pk MBELGA61	0.77 ± 0.02^{C}	0.10 ± 0.01^{C}	0.04 ± 0.00^{C}	1.19 ± 0.04^{C}	0.00 ± 0.66^{B}	0.00 ± 0.00^{B}
Starter M6	0.77 ± 0.02^{C}	0.08 ± 0.01^{C}	0.04 ± 0.00^{C}	1.19 ± 0.03^{C}	0.00 ± 0.62^{B}	0.00 ± 0.00^{B}
T-58	2.81 ± 0.02^{A}	0.33 ± 0.01^{A}	0.08 ± 0.00^{A}	7.05 ± 0.04^{A}	0.00 ± 0.71^{B}	0.00 ± 0.00^{B}
Unfermented brewing wort	0.17 ± 0.04^{D}	0.07 ± 0.01^{C}	0.03 ± 0.00^{D}	0.00 ± 0.07^{D}	15.22 ± 1.24^{A}	4.46 ± 0.00^{A}

*Micro fermentations were carried out in brewing wort of 17.0 °Bx and pH 4.5 ± 0.1, at 20 °C in a volume of 70.0 mL, with stirring (140 rpm). Results are expressed as the media of quantified metabolites of three replicas for each strain ± standard deviation. Each replica was analyzed twice by HPLC. Different capital letters indicate significant differences ($P < 0.05$) among values within a column. *Sc*: *S. cerevisiae*. *Pk*: *P. kudriavzevii*. T-58: Safbrew T-58 (Fermentis, France), commercial lyophilized starter used as positive control of fermentation.

strongest effect, extending *Aspergillus* sp. lag phase more than 48 h. The same behavior was observed for *A. flavus* CMUNLPI5 cultured in a micro atmosphere of VOCs of the starter M6, that is also composed by *P. kudriavzevii* MBELGA61.

Furthermore, *Aspergillus* sp. was unable to produce conidia after 240 h of incubation in the presence of VOCs. Therefore, the volatile bioactive compounds produced by brewer's yeasts had a major impact in the biocontrol of *Aspergillus* sp., preventing the dissemination of fungi through the synthesis and release of conidia. The VOCs would be produced by the live yeasts of the BFRs, associating its antifungal properties to the brewing residue.

Among these metabolites, ethanol and 2,3-butanediol have been reported as bioactive compounds with antimicrobial properties. Ethanol is a disinfectant and antiseptic agent used worldwide, that antagonizes bacteria through the denaturalization of its proteins and the dissolution of the lipids of the cellular membrane, inducing the bacterial lysis (McDonnell & Russell, 1999).

The 2,3-butanediol is produced in the second step of beer fermentation, known as the maturation, by yeasts capable to perform the enzymatic conversion of the diacetyl produced during the main fermentation, in order to decrease the concentration of the off-flavor diacetyl below the taste threshold for organoleptic purposes (Strehaiano et al., 2006). In addition to its technological interest, this compound was reported as a bioactive compound that inhibited the development of Gram-positive and Gram-negative bacteria as well as several fungi (Madasu et al., 2017).

The presence of ethanol and 2,3-butanediol in the fermented products of brewer's yeasts partly explains the bacteriostatic and antifungal properties of the BFRs and its supernatants. Furthermore, a fraction of these bioactive compounds would be present in the micro atmosphere of yeasts' s VOCs, exerting the observed antifungal effect against *Aspergillus* sp.

In this context, *P. kudriavzevii* MBELGA61 produces either certain organic compounds or similar substances at higher concentration than the other yeasts. The low production of ethanol and 2,3-butanediol detected in the wort fermented by that yeast demonstrated that it directs part of the carbon flux to the anabolism of bioactive compounds, different from ethanol and 2,3-butanediol, with antagonic effect against *Aspergillus* sp. and enteropathogenic bacteria.

These findings encourage the application of the brewing residue as an additive with probiotic and biocontrolling potential in beverages, food and feed. The presence of bacteriostatic and antifungal bioactive compounds in the supernatants of the BFRs indicate that those compounds are present in the beer produced with these yeast strains, and raise new questions and hypotheses about the benefits of the consumption of beer with probiotic and prebiotic traits. Interestingly, the production of functional beers is a growing economic activity potentiated in the trends of healthier lifestyles. Secondly, the BFRs, including the supernatants of immature beer and the flocculated yeasts, can be used as additives with biocontrolling properties in feed production. Since most diets elaborated for livestock are based on cereals, it tends to be contaminated by filamentous fungi, especially when not properly conserved. The supplementation of feed with the BFRs can prevent the contamination and mycotoxin synthesis by *Aspergillus* sp., thus avoiding important economic losses for this productive activity.

5. Conclusions

Previous studies discussed in the introduction proved the capacity of these brewer's yeasts to secrete bioactive compounds during the

fermentation of the brewing wort that, even in absence of live yeasts, have antagonic effect on the germination of *Aspergillus* sp. conidia. *S. cerevisiae* MBELGA62 and the starter M6 were the yeasts that produce the major antifungal effect in those assays. Moreover, the BFRs obtained from wort fermentations performed by these yeasts are highly effective in the binding of aflatoxin B_1 (AFB_1), a carcinogenic mycotoxin produced by *Aspergillus* genera.

This investigation continues the characterization of these BFRs with the perspective of its valorization for different applications, contemplating its potential as a source of bioactive compounds. The BFRs, containing physiological active yeasts and a supernatant of immature beer with secreted metabolites, are highly effective in the inhibition of conidia synthesis by *Aspergillus* sp., thus preventing fungal dissemination throughout the environment and food matrices. Further, the BFRs significantly reduce the fungal growth rate and extend *Aspergillus* sp. lag phase more than 100%. Moreover, the cell free supernatant obtained from the wort fermentations carried out with the selected yeasts have a bacteriostatic effect over *S. Enteritidis* and enterohemorrhagic *E. coli*. The analysis of the CFSs allowed the identification of the antimicrobial analytes ethanol and 2,3-butanediol in *S. cerevisiae* MBELGA62 fermentations. Surprisingly, those metabolites were not found in substantial quantities in *P. kudriavzevii* MBELGA61 fermentations, so the identity of the potent antimicrobial bioactive compounds produced by this strain remains unknown, and further investigation is required.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET, Argentina) [grant numbers: Res. 4724 (9/12/2014) - BECA DOC 14 TEMAS ESTRATÉGICOS] and the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA, Argentina). The authors are grateful to Professor Amparo Querol and to Ph.D. Laura Pérez-Través for her assistance in the chromatographic analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100193.

References

Armando, M. R., Dogi, C. A., Poloni, V., Rosa, C. A. R., Dalcero, A. M., & Cavaglieri, L. R. (2013). *In vitro* study on the effect of *Saccharomyces cerevisiae* strains on growth and mycotoxin production by *Aspergillus carbonarius* and *Fusarium graminearum*. *International Journal of Food Microbiology, 161*(3), 182–188.

Bajaj, B. K., Raina, S., & Singh, S. (2013). Killer toxin from a novel killer yeast Pichia kudriavzevii RY55 with idiosyncratic antibacterial activity. *Journal of Basic Microbiology, 53*(8). https://doi.org/10.1002/jobm.201200187

Bevan, E. A., & Somers, J. M. (1969). Somatic segregation of the killer (k) and neutral (n) cytoplasmic genetic determinants in yeast. *Genetical Research, 14*(1), 71–77. https://doi.org/10.1017/S0016672300001865

Bleve, G., Grieco, F., Cozzi, G., Logrieco, A., & Visconti, A. (2006). Isolation of epiphytic yeasts with potential for biocontrol of *Aspergillus carbonarius* and *A. niger* on grape. *International Journal of Food Microbiology, 108*(2), 204–209. https://doi.org/10.1016/j.ijfoodmicro.2005.12.004

Chichester, F., & Tanner, F. W. (1972). Antimicrobial food additives. In T. E. Furia (Ed.), *Handbook of Food Additives* ((2nd ed.), p. (p. 115).). CRC Press.

Colen, L., & Swinnen, J. (2016). Economic growth, globalisation and beer consumption. *Journal of Agricultural Economics, 67*(1), 186–207. https://doi.org/10.1111/1477-9552.12128

Costa, A. G., Magnani, M., & Castro-Gómez, R. J. H. (2012). Obtenção e caracterização de manoproteínas da parede celular de leveduras de descarte em cervejaria. *Acta Scientiarum. Biological Sciences, 34*(1), 77–84. https://doi.org/10.4025/actascibiolsci.v34i1.7124

da Silva Araújo, V. B., de Melo, A. N. F., Costa, A. G., Castro-Gomez, R. H., Madruga, M. S., de Souza, E. L., & Magnani, M. (2014). Followed extraction of β-glucan and mannoprotein from spent brewer's yeast (*Saccharomyces uvarum*) and application of the obtained mannoprotein as a stabilizer in mayonnaise. *Innovative Food Science & Emerging Technologies, 23*, 164–170. https://doi.org/10.1016/j.ifset.2013.12.013

Dashko, S., Zhou, N., Compagno, C., & Piškur, J. (2014). Why, when, and how did yeast evolve alcoholic fermentation? *In FEMS Yeast Research, 14*(6), 826–832. https://doi.org/10.1111/fyr.2014.14.issue-610.1111/1567-1364.12161

Druvefors, U.Å., Passoth, V., & Schnürer, J. (2005). Nutrient effects on biocontrol of *Penicillium roqueforti* by *Pichia anomala* J121 during airtight storage of wheat. *Applied and Environmental Microbiology, 71*(4), 1865–1869. https://doi.org/10.1128/AEM.71.4.1865-1869.2005

Fredlund, E., Blank, L. M., Schnürer, J., Sauer, U., & Passoth, V. (2004). Oxygen- and glucose-dependent regulation of central carbon metabolism in *Pichia anomala*. *Applied and Environmental Microbiology*, *70*(10), 5905–5591. https://doi.org/10.1128/AEM.70.10.5905-5911.2004.

Gamba, R. R., Ni Colo, C., Correa, M., Astoreca, A., Alconada, T., De Antoni, G., & León Peláez, A. (2015). Antifungal activity against *Aspergillus parasiticus* of supernatants from whey permeates fermented with kefir grains. *Advances in Microbiology, 5*(6), 479–492. https://doi.org/10.4236/aim.2015.56049

Golubev, W. I. (2006). In *The Yeast HandbookBiodiversity and Ecophysiology of Yeasts* (pp. 197–219). Berlin/Heidelberg: Springer-Verlag. https://doi.org/10.1007/3-540-30985-3_10.

Hellborg, L., & Piskur, J. (2009). Yeast diversity in the brewing industry. In V. R. Preedy (Ed.), *Beer in health and disease prevention* (pp. 1068–1073). Elsevier.

Holt, S., Mukherjee, V., Lievens, B., Verstrepen, K. J., & Thevelein, J. M. (2018). Bioflavoring by non-conventional yeasts in sequential beer fermentations. *Food Microbiology, 72*, 55–66. https://doi.org/10.1016/j.fm.2017.11.008

Hua, S. S. T., Beck, J. J., Sarreal, S. B. L., & Gee, W. (2014). The major volatile compound 2-phenylethanol from the biocontrol yeast, *Pichia anomala*, inhibits growth and expression of aflatoxin biosynthetic genes of *Aspergillus flavus*. *Mycotoxin Research, 30*(2), 71–78. https://doi.org/10.1007/s12550-014-0189-z

IARC, International Agency for Research on Cancer- World Health Organization. (2002). *IARC Monograph on the Evaluation of Carcinogenic Risk to Humans*, *96*, 171.

Madasu, R. H. B., Muvva, V., Munaganti, R. K., Dorigondla, K. R., & Yenamandra, V. (2017). Bioactive-guided fractionation of diols from *Streptomyces* sp. MSL. *3. Biotech, 7*(1), 59. https://doi.org/10.1007/s13205-017-0649-1

Martins, Z. E., Pinho, O., & Ferreira, I. M. P. L. V. O. (2018). Impact of new ingredients obtained from brewer's spent yeast on bread characteristics. *Journal of Food Science and Technology*, *55*(5), 1966–1971. https://doi.org/10.1007/s13197-018-3107-0.

Masih, E. I., & Paul, B. (2002). Secretion of 1,3-glucanases by the yeast *Pichia membranifaciens* and its possible role in the biocontrol of *Botrytis cinerea* causing grey mold disease of the grapevine. *Current Microbiology, 44*(6), 391–395. https://doi.org/10.1007/s00284-001-0011-y

Mathias, T. R. D. S., Alexandre, V. M. F., Cammarota, M. C., de Mello, P. P. M., & Sérvulo, E. F. C. (2015). Characterization and determination of brewer's solid wastes composition. *Journal of the Institute of Brewing, 121*(3), 400–404. https://doi.org/10.1002/jib.v121.310.1002/jib.229

McDonnell, G., & Russell, A. D. (1999). Antiseptics and disinfectants: Activity, action, and resistance. *Clinical Microbiology Reviews, 12*(1), 147–179.

Muccilli, S., & Restuccia, C. (2015). Bioprotective role of yeasts. *Microorganisms, 3*(4), 588–611. https://doi.org/10.3390/microorganisms3040588

Muccilli, S., Wemhoff, S., Restuccia, C., & Meinhardt, F. (2013). Exoglucanase-encoding genes from three *Wickerhamomyces anomalus* killer strains isolated from olive brine. *Yeast, 30*(1), 33–43. https://doi.org/10.1002/yea.v30.110.1002/yea.2935

Mussatto, S. I. (2009). In *Biotechnology for Agro-Industrial Residues Utilisation* (pp. 313–326). Dordrecht: Springer Netherlands. https://doi.org/10.1007/978-1-4020-9942-7_16.

Pérez-Través, L., Lopes, C. A., Barrio, E., & Querol, A. (2015). Stabilization process in *Saccharomyces* intra- and interspecific hybrids in fermentative conditions. *International Microbiology, 17*(4), 213–224. https://doi.org/10.2436/20.1501.01.224

Piotrowska, A., Waszkiewicz-Robak, B., & Swiderski, F. (2009). Possibility of beta-glucan from spent brewer's yeast addition to yoghurts. *Polish Journal of Food and Nutrition Sciences, 59*(4), 299–302.

Pretscher, J., Fischkal, T., Branscheidt, S., Jäger, L., Kahl, S., Schlander, M., … Claus, H. (2018). Yeasts from different habitats and their potential as biocontrol agents. *Fermentation, 4*(2), 31. https://doi.org/10.3390/fermentation4020031

Puligundla, P., Mok, C., & Park, S. (2020). Advances in the valorization of spent brewer's yeast. *Innovative Food Science & Emerging Technologies, 62*, 102350. https://doi.org/10.1016/j.ifset.2020.102350

Sampaolesi, S., Gamba, R. R., De Antoni, G. L., & León Peláez, Á. M. (2019). Potentiality of yeasts obtained as beer fermentation residue to be used as probiotics. *LWT Food Science and Technology, 113*, 108251. https://doi.org/10.1016/j.lwt.2019.108251

Strehaiano, P., Ramon-Portugal, F., & Taillandier, P. (2006). In *Yeasts in Food and Beverages* (pp. 243–283). Berlin, Heidelberg: Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-28398-0_9.

Suzuki, C., Ando, Y., & Machida, S. (2001). Interaction of SMKT, a killer toxin produced by *Pichia farinosa*, with the yeast cell membranes. *Yeast, 18*(16), 1471–1478. https://doi.org/10.1002/(ISSN)1097-006110.1002/yea.v18:1610.1002/yea.791

Thammakiti, S., Suphantharika, M., Phaesuwan, T., & Verduyn, C. (2004). Preparation of spent brewer's yeast β-glucans for potential applications in the food industry. *International Journal of Food Science & Technology, 39*(1), 21–29. https://doi.org/10.1111/j.1365-2621.2004.00742.x

Vero, S., Garmendia, G., González, M. B., Bentancur, O., & Wisniewski, M. (2013). Evaluation of yeasts obtained from Antarctic soil samples as biocontrol agents for the

management of postharvest diseases of apple (*Malus* × *domestica*). *FEMS Yeast Research, 13*(2), 189–199. https://doi.org/10.1111/1567-1364.12021

Vieira, E. F., Melo, A., & Ferreira, I. M. P. L. V. O. (2017). Autolysis of intracellular content of brewer's spent yeast to maximize ACE-inhibitory and antioxidant activities. *LWT Food Science and Technology*, *82*, 255–259. https://doi.org/10.1016/j.lwt.2017.04.046.

Vieira, E., Cunha, S. C., & Ferreira, I. M. P. L. V. O. (2019). Characterization of a potential bioactive food ingredient from inner cellular content of brewer's spent yeast. *Waste and Biomass Valorization*, *10*, 3235–3242. https://doi.org/10.1007/s12649-018-0368-9.

Waema, S., Maneesri, J., & Masniyom, P. (2009). Isolation and identification of killer yeast from fermented vegetables. *Asian Journal of Food Agro-Industry, 2*(04), 126–134.

Waszkiewicz-Robak, B., & Bartnikowska, E. (2009). Effects of spent brewer's yeast and biological β-glucans on selected parameters of lipid metabolism in blood and liver in rats. *Journal of Animal and Feed Sciences, 18*(4), 699–708. https://doi.org/10.22358/jafs/66443/2009.

White, C., & Zainasheff, J. (2010). Yeast, the practical guide to beer fermentation (Parts 1st and 6th). *Brewers*. Publications.

Yiannikouris, A., Francois, J., Poughon, L., Dussap, C. G., Bertin, G., Jeminet, G., & Jouany, J. P. (2004). Alkali extraction of β-D-glucans from *Saccharomyces cerevisiae* cell wall and study of their adsorptive properties toward zearalenone. *Journal of Agricultural and Food Chemistry, 52*(11), 3666–3673. https://doi.org/10.1021/jf035127x