Research article

Postbiotic characterization of a potential probiotic yeast isolate, and its microencapsulation in alginate beads coated layer-by-layer with chitosan

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A B S T R A C T

Considering biosafety concerns and survivability limitations of probiotics (PRO) under different stresses, application of postbiotics and encapsulated PRO has received considerable attentions. Accordingly, the objective of the present study was to investigate the postbiotic capabilities of a potential PRO yeast isolate and the effect of encapsulation with alginate (Alg) and chitosan (Ch) on its survival under SGI conditions. Sequencing results of the PCR products led to the identification of *Saccharomyces cerevisiae* as the selected potential PRO yeast isolated from wheat germ sourdough. High survival of the isolate under simulated gastrointestinal (SGI) conditions (95.74%), its proper adhesion abilities, as well as its potent inhibitory activity against *Listeria monocytogenes* (75.84%) and *Aspergillus niger* (77.35%) were approved. Interestingly, the yeast cell-free supernatant (CFS) showed the highest antioxidant (84.35%) and phytate-degrading (56.19%) activities compared to the viable and heat-dead cells of the isolate. According to the results of the HPLC-based assay, anti-ochratoxin A (OTA) capability of the dead cells was also significantly ($P < 0.05$) higher than that of the viable cell. Meanwhile, the yeast CFS had no anti-OTA and antimicrobial activities against the foodborne bacteria and fungi tested. Further, microencapsulation of the yeast isolate in Alg beads coated layer-by-layer with Ch (with 77.02% encapsulation efficacy and diameter of 1059 μm based on the field emission scanning electron microscopy analysis) significantly enhanced its survivability under SGI conditions in comparison with the free cells. In addition, electrostatic cross-linking between negatively charged carboxylic groups of Alg and positively charged amino groups of Ch was verified in accordance with Fourier transform infrared and zeta potential data. Human and/or industrial food trials in future are needed for practical applications of these emerging ingredients.

1. Introduction

Probiotics (PRO) as viable microorganisms have promising beneficial effects on the host when consumed in adequate amounts. Study of PRO has focused primarily on lactic acid bacteria (LAB); meanwhile, some yeasts such as *Saccharomyces boulardii* are known for their PRO, health-promoting, and pro-functional potentials. Yeasts have bigger size, inherent resistance towards antibiotics, proper adhesion properties and survivability under environmental stresses. Furthermore, PRO yeasts (PYs) as starter or adjunct cultures play a

crucial role in end product safety, shelf-life, and techno-functional features [1,2]. There are some reports about PRO, antimicrobial, and biotechnological capabilities of yeasts isolated from fermented cereals. For example, Sakandar et al. [3] investigated antibacterial and PRO characteristics of yeasts isolated from wheat sourdough (SD). Yeast diversity of traditional Tuscan SD was also investigated by Palla et al. [4]. These researchers revealed pro-technological, nutritional, and functional traits of the selected isolates such as their antioxidant and phytase activities, as well as their resistance to simulated gastric (SG) and simulated intestinal (SI) conditions. Postbiotic term refers to the positive potential effects of microbial dead cells, structural components or their cell-free supernatant (CFS). Postbiotic attributes are not dependent to viability of beneficial microorganisms. These capabilities are including antimicrobial, anti-mycotoxigenic, health-promoting and nutritional activities (like antioxidant and phytate-degrading abilities). Although viable cells of beneficial microorganisms like PRO have undeniable emerging potentials and different applications in food/pharmaceutical sectors, while considering biosafety concerns and maintenance of PRO survivability under different stresses during their journey to colon as their final destination, postbiotics have gained interesting applications as promising alternative for PRO in a wide range of area. Postbiotic capabilities of PYs associated with their CFS, cell-wall components, or their dead cells were also investigated. Recently, Xu et al. [5] reported that the yeast cell-wall, yeast cell-wall extract, and a postbiotic yeast cell-wall-based blend product adsorbed 32.28, 23.55 and 36.86% of ochratoxin A (OTA), respectively. De Marco et al. [6] studied *in vitro* antioxidant activity of PYs CFS and revealed that their 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was about 10% at different concentrations of the CFS.

Although survival of PRO under gastrointestinal conditions is a key factor for their selection, strategies like microencapsulation enabled us to protect PRO under harsh environments such as gastrointestinal transit. Microencapsulation of PRO also leads to their release in a controlled manner at the target site. Moreover, this approach provides promising potential applications for PRO in a food/medicine matrix to improve their survivability and/or functionality compared to the free cells through efficient fabrication and/or functionalization of PRO. Accordingly, other potentials of PRO, such as their techno-functional capabilities, have become more important for their application. In this regard, encapsulated yeasts showed better survival under simulated gastrointestinal (SGI) conditions compared to the free cells according to the results of Suvarna et al. [7]. It has also been noted that the microencapsulated *S. boulardii* showed greater resistance to environmental factors, allowing its application in value added products [8]. Graff et al. [9] found that encapsulation resulted in greater protection from yeasts and enhanced their delivery to/viability in colon.

Postbiotic capabilities have gained great interest to the scientific community for industrial applications in food/pharmaceutical sectors. Accordingly, the aims of the present study were to investigate PRO and postbiotic capabilities of the selected yeast isolated from wheat germ SD (WGS), as well as its encapsulation in alginate (Alg) beads coated layer-by-layer (LBL) with chitosan (Ch) to improve its viability under SGI conditions. To the best of our knowledge, there are limited reports about postbiotic capabilities of potential PYs such as their anti-OTA, phytate-degrading, and antioxidant activities.

2. Materials and methods

2.1. Raw materials

All chemical reagents and microbial media used in this study were purchased with analytical grade. The studied foodborne bacteria and fungi were also supplied from American type culture collection (ATCC). Wheat germ flour (containing 26.39, 2.82, 11.17, 8.90 and 50.72% protein, ash, moisture, fat and total carbohydrates, respectively) as a by-product of wheat milling was purchased from a local milling industry. Sodium Alg (with molecular weight of 100 KDa), Ch (with medium molecular weight), DPPH, OTA, acetonitrile, isopropanol, pure phytate, ammonium iron III, 2, 2′-bipyridine, bile salt, pepsin and pancreatin were also supplied from Sigma-Aldrich (Burlington, VT, USA).

2.2. Spontaneous fermentation of WGS and isolation of the predominant yeasts

To prepare SD, wheat germ flour and tap water were mixed with dough yield (DY = flour + water/flour × 100) of 160. Then, the mixture was incubated at 28 °C for 24 h. Subsequently, ten-fold serially diluted SD samples were surface plated on yeast glucose chloramphenicol (YGC; Merck, Darmstadt, Hesse, Germany), wallerstein laboratory nutrient (WLN), and yeast extract peptone dextrose (YEPD; Merck, Darmstadt, Hesse, Germany), with the plates incubated at 28 °C for 48 h to isolate predominant yeasts. Thereafter, the yeast isolates were kept at −80 °C in 25% glycerol until use.

2.3. Hemolytic activity of the yeast isolates

The overnight cultured yeast isolates were spotted on blood agar (Merck, Darmstadt, Hesse, Germany) containing 5% (v/v) defibrinated sheep blood, after which the plates were incubated at 28 °C for 48 h. Subsequently, development of green-hued zones (α-hemolysis) and transparent or yellow lyses zones (β-hemolysis) around the colonies were determined and compared to *Staphylococcus aureus* as positive control [10].

2.4. Screening based on survival in SGI conditions

The predominant yeast isolates with no hemolytic activity were screened based on their survival under SGI conditions. In brief, 10^8 colony forming units (CFU)/mL of each isolate were incubated at 37 °C for 2 h in phosphate buffered saline (PBS) with pH 2.0 (adjusted with 1 N HCl) containing 3 mg/mL pepsin as SG conditions. Then, the pH of the suspension was readjusted to 6.5 (using 1 N NaOH) in

the presence of 0.3% bile salt and 1 mg/mL pancreatin, and it was incubated at 37 °C for 3 h as SI conditions, under agitation (50 rpm) to simulate peristalsis. Subsequently, the survival percentage was determined through surface plating (incubation at 28 °C for 48 h) of the treated isolates in comparison with their control (non-treated) samples [11].

2.5. Molecular identification of the selected yeast

The genomic DNA was extracted (Bioneer; Daejeon, Chungcheong, South Korea) from the fresh pure colony of the selected yeast isolate, after which it was subjected to PCR amplification with *ITS1* (5′-TCCGTAGGTGAACCTGCGG-3′) and *ITS4* (5′-TCCTCCGCTTATTGATATGC-3′) primers. The PCR reagents (Ampliqon, Odense, Funen, Denmark) and the thermal cycling (Corbett, Sydney, New South Wales, Australia) were implemented in accordance with White et al. [12] to amplify a 620 bp target sequence from (internal transcribed spacer) *ITS* region. To confirm the identity of the PCR products, they were checked through sequencing (Bioneer; Daejeon, Chungcheong, South Korea) assay. Accordingly, the data were analyzed (BLASTn algorithm), and the phylogenetic evolutionary tree was drawn using MEGA6 software (2013, from Pennsylvania State University, PA, USA) through the neighbor-joining method with 1000 bootstrap replicates [13].

2.6. Adhesion abilities of the selected yeast

The absorbance-based methods described by Fernandez-Pacheco et al. [14] were used to determine auto-aggregation of the selected yeast isolate and its co-aggregation ability with the tested foodborne bacteria. The cell-surface hydrophobicity of the isolate towards xylene was investigated following the procedure reported by Rodríguez et al. [15]. Biofilm formation ability of the isolate on sterile glass slide was also studied according to Speranza et al. [16].

2.7. Antibiotic and anti-mycotic susceptibility of the selected yeast

The disc diffusion bioassay was used to investigate antibiotic and anti-mycotic susceptibility of the selected yeast isolate. The overnight cultured yeast (10^8 CFU/mL) and YGC agar mixture was poured in the plates. Then, the discs were placed on the plates, and the diameter of inhibition zone was determined after 24 h incubation at 28 °C. The results were also reported as sensitive, intermediate, and resistance with diameter of inhibition zone higher than 10 mm, within 10–20 mm, and lower than 10 mm, respectively [17].

2.8. Antibacterial activities

The antibacterial activity of the selected yeast isolate against foodborne bacteria including *Listeria monocytogenes* ATCC 19115, *S. aureus* ATCC 6538, *Escherichia coli* ATCC 25922, and *Salmonella enterica* serovar *Typhimurium* ATCC 14028 was also assayed through ten-fold serially diluted samples of their mixed-culture on chromogenic media (CHROMagar; Paris, Île-de-France, France) according to Zarali et al. [18]. To prepare the yeast CFS, overnight cultured isolate was centrifuged (Sigma; Osterode, Niedersachsen, Germany; 4500 g, 10 min at 4 °C), and then the supernatant was micro-filtered (0.45 μm). The inhibitory effect of the yeast CFS was also determined on the foodborne bacteria through broth microdilution method [19].

2.9. Antifungal activities

The antifungal activity of the selected yeast isolate and its CFS against *Aspergillus niger* ATCC 1004 and *Aspergillus flavus* ATCC 96044 was also analyzed through microtiter bioassay according to the Ruggirello et al. [20] procedure with slight modifications. In brief, fungal spores were enumerated with a hemocytometer and were resuspended in brain heart infusion (BHI) broth medium at a concentration of 10^6 spores/mL. 200 μL of inoculum containing 180 μL of yeast CFS with 20 μL of fungal spores was added into each well of a 96-well microtiter plate. The microplates were then incubated at 30 °C for 72 h, and the absorbances measured at 490 nm compared to values found in positive control (fungal spore suspension in BHI broth without yeast CFS) and blank (fresh sterile medium without fungal spore suspension) wells.

2.10. Anti-OTA activities

The high-performance liquid chromatography (HPLC- Hitachi; Osaki, Tokyo, Japan, L-7100 coupled with L-2480 Fluorescence detector)-based method was used for OTA detection. The separation of OTA was performed using a Prosphere 100C18 (150 × 4.6 mm, 5 μm) column with a solution of acetonitrile/water/isopropanol/acetic acid (46/46/6/2 v/v) as the mobile phase. The flow rate and the oven temperature were set at 1 mL/min and 40 °C, respectively. The excitation and emission wavelengths to detect OTA content in the samples were also equal to 333 and 460 nm, respectively [21]. Accordingly, anti-OTA activity of the viable and heat-dead (autoclaved at 121 °C for 15 min) yeast cells, as well as its CFS were determined and compared to the control (mixture of culture medium and OTA) sample.

2.11. Phytate-degrading abilities

The phytate-degrading ability of the selected yeast isolate as viable, heat-dead cells, and its CFS was determined through the

method of Haug and Lantzsch [22] with some modifications. In brief, each sample was inoculated to the pure phytate and incubated for 24 h at room temperature. Then, ammonium iron III sulfate solution was added (30 min in boiling water), and the mixture was centrifuged at 3000 g for 30 min. Subsequently, the supernatant was added to 2, 2′-bipyridine solution, where the absorbance of the mixture was measured at 519 nm. The amount of the residual phytate was also calculated using a standard curve calibrated with known concentrations of the pure phytate.

2.12. DPPH radical scavenging activities

The DPPH radical scavenging activity of the yeast isolate (as viable and heat-dead cells, as well as its CFS) was investigated in accordance with Kao and Chen [23] with some modification. Briefly, the yeast cells with 10^8 CFU/mL were added into ethanolic DPPH solution. The scavenging ability was also measured at 517 nm after 30 min incubation at room temperature in comparison with the control (deionized water and DPPH solution), blank (ethanol and the cells), and butylated hydroxyanisole (BHA) as the reference.

2.13. Microencapsulation and characterization of the yeast-loaded microcapsules

2.13.1. Yeast encapsulation in Alg beads coated LBL with chitosan
Overnight cultured yeast isolate was centrifuged (5000 g at 4 °C for 10 min), and then 9 mL of sodium Alg (2% w/v) solution was mixed with 1 mL of the cell suspension (10^8 CFU/mL). Subsequently, the mixture was dropped slowly into a solution containing 0.05 M CaCl$_2$. The beads were then left to harden for 45 min. For additional coating using LBL method, Ch (0.4% w/v) was acidified with glacial acetic acid, and its pH was adjusted to 5.7–6.0 using 1 N NaOH. Thereafter, Alg beads prepared by extrusion method were suspended in Ch solution and shaken for 30 min at 100 rpm [24].

2.13.2. Encapsulation efficiency
In order to enumerate viable cells entrapped in the produced microcapsules, 1 g of the capsules was mixed with 9 mL of sodium citrate solution (3 g/L) with gentle agitation until the beads were completely released. Then, ten-fold serially diluted samples were spread plated on YGC agar. Encapsulation efficiency was also determined as the number of the entrapped viable cells divided by the amount of the initial cells used for encapsulation [18].

2.13.3. Morphology and zeta potential of the produced microcapsules
The surface morphology and size of the microcapsules were characterized using field emission scanning electron microscopy (FESEM; Mira3 Inc., Brno, Moravia, Czech Republic). For this purpose, a layer of gold was coated on the samples under vacuum before observation [19]. The average zeta potential of the microcapsules was also determined using a zeta-sizer (Horiba, Fukuchiyama, Kyoto, Japan) in accordance with Tan et al. [25].

2.13.4. FTIR spectroscopy analysis
Fourier transform infrared (FTIR) spectroscopy was performed using an FTIR spectrophotometer (Spectrum RX I; PerkinElmer, Waltham, MA, USA) to investigate how Alg and Ch interact in the microcapsules. Peak adsorptions also ranged between 400 and 4000 cm^{-1} [8].

2.14. In vitro survival of the microencapsulated yeast under SGI conditions

Viability of the microencapsulated yeast under SG, SI, and SGI conditions was determined as mentioned earlier compared to the free cells [7].

2.15. Statistical analysis

All experiments were done in triplicate. One way analysis of variance (ANOVA) with the least significant difference (LSD) post-hoc at $P < 0.05$ was used for statistical analysis of the data. Comparison between two different samples was performed using independent t-test. SPSS20 software (2011, from IBM Co., Louisville, KY, USA) and Office Excel 2019 were employed to analyze the data and draw the charts, respectively.

3. Results and discussion

### 3.1. Screening of the predominant yeast isolates

Eleven different yeasts were isolated from WGS as the predominant. Meanwhile, only one of the isolates showed viability higher than 95.74% after exposure to SGI conditions, while the other isolates had survival lower than 61%. Thus, the isolate with the highest survival, which had no hemolytic activity, was selected for further study. Hemolytic activity is the common *in vitro* biosafety assay in PRO characterization. No-hemolytic or gama hemolytic activity means the lack of the pathogenicity factors in the potential studied PRO. The viability of the selected yeast isolate under SGI conditions was significantly ($P < 0.05$) higher than that of the others (Fig. S1).

Proper survival (100% or more) of *Candida norvegensis* f2 fp21 isolated from Fura (spontaneously fermented cereal in west of Africa)

was verified after exposure to pH 2.5 and 0.3% bile salt in the study of Pedersen et al. [26]. In Palla et al. [4] survey, all strains of *Saccharomyces cerevisiae* and *Kazachstania humilis* G23Y selected among the autochthonous Tuscan SD yeasts showed a high resistance (about 95% survival in SG and 87% survival in SI conditions) to SGI conditions. The ability to survive under SGI conditions is a strain-dependent activity, while it is a pre-requisite for the selection of PRO, and 70% viability in these conditions has been established to select yeast isolates as potential PRO. There are several mechanism in PRO tolerance to low pH, bile salts, digestive enzymes and peristaltic. In general, harsher conditions of the SG than those of the SI conditions lead to the destruction of the microbial membrane proteins by pepsin especially at pH = 2. In addition, high concentrations of bile salts can dissolve the cell membrane, while its lower concentrations can increase flux of divalent cations [27].

3.2. Identification of the selected yeast

Sequencing results of the PCR products led to the identification of *S. cerevisiae* RWGS07 (with the similarity levels over 97%) as the selected yeast isolate. The nearest yeast to the isolate in the phylogenetic evolutionary tree (Fig. 1) was also *S. cerevisiae*.
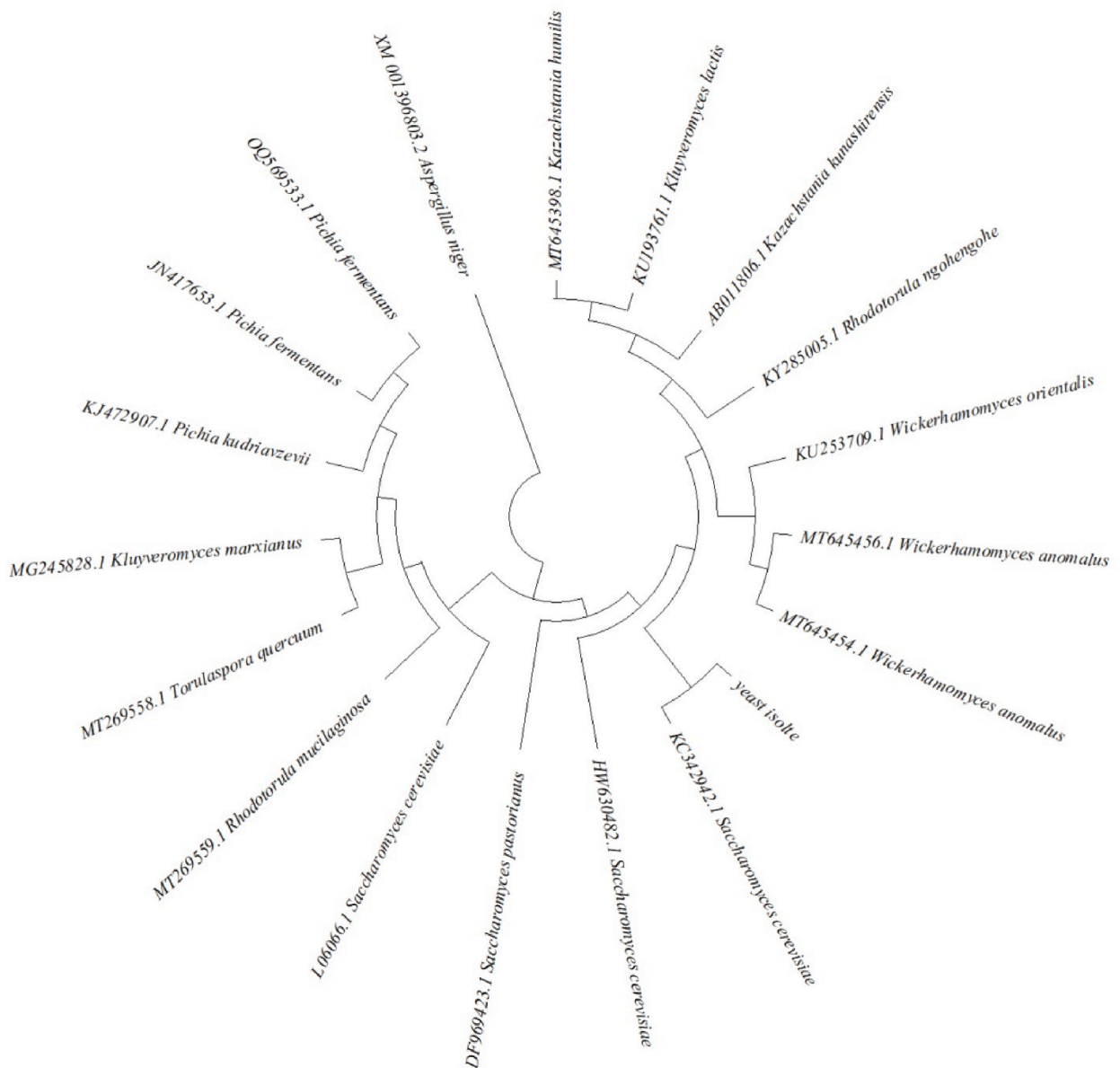
Fig. 1. The phylogenetic tree reveals the evolutionary position of the selected yeast isolated from wheat germ sourdough. This tree was derived from the neighbor-joining analysis of internal transcribed spacer genes with 1000 bootstrap replicates using MEGA6 software. *Aspergillus niger* was used as out-group, with the bar indicating 1% differentiation.

In agreement with our findings, *S. cerevisiae* and *Candida* sp. were the most frequently identified yeasts in traditional SD as stated by Minervini et al. [28]. Furthermore, Zhang et al. [29] illustrated that *S. cerevisiae* was the predominant species in Chinese traditional SD. The yeast diversity in SD is usually limited to specific genera at a given time. In addition, *S. cerevisiae* is often found in SDs due to its ubiquitous occurrence and the adaptation of its specific strains to the environmental conditions of the SD ecosystem. Furthermore, it is hypothesized that the yeasts adapted to SD as a stressful environment with low pH, low oxygen tension, and high osmotic conditions are good potential PRO. Application of industrial by-products like wheat germ as a valuable and interesting source for isolation of beneficial microorganisms like PYs has received increasing attention. As mentioned, considering the potential of SD fermentation due to its acidic and osmotic stresses for isolation of PRO, it is also a proper approach to revalorize industrial wastes and by-products [30, 31].

3.3. Adhesion properties of the selected yeast

In accordance with our data, *S. cerevisiae* isolate had 83.54 ± 6.38% auto-aggregation ability. Co-aggregation capability of the isolate with foodborne bacteria has also been shown in Fig. 2. Accordingly, co-aggregation percentage of the yeast isolate with *S. enterica* (70.71 ± 5.52%) was significantly ($P < 0.05$) higher than that of the other foodborne bacteria tested. Furthermore, co-aggregation ability of the yeast isolate with Gram-negative bacteria was higher than that of the Gram-positive bacteria studied. The cell viability in biofilm formation assay and the cell-surface hydrophobicity of the selected yeast isolate were also equal to 4.77 ± 0.31 Log CFU/cm^2 and 55.09 ± 2.3%, respectively.

Among the autochthonous yeasts isolated from wheat SD (khamir) in the study of Sakandar et al. [3], *Wickerhamomyces anomalus* QAUWA04 had the highest auto-aggregation (64.34%) capability. Isolated *S. cerevisiae* AKP1 was also able to co-aggregate with *E. coli* (75.87%) and *Vibrio cholerae* (24.31%) in Banik et al. [32] survey. Perricone et al. [33] reported relatively lower viabilities (2.96–4.63 Log CFU/cm^2) in biofilm formation assay with 60–96% cell-surface hydrophobicity for the yeast strains isolated from Altamura SD. Auto-aggregation as prerequisite for colonization and adhesion ability of the PRO to the intestinal epithelial cells is one the main steps of biofilm formation. The 50% auto-aggregation ability or more can increase withstanding of PYs in the gastrointestinal tract [7]. The cell-surface hydrophobicity of the PRO is also necessary for their attachment and maintenance in the human intestinal mucosa. Furthermore, co-aggregation properties of PRO with foodborne bacteria are important for their therapeutic applications and prevention from the invasion of different pathogens. Accordingly, adhesion capabilities are associated with colonization of PRO in the colon, their attachment with intestinal mucosa, pathogenic microorganisms or covering the gastric wounds as a treating shield [34, 35].

3.4. Antibiotic and anti-mycotic susceptibility of the selected yeast

The yeast isolate was resistant to all of the tested antibiotics as no inhibition zone was found; meanwhile, it was sensitive to fluconazole and ketoconazole (Table S1). These results are in agreement with those reported by Fekri et al. [36] about anti-mycotic susceptibility of the yeasts isolated from Arasbaran SD. Fernandez-Pacheco et al. [14] studied antibiotic susceptibility of the *S. cerevisiae* strains, and found that all yeasts showed resistance to the studied antibiotics. Banik et al. [32] reported that *S. cerevisiae* AKP1 was resistant to antibacterial compounds, while it was sensitive to anti-mycotic agents such as fluconazole and clotrimazole with
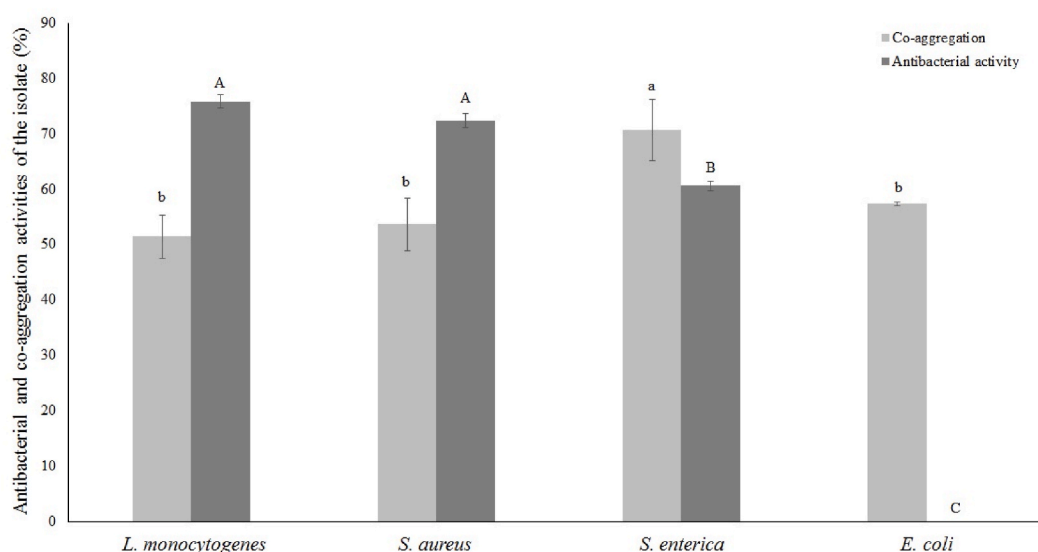
Fig. 2. Co-aggregation ability and antibacterial activity of the *S. cerevisiae* isolate against foodborne bacteria. The different lowercase and uppercase letters show significant difference ($P < 0.05$) among co-aggregation or antibacterial activities of the isolate, respectively.

23- and 20-mm inhibition zones, respectively. In general, PRO must be resistant against antibiotics without the ability to transfer resistance genes. PYs are usually resistant to antibacterial agents such as antibiotics, and their chromosomal resistance genes are not transferrable especially to prokaryotic cells. Accordingly, these eukaryotic microorganisms are proper potential PRO. Resistance of PRO to antibiotics and anti-mycotic agents allow their applications in combination with these components in treatments of diseases or control of foodborne pathogens in food matrices [37].

3.5. Antibacterial activities

The selected yeast isolate exhibited the highest antibacterial activity against *L. monocytogenes* followed by *S. aureus* and *S. enterica*, respectively. Meanwhile, the isolate had no inhibitory effect on *E. coli* (Fig. 2). Accordingly, the antibacterial effect of the isolate on Gram-positive foodborne bacteria was significantly ($P < 0.05$) higher than that of the Gram-negative bacteria tested. Furthermore, the yeast CFS did not show inhibitory activity against these foodborne bacteria.

In contrast to our findings, *S. cerevisiae* IFST 062013 revealed moderate inhibitory activity against bacteria studied, and its cell lysate had higher antimicrobial activity than the whole cell in the study of Fakruddin et al. [38]. Furthermore, the isolate showed higher antibacterial activity against Gram-negative bacteria than the Gram-positive bacteria tested. It is also reported that the formation of a "yeast-bacteria complex" in the cell-wall of *Saccharomyces* genus with pathogenic bacteria increases the sensitivity of pathogens to elimination mechanisms. Antimicrobial activity is one of the most important aspects for PRO applications in food and/or medicine sectors. Considering concerns about antibiotics and synthetic preservatives, introduction of the efficient bio-alternatives for antibiotics have received considerable importance. In general, antimicrobial activities of PRO are associated with their competition for foods or adhesion sites, production of a variety of inhibitory metabolites, their aggregation potential with pathogens or their effects on growth curve of the unwanted microorganisms. This hurdle is a promising practical approach to control foodborne pathogens. Antibacterial activities of the yeasts are also attributed primarily to competition for nutrients and their co-aggregation (physical elimination) ability, as well as production of antibacterial metabolites. Postbiotics have also emerging antimicrobial activities against foodborne pathogens. Meanwhile, one of the most important benefits of postbiotics compared to PRO is the possibility to utilize the higher dosage of the effective compounds. Furthermore, purified specific metabolite can be used in specific formulation or processing condition [39,40].

3.6. Antifungal activities

The inhibitory activity of the yeast isolate against *A. niger* (77.35 ± 0.16%) was significantly ($P < 0.05$) higher than that of *A. flavus* (60.99 ± 1.20%). Meanwhile, the yeast CFS had no antifungal effect on the studied fungi. These findings reaffirm that the antimicrobial activities of the *S. cerevisiae* isolate were mainly associated with its competition for nutrients or adhesion abilities rather than the production of inhibitory metabolites. In the same vein, Goktas et al. [41] reported that the high antifungal activity (77.03%) of *S. cerevisiae* KY-7 against *Penicillium rubens* was due to its adaptation ability to the same environment. Ng et al. [42] also revealed that the antimicrobial activity of *S. cerevisiae* strain Y26 compared to the negative control (wild-type BY4741) was solely attributable to the engineered biosynthetic pathway of the produced metabolites. Antifungal activity of PRO enabled us to apply these beneficial microorganisms as protective adjunct, starter or co-cultures in processing of fermented foods. Accordingly, simultaneous improvement of the product safety, shelf-life and functionality will be possible.

3.7. Anti-OTA capabilities

The heat-dead and viable cells of *S. cerevisiae* isolate diminished OTA by 28.14 and 11.96%, respectively, while its CFS had no effect on OTA removal as compared to the control (Table 1 and Fig. 3). Although several modes of action such as degradation of toxins into the less toxic metabolites, adsorption (physical binding) of toxins to the cell-wall or inhibition of toxin synthesize have been reported for detoxification of mycotoxins by yeasts [37], it is hypothesized that the main phenomenon associated with OTA removal by the yeast isolate in the present study was toxin adsorption due to the expanded surface of the dead cells compared to the viable cells. Likewise, Pinheiro [43] concluded that PRO *S. cerevisiae* reduced OTA efficiently (up to 31.8%) through its adsorption to the yeast cell-wall. *S. cerevisiae* Y33 as a potent biological agent reduced OTA by about 60% in the study of Tryfinopoulou et al. [44]. Armando et al. [45] revealed that all *S. cerevisiae* strains were able to remove OTA up to 82.3% under different conditions including PBS (with pH 7), at pH = 2 and presence of 0.5% bile salts. Physical adsorption as a strain-specific activity was also introduced as the main mechanism involved in the OTA removal in the aforementioned study. Although almost all of the molds are mesophile and sensitive to

Table 1
Postbiotic capabilities of the selected yeast isolated from wheat germ sourdough.

	DPPH scavenging activity (%)	Phytate-degrading ability (%)	OTA removal (%)
CFS	84.35 ± 0.12[a]	56.19 ± 0.40[a]	–
Dead cells	8.81 ± 0.61[b]	11.83 ± 2.86[b]	28.14 ± 2.31[a]
Viable cells	3.60 ± 0.65[c]	7.29 ± 0.58[c]	11.96 ± 0.77[b]

Values with different superscript letters within a column are significantly different ($P < 0.05$). CFS: cell-free supernatant, DPPH: 2,2-diphenyl-1-picrylhydrazyl, OTA: ochratoxin A.
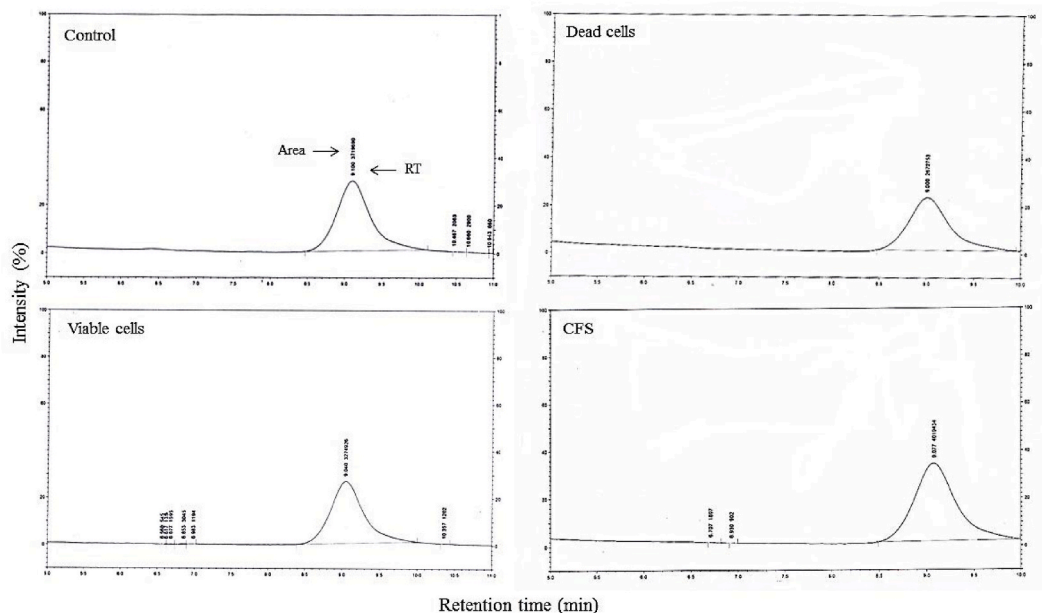
Fig. 3. Anti-OTA activities of the viable and heat-dead cells of the yeast isolate, as well as its CFS compared to the control sample based on the results of a HPLC-based analysis. The percentage of OTA removal was calculated as [1- (sample area/control area)] × 100. OTA: ochratoxin A, CFS: cell-free supernatant, HPLC: high performance liquid chromatography, RT: retention time.

thermal processing unit operations, while their mycotoxins as high risk, heat stable hazardous secondary metabolites have fundamental importance from health and economic viewpoints. Accordingly, anti-mycotoxigenic activity of PRO or their postbiotics have crucial impact on human health and safety aspects of the food processing chain.

3.8. Phytate-degrading abilities

The phytate-degrading ability of the yeast isolate was equal to 7.29%. According to Table 1, the highest phytate-degrading ability was associated with the yeast CFS, which was significantly ($P < 0.05$) higher than the ability of viable or dead cells. This further corroborated the hypothesis that the extracellular phytase activity is responsible for reducing phytate content by the yeast isolate.

These results are in line with those found by Karaman et al. [46], who studied phytate-degrading capability of *S. cerevisiae* strains isolated from SD and revealed their extracellular phytase activity. Sakandar et al. [47] also reported the phytate-degrading ability of *W. anomalus* WAQAU03 isolate, which had the highest orthophosphate release ability than the other isolates in the aforementioned study. The most important mechanisms involved in phytate-degrading ability of the yeasts (as strain-dependent activity) include production of phytase and utilization of phytic acid as the sole phosphorus source. Phytate-degrading ability of PRO or their postbiotics is a promising pro-functional potential for their application to improve nutritional quality of the product through removal or reduction of phytic acid as an anti-nutritional chelating agent [48].

3.9. Antioxidant activities

As outlined in Table 1, the DPPH scavenging activity of *S. cerevisiae* CFS was significantly ($P < 0.05$) higher than that of the viable and dead cells of the isolate, as well as the reference (0.5 mg/mL BHA, with ca. 70.44% scavenging activity). Furthermore, the dead cells showed significantly higher scavenging ability than the viable cells did.

One of the emerging health-promoting capabilities of PYs is their antioxidant activity, which is associated with immune modulation. Antioxidant activity of posbiotics is also very important in human health via alleviation of oxidative stresses. The antioxidant activities of the *S. cerevisiae* isolates, their CFS, and whole-cell extract were revealed in the study of Sourabh et al. [49]. Accordingly, the whole-cell extract of *S. cerevisiae* Sc02 showed the highest antioxidant activity (42.51%). A broad spectrum of antioxidant capacity for PRO *S. boulardii* has also been reported due to the production and/or release of polyphenolic compounds [50]. Other phenomena involved in antioxidant activity of the yeasts are their cell-wall glucomannan and production of antioxidant peptides which exhibit their activity through scavenging free radicals and chelating iron ions [51].

*3.10. Characteristics of the microencapsulated yeast*

3.10.1. Encapsulation efficiency

Encapsulation efficiency of *S. cerevisiae* isolate in Alg-Ch microcapsules (Alg-Ch-m) was equal to 77.02 ± 0.25%. According to Santos and Machado [8], the encapsulation efficiency of *S. boulardii* in Alg-Ch-m via external ionic gelation technique was 95.35%. According to Benucci et al. [52], double layer calcium Alg–Ch-m enhanced the survival of S. *cerevisiae bayanus* Lalvin EC-1118 cells for sparkling wine production, and reported that the encapsulation efficiency was about 87%. Alg and Ch as polysaccharide biopolymers have great potential to be used in microencapsulation due to their easy preparation and handling, as well as nontoxic and cost-effective properties. The encapsulation efficiency in Alg-Ch-m also depends on the utilized technique and the initial population of the yeast cells [7].

3.10.2. Morphology of the produced microcapsules

As displayed in Fig. 4, the size of the Alg microcapsules (Alg-m) and Alg-Ch-m was equal to 0.85 ± 0.05 and 1.05 ± 0.02 mm, respectively. Accordingly, there was significant difference ($P < 0.05$) between size of Alg-m and Alg-Ch-m. The shape of Alg-m and Alg-Ch-m was more spherical (uniform) and rough in nature, respectively. Hills et al. [53] observed that the matrix structure of Alg contained a uniform array of small pores less than a few tens of micrometers in diameter which was suitable to entrap microorganism and to maintain their viability during environmental changes. Suvarna et al. [7] reported that the size of Alg beads and Alg-Ch-m that were used for encapsulation of PY strains had no significant differences (2.94–2.96 mm). The mean size of encapsulated commercial PRO *S. boulardii* in whey protein and Alg beads was also 0.96 ± 0.19 mm in the study of Hébrard et al. [54].

3.10.3. Zeta potential

The zeta potential of Alg-m and Alg-Ch-m was −5.7 and + 14.6 mV, respectively. The zeta potential of the Alg and Ch solutions was also equal to −39.3 and + 58.6 mV, respectively. $CaCl_2$ solution also showed positive charge (+0.70 mV). In line with our findings, Tan et al. [25] demonstrated that Alg and Ch at different pH and concentrations showed different zeta potentials ranging between −29.5 and −42.2 mV and from 18.5 to 32.1 mV, respectively. Xie et al. [55] reported that the microcapsules made of Alg had a negative charge between −2.1 and −6.5 based on their molecular weight. The negative zeta potential indicated the presence of more carboxylic groups, suggesting the positively charged Ch had completely reacted with the Alg as reported by Wang et al. [56]. Accordingly, a membrane for Alg beads coated with Ch was constructed through electrostatic interaction between negatively charged carboxylic groups of Alg and positively charged amino groups of Ch, followed by neutralizing excess positively charged Ch with Alg solution.
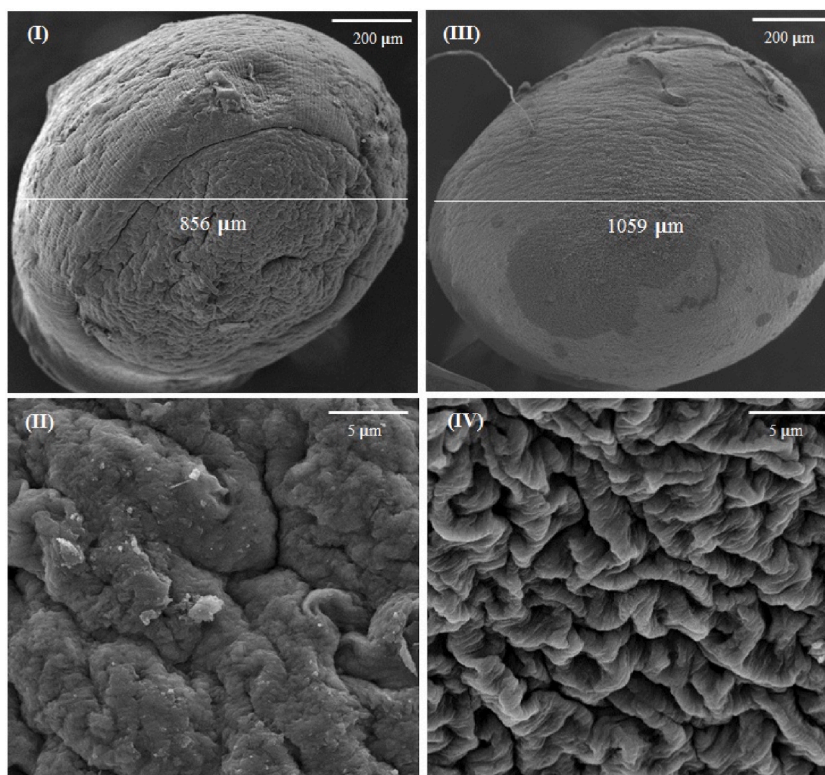
Fig. 4. Field emission scanning electron photomicrograph of *S. cerevisiae* isolate encapsulated in alginate (Alg) microcapsules (I and II), and in Alg beads coated layer-by-layer with chitosan (III and IV), respectively.

3.10.4. FTIR spectrum

Fig. 5 (I) indicates the transmission spectra of the wall materials including Alg-m and Alg-Ch-m (as well as Alg and Ch solutions) in the infrared region. In the spectrum of Alg solution, the wave numbers of 1418 and 1637 cm^{-1} belonged to the symmetric and asymmetric deformation of carboxylic group (COO-), respectively, while 1033 cm^{-1} was related to asymmetric elongation of –COC– [57]. The bands at 687 cm^{-1} were also attributed to the -C–O- stretching with the contributions of -C–C–H- and -C–O–H- deformation [58]. Typical hydrogen bonds (OH) were also identified between 3100 and 3900 cm^{-1} [59]. In the spectrum of Ch solution, bands at 1024 cm^{-1} were associated with the stretching of the -N–H- bond, while bands at 1637 and 2081 cm^{-1} corresponded to the primary and secondary amides, respectively [57]. Furthermore, the same bands of Alg solution were observed in the spectrum of Alg-m, as well as Alg-Ch-m. In the spectrum of Alg-Ch-m, the bands related to –COC– stretching had a slight shift to 1026 cm^{-1}, and the band intensity was reduced. In addition, the bands appearing at 1639 and 2108 cm^{-1} were related to effective ionic bonds between the amino group of Ch and the carboxylate group of Alg, respectively [59]. These findings also concurred with those reported by Santos and Machado
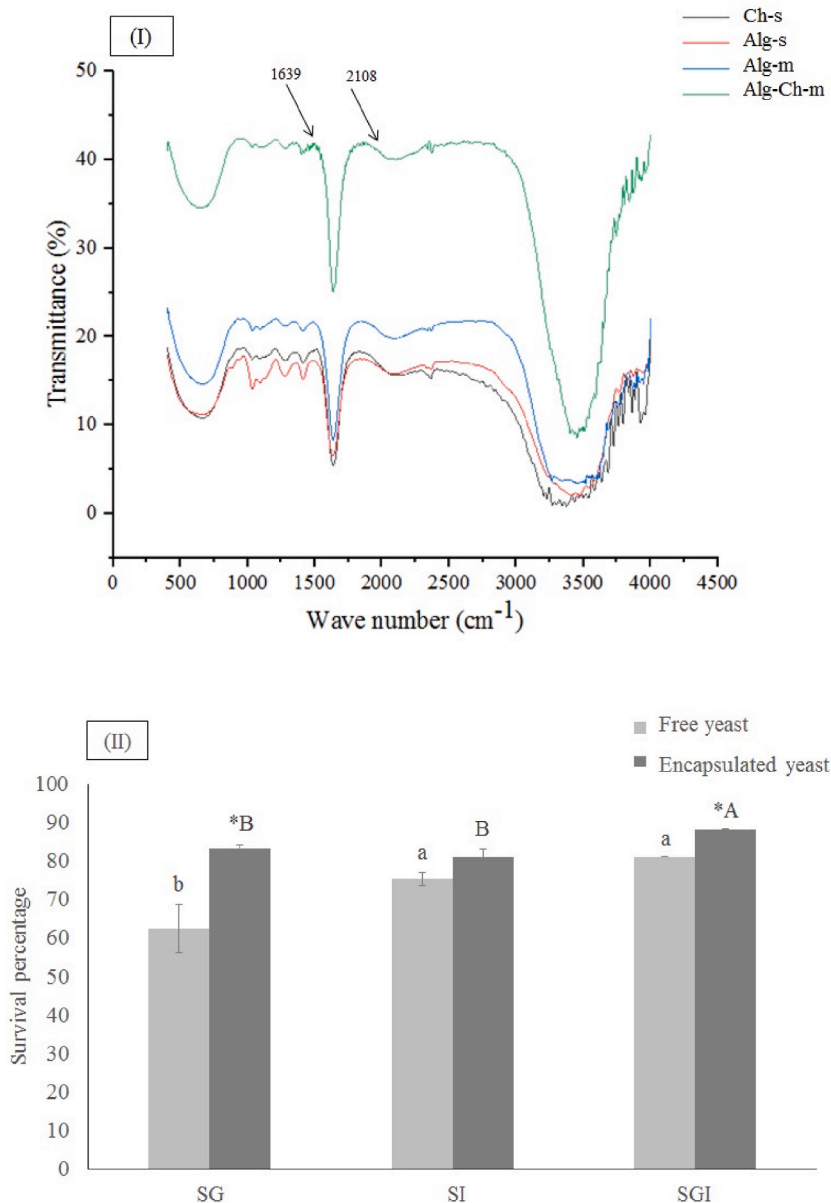

Fig. 5. (I): Fourier transform infrared spectrum of alginate solution (Alg-s), chitosan solution (Ch-s), Alg microcapsules (Alg-m) and Alg beads coated layer-by-layer with Ch (Alg-Ch-m). (II): Survivability of the microencapsulated *S. cerevisiae* isolate under simulated gastric (SG), simulated intestinal (SI) and simulated gastrointestinal (SGI) conditions compared to the free cells. Different uppercase and lowercase letters indicate significant differences ($P < 0.05$) among the encapsulated yeast and free yeast under different treatments, respectively. The star symbol also shows significant differences between free and encapsulated yeast under the same treatment.

[8], and reaffirmed the interaction between Alg and Ch in the produced microcapsule.

3.11. Survival of the microencapsulated yeast under SGI conditions

The survivability of the microencapsulated yeast isolate under SG, SI, and SGI was equal to 83.33, 80.98, and 88.23%, respectively. Furthermore, viability of the microencapsulated yeast in SG and SGI was significantly ($P < 0.05$) higher than that of the free cells (Fig. 5-II).

In the same vein, Alg beads coated with Ch resulted in the highest survival of *S. boulardii* compared to the free cells or to those encapsulated by Alg alone, in the study of Thomas et al. [60] due to its resistance to gastric fluid after addition of Ch layer. Suvarna et al. [7] reported that the microencapsulated *S. cerevisiae* VIT-ASN03 in Alg-Ch-m had 70% survival under SGI conditions. Santos and Machado [8] reported that the survival percentage of *S. boulardii* as free and microencapsulated in Alg-Ch-m under SGI conditions was equal to 80.62% and 100%, respectively. Although Alg has excellent properties of gel formation and PRO trapping, due to the high porosity of the Alg, it should be coated with another layer for better protection from microencapsulated PRO. Further, Alg and Ch are sensitive to more acidic media, which could cause the release of encapsulated cells even in the gastric phase. Meanwhile, Ch exhibits cationic behavior with proper resistance to acidic media. It is also able to cover the pores of the Alg beads to improve the viability of the PRO under stresses such as gastrointestinal transit [61]. The interaction between Alg and Ch (which was verified through FTIR and zeta potential analyses in the present study) is responsible for suppression of the release of the entrapped PRO as a good barrier. It is assumed that the partial destruction of Alg beads and release of Ch (as an antimicrobial agent) in SG and SI, as well as entrance of the bile salts from the created pores into the microcapsules in SI reduced survivability of the yeast isolate under these conditions. Meanwhile, in continuous SG and SI (SGI) conditions, probably the acidic pH of SG led to stronger cross-links between Alg and Ch which was maintained in SI, and finally resulted in better protection from the yeast under SGI conditions compared to SG or SI alone. Between encapsulating materials studied, Ch has higher resistance than those of the Alg towards acidic pH of the gastric fluid. Moreover, Ch can cover Alg and seal the pores of the Alg microcapsule for efficient protection from PY during gastrointestinal transit. Considering sensitivity of Alg and Ch in alkaline pH, the produced microcapsule is useful for colon-targeted delivery of the encapsulated PY. In addition, Ch is also an antimicrobial compound with proper muco-adhesion properties which are emerging key factors for PRO encapsulation.

4. Conclusions

Promising capabilities of the SD yeasts have made these eukaryotic microorganisms proper PRO candidates. In the present study, postbiotic capabilities of a potential PY isolated from WGS including its anti-OTA, antioxidant, and phytate-degrading activities were verified. Interestingly, some functionalities of the yeast CFS or its dead cells were significantly higher than those of viable cells. Furthermore, microencapsulation of the yeast isolate in Alg beads coated LBL with Ch significantly improved its survivability under SGI conditions compared to the free cells. Considering these potentials, it is possible to apply this PY as free or encapsulated in Alg-Ch-m, as well as its postbiotics in different food products. Human applications of postbiotics as the next-generation of personalized medicine have received considerable attention for near future. These components as promising alternatives for synthetic preservatives for food industries have also emerging potential applications to control foodborne pathogenic agents.

Funding statement

Data availability statement

All data generated or analyzed during this study are included in this article and the *16S rDNA* gene sequence of the yeast isolate is deposited in the GenBank database under PP479936 accession number.

CRediT authorship contribution statement

Delasa Rahimi: Formal analysis. **Alireza Sadeghi:** Writing – review & editing, Validation, Project administration. **Mahdi Kashaninejad:** Validation. **Maryam Ebrahimi:** Writing – review & editing, Validation.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28452.

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