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MINI-REVIEW

New insights on yeast and filamentous fungus adhesion in a natural co-immobilization system: proposed advances and applications in wine industry

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Abstract

Fungi possess extraordinary strength in attachment to biotic and abiotic surfaces. This review focuses on adhesion mechanisms of yeast and filamentous fungi and the proposed combination of the adhesive forces of both organisms in an immobilization system called yeast biocapsules, whereby *Saccharomyces cerevisiae* cells are attached to the hyphae of *Penicillium chrysogenum*. The natural adherent properties of each organism, one multicellular and another unicellular, allow yeast to be fixated securely on the filamentous fungi and complete alcoholic fermentation. Following alcoholic fermentation, the hyphae become an inert support for yeast cells while maintaining shape and integrity. Biocapsules have been used successfully in both wine and bioethanol production. Investigation of the potential genes involved in fungal-yeast fusion suggests that natural hydrophobic interactions of both organisms play a major role. Analysis of the possible mechanisms involved in fungus and yeast adhesion, future perspectives on improving yeast immobilization, and proposed applications of the biocapsules are explored.

Keywords Adhesion · Fungal cell wall · Immobilization system · Filamentous fungus · Yeast · Yeast biocapsules

Introduction

Adhesion properties of fungi enable multifunctional capabilities. By attaching to each other or other surfaces, fungi cells gain a powerful ability to colonize, develop into multicellular structure, and survive long-term in environments that could otherwise be unfavorable (Herker et al. 2004; Vallejo et al. 2013). Fungal attachment is largely mediated by hydrophobic interactions (Epstein and Nicholson 2016). These interactions enable attachment to plant and other biotic surfaces and are protein-mediated, are stable in aqueous environments, and have been referred to as fungal "glue" (Epstein and Nicholson 2016). This natural property has been exploited in the biotechnology field by intentionally locking active cells to a particular domain or carrier for easy control and reuse (reviewed in Moreno-García et al. 2018a).

Yeasts also display adhesive properties (reviewed in Brückner and Mösch 2012). The experimental model yeast *Saccharomyces cerevisiae* displays both self- and non-self-adhesion. Self-adhesion may be sexual or asexual. Specific mating lectins mediate the association of opposite mating types and is highly specific to species. Asexual adhesion leads to the formation of flocs and sediments of vegetative cells, depending upon the amount to air trapped within the structure. Non-self-adhesions can be biotic (attachment to other species) or abiotic (attachment to surfaces). Generally, different proteins and mechanisms are involved in these multiple types of attachments, but in more complex structures such as biofilms, both self- and non-self-adhesion may be involved. Yeast cells are commonly immobilized, and many advantages have been found compared to non-immobilized, planktonic cells. These include protection within a toxic environment, invasive growth, increased ethanol tolerance, increased ethanol productivity, and resistance to contamination (Kourkoutas et al. 2004; Moreno-García et al. 2018a).

A natural form of yeast immobilization, called yeast biocapsules, is an emerging form of yeast immobilization that utilizes another fungus—filamentous fungus (ff)—as a carrier

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(Peinado et al. 2004) (Fig. 1). The combination of a fermentative yeast with a ff utilizes the innate adhesive properties of both organisms and confers a strong attachment (Peinado et al. 2004). The natural formation of spherical bodies from the adhesion process in aqueous solutions enables study of cross-species attachment in a more complex, yet controllable, setting. García-Martínez et al. (2011) investigated the interaction of *Saccharomyces cerevisiae* yeast with *Penicillium chrysogenum* hyphae within the biocapsules using electron microscopy and confirmed that the yeast cells were directly attached to the surface of ff hyphae. In fermentative media, the ff lose viability and remain as a highly inert form of support for the yeast. Even after complete fermentation, the biocapsules maintain their integrity which makes possible to reuse them for subsequent fermentations (Peinado et al. 2006). Further, the biocapsules have been investigated to have high immobilization efficiency up to 84% yeast cell immobilized (Moreno-García et al. 2018b) and unlike most immobilization methods, yeast biocapsules can prevent loss of cells from the carrier because of potential attachment of any newly formed daughter cells using the same natural process as the attached parental cells (Moreno-García et al. 2018c). What determines the specific mechanism of attachment is still in question, but studies have shown that different yeast strains affect immobilization ability. After forming biocapsules with yeast displaying different patterns of flocculent/biofilm formation, Moreno-García et al. (2018b) found that those yeasts that were able to form biofilm produced biocapsules with high immobilization yields and high resistance to compression. Other similar types of co-immobilization have been achieved by Nyman et al. (2013) between *Rhizopus* sp. and *S. cerevisiae*.

This review aims to focus on (i) mechanism of yeast and ff adhesion properties, (ii) how these properties can activate under biocapsule formation conditions and form biocapsules, (iii) how to improve co-adhesion in biocapsules, and (iv) application of biocapsules to the wine industry. Specifically, we will focus on adhesion properties that can be activated during the formation of the biocapsules which are asexual adhesion properties in submerged aqueous cultures.

Mechanism of yeast and fungus adhesion

Yeast adhesion

Adhesion in yeast can be self- or non-self. Self-adhesion of vegetative cells is a common property of *S. cerevisiae* and is also called flocculation. In *S. cerevisiae*, flocculation is known to be mediated by several genes. Among them, the *FLO* or flocculation genes encode cell surface proteins, called flocculins, that directly participate in adhesion of cells to each other or other substrates (Verstrepen and Klis 2006). The *FLO* gene family can be divided into two groups. The first group—*FLO1*, *FLO5*, *FLO9*, and *FLO10*—share considerable sequence homology and are subtelomeric genes that code for proteins responsible for cell to cell adhesion and form aggregates of many cells known as flocs (Soares 2011; Di Gianvito et al. 2017). These lectin-like proteins recognize and bind to α-mannan residues (receptors) of neighboring cells (Verstrepen et al. 2003). Calcium ions confer the active conformation of these proteins (Miki et al. 1982; Stratford 1989). Although the active flocculins only exist on flocculent yeast cells, the receptors may be found on non-flocculent cells since the cells walls of *Saccharomyces* like other fungi are composed of mannans; hence, the prerequisite of flocculation is the presence of the flocculins.

Non-self-adhesion can involve these proteins if the foreign surface to be adhered to also contains the specific mannose

Fig. 1 Macroscopic (left) and microscopic 40× objective (right) pictures of yeast biocapsules

polymers that are binding substrates for the flocculins. However, non-self-adhesion can also be mediated by hydrophobic interactions that involve a different group of cell surface proteins, principally *FLO11/MUC1* (reviewed in Brückner and Mösch 2012). These hydrophobic interactions are calcium independent, not inhibitable by mannose, and stable in aqueous and non-aqueous environments. In contrast to the mannose-polymer-specific flocculins, *FLO11* is non-subtelometric and, in addition to flocculation, is primarily responsible for biofilm formation, pseudohyphal growth, and invasive growth (Lambrechts et al. 1996; Van Mulders et al. 2009; Kraushaar et al. 2015). Biofilm formation is a biological process where yeast cells adhere in a matrix of extracellular polymers, allowing them to stick to inanimate objects and in liquid-air interfaces (Kuchin et al. 2002). Other known genes that transcribe for biofilm are *NRG1*, *NRG2*, *SNF1*, *SDS3*, *CCW14*, and *YGP1* (Kuchin et al. 2002; Ishigami et al. 2006; Barrales et al. 2008; Moreno-García et al. 2018d). Invasive growth and pseudohyphal growth are biological processes where budding haploid cells elongate and fail to separate after division, resulting in physical penetration of cells into an agar medium (Madhani and Fink 1998). These additional biological processes allow attachment that is difficult to remove, where intense washing nor physical rubbing can undo. The exact mechanism by which Flo11p adheres is unconfirmed but it involves homotypic binding (Kraushaar et al. 2015).

Besides the lectin interactions and homotypic binding, other forces such as cell surface hydrophobicity (CSH) are thought to initiate the attraction of cells and reinforce the adhesive interactions. A positive increase in CSH is observed when cells go through exponential phase and then reach high, stable values of CSH at stationary phase where the onset of flocculation is seen (Amory and Rouxhet 1988; Smit et al. 1992; Straver et al. 1993; Jin et al. 2001; Speers et al. 2006). The hydrophobicity has been speculated to be from hydrophobic oxylipins, or bioactive lipid metabolites, on cell surfaces and also from the flocculins conferred by the *FLO* genes (Vidgren and Londesborough 2011). All flocculins have structurally similar organization consisting of three domains where the C- terminal domain harbors the glycosyl phoshatidylinositol anchoring sequence, the central domain with tandem repeats (TR) of serine and threonine-rich sequence, and the N-terminal that contains the carbohydrate-binding domain (Kobayashi et al. 1998; Verstrepen et al. 2004). The apical N and C terminals are known to be more hydrophobic than the rest of the protein but El-Kirat-Chatel et al. (2015) observed TR regions to be hydrophobic and lead to microscale cell adhesion. The TR regions are exposed by large unfolding forces when Flo1p is subjected to strong mechanical stress. It was also confirmed that both lectin binding and the unfolding forces strengthen with time and strongly influence cell adhesion.

Filamentous fungus adhesion

Filamentous fungi adhere to many different types of surfaces. These attachments are largely thought to be mediated by hydrophobic mechanisms (Epstein and Nicholson 2016). Many biotic surfaces such as plant cuticle are hydrophobic and fungal "glues" are mannoprotein in nature that can become cross-linked to target substrates extracellularly (Epstein and Nicholson 2016). This strong adhesion is important in both beneficial and pathogenic relationships (de Groot et al. 2013; Tunlid et al. 1992). The role of fungal adhesion in the formation of mycorrhizal symbiosis is well-known (Finlay 2008) as is the simultaneous and often beneficial attachment of bacteria to the mycorrhizal fungi (Leveau and Preston 2008; Bonfante and Anca 2009; Hodge 2014). These complex interactions form a stable system of attachment of different beneficial species within soil and similar interactive processes may exist in non-soil environments as well. Bacterial pathogens of fungi can use similar attachment processes (Stanley et al. 2014). Bacterial attachment to fungi may be specific to viable cells (Toljander et al. 2006) or to a subset of hyphal cell types (Stanley et al. 2014). Given the similarities of fungal wall composition across yeast and filamentous fungi, attachment could be mediated by flocculins, a Flo11-dependent manner, or require both mechanisms. Yeast-fungal attachment in biocapsules is seen under submerged aqueous co-cultivation and growth.

When grown in pure culture in a submerged state under agitation, ff aggregate and form a spherical mass of hyphae called a pellet (Papagianni 2004). The initial formation of the pellet largely depends on the adhesion properties or forces of the spores: hydrophobicity, electrostatic interaction, and salt bridging. A very interesting feature of some ff are the hydrophobins, which are amphipathic proteins that are located on the surface of the cell walls or secreted into the medium in liquid fungal cultures (Wessels 1994). The hydrophobic and hydrophilic parts of the protein allow firm adhesion to solid surfaces and water-solid interfaces, where the hydrophobic patch would face towards the hydrophobic area. It has been speculated that this creates an ordered structure to form, causing hydrophobins to self-assemble and lock onto each other and also melanin (Linder et al. 2005; Linder 2009) forming a layer of tightly packed rods. This rodlet layer is cross-linked to polysaccharides of the spore wall (Eisenman and Casadevall 2012). Fungal pathogens rely on this hydrophobic interaction by using them as structural components of the appressorium cell wall to attach and invade their host (Talbot et al. 1993; Doyle 2000). Hydrophobicity could also arise from oxylipins, which the ff produce in response to injury as a defense mechanism (Hernández-Oñate et al. 2012).

Electrostatic interaction includes van der Waal forces and net negative charge of the spore surface from the carboxyl groups. Generally, most cell walls of microorganisms are

negatively charged at pH values above 5.5 (Papagianni 2004). In the spore wall, the acidic group that facilitates the negative charges is the carboxyl-rich group (Zhang and Zhang 2016). The similar charge of the spore surface causes a repulsion effect, recognized as the Derjaguin, Landau, Verwey, and Overbeek (DVLO) theory. Increasing the pH of the solution increases the strength of the negative charges or electrophoretic mobility (EPM); thus, aggregation of spores is greater at low pH values (Lytle et al. 2002). Other factors affecting EPM are ionic strength and valence and cation concentration (Zhang and Zhang 2016).

Aside from the hydrophobins and electrostatic interactions, specific interactions between protein content in spore walls enhance attachment through salt bridging of polysaccharides (Zhang and Zhang 2016). As spores undergo germination and start to swell, the protective outer melanin-hydrophobin rodlet layer is broken away and the polysaccharides are exposed, allowing spore-specific interactions to increase as hydrophobic forces decrease (Gerin et al. 1993). Fontaine et al. (2010) demonstrated that when adding α-1,3-glucanase, spore aggregation is prevented and only swollen spores attach to α-1,3-glucan chains. Since no other cell wall component was seen in this interaction, the α-1,3-glucan interactions can be considered an additional force that exclusively confers adhesion.

Yeast biocapsule formation

The combined adhesive forces of yeast and ff give rise to the formation of yeast biocapsules. The biocapsules are formed in a liquid medium comprised of yeast nitrogen base medium without amino acids and gluconic acid as a carbon source. Mainly, the lack of sugars (mannose, glucose, sucrose, maltose, maltotriose, galactose) can be thought to activate the adhesion phenotypes of yeast, where induction of flocculation and biofilm formation have been reported when fermentable carbon sources are lacking (Moreno-García et al. 2018b). Similarly, the lack of any nutrient in a condition of submerged state induces pellet formation (Hermersdörfer et al. 1987). Another prerequisite to form biocapsules is continuous agitation. Agitation is required for yeasts to flocculate (Stratford 1992) because, in part, it acts a colloidal force for yeast cells to make contact and overcome the natural repulsion effect from the cell surface charges. Ff also require agitation to form pellets in submerged culture (Papagianni 2004).

Initial formation of biocapsules hypothetically begins with the hydrophobic attraction of ff spores and yeast in liquid medium (Fig. 2). The hydrophobins that cover the spore walls are drawn to the hydrophobicity of the flocculins and oxylipin on yeast cell walls and form an agglomeration of yeast cells and ff spores. The spores and yeast cells generally have negative charges which causes a repulsion effect, represented by arrows. However, this force is overcome by the hydrophobic interactions. Once germination begins, the spores will lose their melanin and hydrophobin coating and polysaccharides are exposed, leading to salt bridging between polysaccharides on ff and yeast cell walls. After germination, hyphal elongation begins and yeast flocculins can bind to α-mannan residues on hyphal cell walls, similar to the mechanism of yeast flocculation. Hydrophobic forces between oxylipins on ff and yeast flocculins may contribute to additional attachment.

The formation of biocapsules occurs under specific co-cultivation conditions in the laboratory. An intriguing question is if this attachment can occur in the wild or is an artifact of laboratory culture. Yeast can form attachments to fungi in nature. The antagonism of the grape pathogen *Botrytis cinerea* by the yeast *Rhodotorula glutinis* is thought to be mediated by attachment of the yeast to hyphae and spores (Li et al. 2016). In the case of bacterial attachment to fungal hyphae, possible benefits include access to nutrients in the case of mycorrhizal bacteria and the coordination of diverse metabolic capacities among specialist organisms can lead to more efficient acquisition of nutrients and removal of toxins (Bonfante and Anca 2009). Binding to more rapidly growing fungal hyphae may be of benefit to unicellular organisms simply for dispersal (Warmink et al. 2011). Adhesion to an invasive and rapidly growing mat of cells enables spread of the attached species within the environment and to new environments. Attachment of yeast to fungi could perform the same function. Expression of the *FLO11* gene displays complex regulation including epigenetic and post-transcriptional in addition to induction and repression (Brückner and Mösch 2012) suggesting that expression of adhesive ability occurs under a variety of environmental conditions. Nutrient starvation induces *FLO11* (Brückner and Mösch 2012) and it seems counterintuitive to adhere to a solid surface in a nutrient-limited environment. However, if in the wild the adherence is to a rapidly growing hyphal adherence would serve to spread the cells to new environments.

Current and future perspectives on co-adhesion improvement of biocapsules

Research on improving the adhesion of yeasts to ff have been conducted over the last few years. One of the first studies about biocapsules observed that flor yeast—*S. cerevisiae* strains used in sherry winemaking that are capable of forming a thick biofilm on the wine-air interface—are high in immobilization efficiency versus non flor yeast (Peinado et al. 2004). This observation is supported by another study where yeasts displaying different patterns of flocculating/biofilm formation were utilized to study biocapsule parameters (Moreno-García et al. 2018b). What was found was that biofilm-forming yeast strains displayed higher rates of immobilization and larger in size but few in number of biocapsules than flocculating strains.

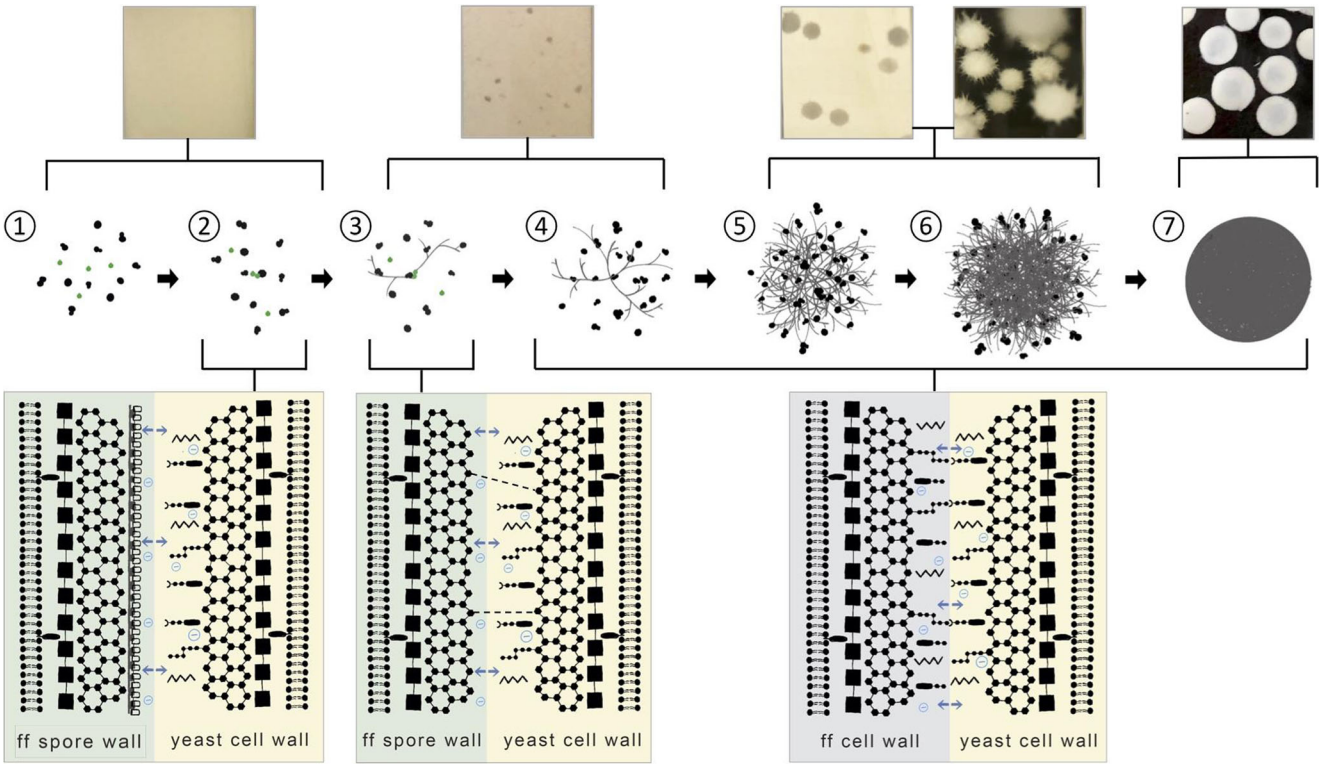

Fig. 2 Formation of a yeast biocapsule and proposed mechanism of attachment. Top most pictures are photographs of samples ① Dispersed free yeast ( ) and fungal spore ( ) in biocapsule formation medium. ② Agglomeration of spore and yeast cell by hydrophobic interaction between hydrophobin-melanin ( ) layer on spore wall with oxylipins ( ) and hydrophobic parts of Flo proteins ( ). Arrows ( ) represent negative electrostatic forces ( ). ③ Start of hyphal ( ) growth where germinating spores lose the melanin and hydrophobin layer and polysaccharides ( ) are exposed, leading to salt bridging (- -). ④⑤⑥⑦ Further growth and branching of hyphae. Yeasts adhere to hyphae by Flo proteins on yeast cell wall attaching to α-mannan residues ( ) on hyphal walls and further reinforcement by hydrophobic forces from oxylipins. Expression of *FLO11* can induce pseudohyphal growth and invasive growth into the mass of hyphal matrix

In order to assess the potential roles of flocculins and Flo11-mediated mechanisms in fungal filament attachment overexpression strains of *FLO1*, *FLO5* and *FLO11* genes were evaluated. Overexpression of all three genes enhanced biocapsule formation with *FLO11* showing the strongest effect (Moreno-García et al. 2018b). Deletion of either *FLO1* or *FLO11* resulted in a decrease in attachment ability to ff. *FLO11* is the dominant gene that transcribes for biofilm or flor formation. Strains with overexpressed *FLO11* immobilized the most cells compared to wild-type isogenic strain and strains overexpressing *FLO1* and *FLO5*, which are primarily responsible genes for flocculation and not biofilm formation. Collectively, the data consistently show that expression of *FLO11* significantly impacts biocapsule parameters and the adhesion of yeast to ff. In a future approach, all three co-flocculating genes could be overexpressed, as well as other known biofilm transcribing genes (*NRG1*, *NRG2*, *SNF1*, *SDS3*, *CCW14*, and *YGP1*) for higher rates of cell attachment. These findings suggest that both self- and non-self-adhesion mechanisms operate during biocapsule formation.

There exists a capacity of yet unexplored improvement to advance immobilization efficiency in biocapsules (Table 1). One interesting potential could be to select ff that produce high amounts of hydrophobins to co-immobilize with the yeast cells. There have been studies of hydrophobin of *Trichoderma reesei* for yeast immobilization by successfully fusing the protein to *S. cerevisiae* Flo1p (Nakari-Setälä et al. 2002). The binding affinity was tested on hydrophobic silicone-based material and was found to be two times more than the non-transformed host cells. Contact angle and zeta potential (or negative charge of the cell wall) measurements suggested that the transformed yeast cells became more apolar and slightly less negatively charged. In the case of biocapsules, it would be interesting to use *T. reesei* as the carrier, which is generally-recognized-as-safe (GRAS) by US Food and Drug Administration evaluation (http://www.accessdata.fda.gov/scripts/fdcc/?set=GRASNotices) and can be utilized for food-related purposes. The expression of the hydrophobin genes *HFBI*, *HFBII*, and *HFBIII* in *T. reesei* and the hydrophobic flocculins of *S. cerevisiae* would most likely strengthen the adhesion of cells.

Another simple improvement would be to increase the ionic strength of the medium or to suppress the electrostatic repulsion forces of both the yeast cells and filamentous fungus,

Table 1 Proposed modifications to increase attachment between yeast and filamentous fungus in yeast biocapsules

Modification	Explanation	Reference
Use ff that can synthesize high amount of hydrophobins and oxylipins	Hydrophobins are proteins that are amphiphiles which contain a highly hydrophobic and hydrophilic end. Oxylipins are bioactive lipid metabolites produced by both ff and yeast. Hydrophobic interaction between hydrophobins on ff and Flop on yeast and oxylipin on both yeast and ff mediate attachment.	Vidgren and Londesborough (2011), Nakari-Setälä et al. (2002)
Overexpression of biofilm formation genes	Biofilm formation genes have been proven to enhance immobilization in yeast biocapsules.	Moreno-García et al. (2018b)
Overexpression of FLO genes	FLO proteins are lectin-like receptors that recognize and bind to α-mannan residues found on fungi cell walls.	Verstrepen et al. (2003), Moreno-García et al. (2018c)
Addition of polycations and decrease pH of the biocapsule formation medium	Ca^{2+} ions bridge cells, overcoming zeta potential and activating the conformation of *FLO* proteins. Decreasing the pH of the medium increases the available Ca^{2+} ions.	Soares (2011)

facilitating aggregation. Induction of aggregation occurs with the addition of polycations while polyanions suppress it (Papagianni 2004). Further, bridging cells with Ca^{2+} ions can overcome the zeta potential and also activate the conformation of the lectin-like FLO proteins in *Saccharomyces*. The available calcium ions are influenced by the pH of the medium (Soares 2011) and many studies report on the increase in aggregation both with yeast and filamentous fungus when pH of medium was low than high (Zhang and Zhang 2016). According to Soares (2011), optimum flocculation of yeast is pH 3 to 5 while *P. chrysogenum* decrease in hyphal length in medium above pH 6 (Papagianni 2004). Thus, addition of polycation and shifting the pH of the biocapsule formation medium to raise the number of available Ca^{2+} ions, which with the current protocol is kept at pH 7, would most likely mediate stronger adhesion of cells.

Application of biocapsules in the wine industry

Biocapsules have thus far been studied in lab scale to perform fermentation on white, sparkling, and naturally sweet wine and bioethanol from molasses and starch (Peinado et al. 2005, 2006; García-Martínez et al. 2012, 2013, 2015; Puig-Pujol et al. 2013). The difference in the final wine between biocapsule vs traditional free yeasts use is an increase in acetaldehyde, isobutanol, L-proline, and aspartic acid, without producing off flavors (Peinado et al. 2005). In the elaboration of sweet wine, osmotolerant *S. cerevisiae* strains have been immobilized to overcome osmotic stress (López de Lerma et al. 2012; García-Martínez et al. 2013). After fermentation, the wine contained compounds related to osmoregulation such as glycerol, acetaldehyde, or acetoin.

In sparkling wine production, biocapsules were utilized to speed up the time-intensive process of riddling—the process of collecting and removing the lees to the neck of the wine bottle. Compared to Ca-alginate beads, which are the most commonly used yeast immobilization carrier method for sparkling wine, the use of biocapsules completed riddling in 2 min and produced sparkling wines that were lower calcium ion content and enologically improved (López de Lerma et al. 2018; Puig-Pujol et al. 2013). The use of biocapsules have also been suggested for effective multi-starter fermentation by utilizing non-*Saccharomyces*/*Saccharomyces* yeast strains sequentially to avoid stuck fermentations and make wines that have improved complexity and quality (Moreno-García et al. 2018a). Similarly, different ff strains may be utilized. *Botrytis cinerea* is a well-known ff that is used to produce botrytized sweet wines, known to have aromas such as citrus and sweet nuances (reviewed in González-Barreiro et al. 2015; Reboredo-Rodríguez et al. 2015). Applying different strains of ff, such as *Botrytis*, to make biocapsules could open a new line of study that may produce positive aromas on finished wine.

Application of biocapsules to fermentations depends on parameters such as the number and the size. It is known that ff pellet shape is easily influenced by agitation rate where strong agitation produces smaller and compact pellets (Papagianni 2004). Similarly, biocapsules require moderate agitation to form their spherical shape. Inoculum size of ff spores should be below 10^4 spores/mL for pellet formation (Calam 1987). Yeast strain properties also influence size and number of biocapsules, where different strains can produce biocapsules with diameters from 2 to 6 mm and number of biocapsules from 100 to 1200 when made in 150 mL media depending on the strain used (Moreno-García et al. 2018b, c).

The use of biocapsules in winemaking practices can be regarded as a sustainable winemaking practice. Both components that make up the biocapsules, yeast, and filamentous fungus are renewable, abundant, preservable, and low-cost resources (Moreno-García et al. 2018a). Further, the biocapsules are a natural yeast settling method and eliminates the cost and materials needed for filtration, clarification, and other post-fermentation procedures. Yeasts can also be reused and

recycled for new fermentations without the consequence of unintentional mixing of must or lees. Additionally, the biocapsules are completely natural, non-destructive, food-grade, and categorized as GRAS, making them possible to use in organic winemaking. In the current trend of consumer preference geared towards creating production and products that are environmentally friendly, biocapsules can fit as a novel and completely natural method of sustainable practice in winemaking and other fermentable beverages. Further, the model organisms that have thus far been most utilized to form biocapsules, *P. chrysogenum* and *S. cerevisiae*, are commonly used food and beverage production that are widely accepted by consumers and have a history of safe use.

Conclusion

Yeast biocapsules can be a tool to study the combined adherence mechanisms of the yeast and the filamentous fungus. In addition, the yeast biocapsules have direct application in fermentation products such as beverages and biofuels. Exploring the adherences properties allows better understanding of the combined forces of both organisms and improves biocapsule efficiency when applied to fermentative practices. Increasing hydrophobins, overexpressing *FLO* and biofilm formation genes, and raising the available free Ca^{2+} ion concentration are hypothetical methods to improve immobilization in this co-immobilization system. Further, new ff strains have been suggested as supports depending on their advantageous features and/or usage in winemaking. The use of yeast biocapsules in fermentation practices can be considered sustainable and ecological development, supporting a practice that reuses resources without negatively changing the taste and quality of the original products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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