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Short communications

Lipid droplet levels vary heterogeneously in response to simulated gastrointestinal stresses in different probiotic *Saccharomyces cerevisiae* strains

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ABSTRACT

To exert their therapeutic action, probiotic *Saccharomyces cerevisiae* strains must survive harsh digestive environments. Lipid droplets accumulate in cells which undergo stress-inducing situations, supposedly having a protective role. We assessed lipid droplet levels, either naturally accumulated or induced in response to digestive challenges, of probiotic strains *S. boulardii*, *S. cerevisiae* A-905, *S. cerevisiae* Sc47 and *S. cerevisiae* L11, and of non-probiotic strains *S. cerevisiae* BY4741 and *S. cerevisiae* BY4743. Strains 905 and Sc47 had lower and higher lipid droplet levels, respectively, when compared to the remaining strains, showing that higher accumulation

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of these neutral lipids is not a feature shared by all probiotic *Saccharomyces* strains. When submitted to simulated gastric or bile salts environments, lipid droplet levels increase in all tested probiotic strains, at least for one to the induced stresses, suggesting that lipid droplets participate in the protective mechanisms against gastrointestinal stresses in probiotic *Saccharomyces* yeasts.

1. Introduction

Lipid droplets (LD), the fat reservoirs of eukaryotic cells, are composed mostly of triacylglycerols (TAG) and sterol esters (SE), and are involved in many biological processes, such as inflammation, immune response, antigen presentation and interactions with pathogens (Saka & Valdivia, 2012). These intracellular organelles play a role in the lipid homeostasis and tend to increase when cells undergo stress situations, such as endoplasmic reticulum, oxidative and osmotic stresses (Khor, Shen, & Kraemer, 2013), protecting the cell against the effects of misfolded proteins and toxic lipids (Hapala, Marza, & Ferreira, 2011). In *Saccharomyces cerevisiae*, LD levels have been shown to increase when yeasts are subjected to temperature and secretory stresses (Fei, Wang, Fu, Bielby, & Yang, 2009; Gaspar et al., 2008; Hapala et al., 2011), drug treatment (Garaiová, Zambojová, Šimová, Griač, & Hapala, 2014) and to high saline concentrations and nitrogen starvation (Madeira et al., 2014).

Some *S. cerevisiae* strains have probiotic properties, which can provide health benefits to human [and animal] hosts when administered in adequate amounts (Vieira, Teixeira, & Martins, 2013). To date, *S. cerevisiae* var. *boulardii* (henceforth designed as *S. boulardii*) is the only probiotic yeast approved by the FDA for human consumption (Czerucka, Piche, & Rampal, 2007), although several *S. cerevisiae* strains have proven probiotic potential (Diosma, Romanin, Rey-Burusco, Londero, & Garrote, 2014; Kourelis et al., 2010; Martins et al., 2005; Palma et al., 2015; Perricone, Bevilacqua, Corbo, & Sinigaglia, 2014; Van der Aa Kühle, Skovgaard, & Jespersen, 2005) and some are commercialised as animal feed additives and veterinary probiotics (Ferraretto, Shaver, & Bertics, 2012; Pérez-Sotelo et al., 2005; Zanello et al., 2013). Probiotic *S. cerevisiae* strains are used as therapeutics against several types of diarrhoea, colitis and other gastrointestinal tract (GIT) malaises (Czerucka et al., 2007). To exert their probiotic potential, these yeasts must survive the harsh environments of the GIT, such as gastric acidic pH, bile salts and intestinal proteases (Fietto et al., 2004). It has been proposed that *S. boulardii* resistance to GIT milieus is related with overexpression of genes related to stress responses (Edwards-Ingram et al., 2007). However, the mechanisms responsible for probiotic *S. cerevisiae* survival in the GIT are still scarcely unknown. In this work, due to the cellular protective role of LD against several stress conditions, we sought to investigate if these organelles also play a protective role in probiotic *S. cerevisiae* yeasts when these are submitted to digestive challenges.

2. Materials and methods

2.1. Reagents, strains and growth media

All reagents, unless specified otherwise, were purchased from Sigma-Aldrich (St. Louis, MO, USA).

S. cerevisiae strains used in this work are listed in Table 1. Yeasts were manipulated as previously described (Douradinha et al., 2014; Madeira et al., 2014; Martins et al., 2005) and grown in YPD medium (1% yeast extract, 2% peptone, 2% dextrose) at either 37 °C (probiotics) or 30 °C (non-probiotics). Following overnight growth, yeasts were diluted to the desired working optical density at 600 nm (OD_{600}).

2.2. Doubling time

To determine yeast doubling time, cells were diluted to an OD_{600} of 0.2 and grown in YPD at the referred temperatures and incubated in a Bioscreen C spectrophotometer (Growth Curves, Piscataway, NJ, USA), according to manufacturer's instructions, during 28 h. The OD_{600} was measured every 15 min. All strains reached stationary phase by 24 h (Fig. S1). Doubling time was calculated based on the OD_{600} values of early to mid-log phase with GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA) (Table S1).

Table 1 – *Saccharomyces* strains used in this work.

Strains	Origin	Supplier	Reference
Probiotics			
S. boulardii 17	Floratil®	Merck SA (Rio de Janeiro, Brazil)	(Blehaut et al., 1989)
S. cerevisiae UFMG A-905	Cachaça[a]	UFMG[b] (Belo Horizonte, Brazil)	(Martins et al., 2005)
S. cerevisiae Sc47	Biosaf	Lesaffre Brazil (Penha, Brazil)	(Pérez-Sotelo et al., 2005)
S. cerevisiae L11	Procreatin7	Lesaffre Brazil (Penha, Brazil)	(Ferraretto et al., 2012)
Non-probiotics			
S. cerevisiae BY4741	–	Open Biosystems (Lafayette, CO)	(Winston et al., 1995)
S. cerevisiae BY4743	–	ATCC (Manassas, VA)	(Brachmann et al., 1998)

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