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In vitro bioassessment of the immunomodulatory activity of *Saccharomyces cerevisiae* components using bovine macrophages and *Mycobacterium avium* ssp. *paratuberculosis*

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ABSTRACT

The yeast *Saccharomyces cerevisiae* and its components are used for the prevention and treatment of enteric disease in different species; therefore, they may also be useful for preventing Johne's disease, a chronic inflammatory bowel disease of ruminants caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP). The objective of this study was to identify potential immunomodulatory *S. cerevisiae* components using a bovine macrophage cell line (BOMAC). The BOMAC phagocytic activity, reactive oxygen species production, and immune-related gene (*IL6*, *IL10*, *IL12p40*, *IL13*, *IL23*), transforming growth factor β, *ARG1*, *CASP1,* and inducible nitric oxide synthase expression were investigated when BOMAC were cocultured with cell wall components from 4 different strains (A, B, C, and D) and 2 forms of dead yeast from strain A. The BOMAC phagocytosis of mCherry-labeled MAP was concentration-dependently attenuated when BOMAC were cocultured with yeast components for 6 h. Each yeast derivative also induced a concentration-dependent increase in BOMAC reactive oxygen species production after a 6-h exposure. In addition, BOMAC mRNA expression of the immune-related genes was investigated after 6 and 24 h of exposure to yeast components. All yeast components were found to regulate the immunomodulatory genes of BOMAC; however, the response varied among components and over time. The in vitro bioassessment studies reported here suggest that dead yeast and its cell wall components may be useful for modulating macrophage function before or during MAP infection.

Key words: *Saccharomyces cerevisiae*, *Mycobacterium avium* ssp. *paratuberculosis*, macrophage function

INTRODUCTION

Dairy producers have been using commercially available yeast probiotics and their components as feed supplements for almost 2 decades based on findings that these products improve animal production, promote health, and reduce the need for antibiotic use (Jouany et al., 1998; Salama et al., 2002; Ganan et al., 2009). For instance, studies have demonstrated that supplementing the ruminant diet with specific strains of *Saccharomyces cerevisiae* improves feed intake (Williams et al., 1991; Robinson and Garrett, 1999), weight gain (Salama et al., 2002), and fiber digestion (Wohlt et al., 1998; Kamel et al., 2004). It has been reported that live yeast stabilizes pH (Doreau and Jouany, 1998; Jouany et al., 1998) and the number of anaerobic cellulolytic bacteria in the rumen (Mosoni et al., 2007; Silberberg et al., 2013). Studies have also shown that *S. cerevisiae* cell wall components (**CWC**) adhere to various enteric pathogens, thereby reducing their ability to invade host cells (Ganan et al., 2009). More specifically, we have shown that CWC from specific *S. cerevisiae* strains can reduce attachment of *Mycobacterium avium* ssp. *paratuberculosis* (**MAP**) to different types of epithelial cells (Li et al., 2016). *Mycobacterium avium* ssp. *paratuberculosis* is an intracellular pathogen that invades intestinal epithelial cells (M-cells) and is subsequently transferred to underlying macrophages; because MAP is capable of evading macrophage-killing mechanisms, this innate immune cell is an important target cell for MAP survival and replication (Kuehnel et al., 2001). *Mycobacterium avium* ssp. *paratuberculosis* is the causative agent of Johne's disease, an inflammatory bowel disease of ruminants. Neonatal calves and calves less than 6 mo of age are highly susceptible to MAP infection (Hines et al., 1995; Veterinary Laboratories Agency, 2008), in part because their acquired immune system is underdeveloped during this exposure period (Whitlock and Buergelt, 1996).

In addition to having nutritional and antimicrobial properties, there is evidence that *S. cerevisiae* and its

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CWC have immunomodulatory properties that can affect both the innate and acquired immune systems (Ganner and Schatzmayr, 2012). Cell wall components can be used as an adjuvant to enhance vaccine efficacy, increase passive immunity (Short et al., 2016), and stimulate trained immunity and may stimulate the immune system following sepsis, which—if one survives—can result in long-term immunosuppression (Novakovic et al., 2016). Beta-glucans, for example, which are the major components of the yeast cell wall, have been shown to stimulate immune function, including upregulating macrophage proinflammatory cytokine and chemokine expression and inducing production of reactive oxygen species (**ROS**; Olson et al., 1996; Williams, 1997). For instance, β-glucan and β-glucan-rich zymosan have been found to increase the production of tumor necrosis factor-α by rodent macrophages (Olson et al., 1996). In addition, β-glucans promote leukocyte activity, which increases host survival against pathogenic infections. Rodríguez et al. (2009), for example, showed that an injection of β-glucan before *Aeromonas hydrophila* challenge significantly enhanced survival of zebrafish.

In addition to β-glucan, yeast cell wall mannan-oligosaccharides (**MOS**) are able to modulate the immune system (Staykov et al., 2007; Torrecillas et al., 2007; Castro-Osses et al., 2017). For example, higher IL-8 expression was reported in rainbow trout that were supplemented with MOS and challenged with *Vibrio anguillarum* (Castro-Osses et al., 2017); IL-8 controls the trafficking of immune cells, especially neutrophils (Mukaida et al., 1998). Mannan-oligosaccharides have also been found to significantly increase lysozyme concentration and improve complement activation, both of which mediate host defense against pathogens. Similarly, the immunomodulatory effect of MOS has also been reported in mammals (Newman and Newman, 2001; White et al., 2002). In one study, White et al. (2002) reported that pigs fed MOS tended ($P < 0.10$) to have higher serum IgG levels than controls; in other words, certain humoral immune proteins appear to be enhanced by feeding MOS.

Given these potential immunomodulatory properties of *S. cerevisiae* and its CWC, we hypothesized that CWC from 4 strains of *S. cerevisiae* (A, B, C, and D) and 2 different forms of dead strain A yeast (inactive and autolyzed yeast) would differentially affect macrophage function. To test this hypothesis, bovine macrophage (**BOMAC**) viability, phagocytosis of MAP, production of ROS, and expression of immune-related genes were assessed at various concentrations of CWC from yeast strains A, B, C, and D and inactive and autolyzed yeast strain A.

MATERIALS AND METHODS

Preparation of MAP Infection Stock

The mCherry-labeled MAP that was used in the present study was developed by Mead (2013) using the clinical isolate Gc86 strain previously isolated in the laboratory of Lucy Mutharia by Melinda Raymond (Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada). The plasmid (pMADDOGPM5) containing mCherry was electroporated into the Gc86 strain to construct the mCherry-MAP based on the method described by Dower et al. (1988), and the fluorescent mCherry-MAP was visualized by microscope using a 590-nm excitation filter. The MAP stock was prepared according to the procedure previously described by Li et al. (2016); quantification of mCherry-MAP was based on measurement of fluorescence (excitation 587 nm, emission 610 nm).

BOMAC Cell Line and Culture Conditions

The BOMAC cell line (Stabel and Stabel, 1995), derived from peritoneal macrophages that were transfected with SV40 plasmid DNA, was used in this study to characterize *S. cerevisiae* bioactivity in vitro. The transformed cell line retained the morphology of resident peritoneal macrophages, such as nonspecific esterase activity and positive lysozyme activity. The BOMAC were cultured in T75 tissue culture flasks (Corning, Tewksbury, MA) at 37°C with 5% CO_2 in RPMI-1640 medium (Invitrogen, Burlington, ON, Canada) supplemented with 2.0 m*M* L-glutamine, 10% heat-inactivated fetal bovine serum (FBS; Invitrogen), 2.5 m*M* HEPES buffer (Invitrogen), 1 m*M* sodium pyruvate (Invitrogen), and 1% antibiotic-antimycotic [100 U/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B (Fungizone); Invitrogen].

Assessment of BOMAC Viability Following Exposure to S. cerevisiae CWC

Because *S. cerevisiae* CWC were previously found to reduce bovine epithelial cell viability at higher concentrations (Li et al., 2016), it was necessary to assess BOMAC viability following exposure to ensure that bioactivity of *S. cerevisiae* CWC was assessed at sublethal concentrations. After reaching 80 to 100% confluence, BOMAC were washed with warm PBS (Sigma-Aldrich, St. Louis, MO), dislodged with TrypLE Express (Invitrogen) for 5 min, and counted with 0.4% trypan blue using a hemocytometer chamber slide.

Cells were seeded into black 96-well flat-bottomed plates (Corning; 5×10^4 cells/well) in cell medium