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Beta-glucan-depleted, glycopeptide-rich extracts from Brewer's and Baker's yeast (*Saccharomyces cerevisiae*) lower interferon-gamma production by stimulated human blood cells *in vitro*

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ABSTRACT

Regulation of the human immune system requires controlled pro- and anti-inflammatory responses for host defence against infection and disease states. Yeasts (*Saccharomyces cerevisiae*), as used in brewing and baking, are mostly known for ability to stimulate the human immune-system predominantly reflecting the pro-inflammatory cell wall β-glucans. However, in this study, using food-compatible processing methods, glycopeptide-enriched and β-glucan-depleted products were each prepared from Brewer's and Baker's yeasts, which suppressed production of interferon-γ (IFN-γ) in human whole blood cell assay, signifying that anti-inflammatory factors are also present in yeast. Anti-inflammatory bioactivities of products prepared from Brewer's and Baker's yeast were compared with the commercial yeast product, Epicor®. While unfractionated Epicor was inactive, the C_{18} resin-binding fractions of Brewer's and Baker's yeast products and Epicor dose-dependently lowered IFN-γ, demonstrating that Epicor also contained both pro-inflammatory (β-glucans) and anti-inflammatory components. Anti-inflammatory activity was attributed to C_{18} resin-binding species glyco-peptides in Epicor and experimental yeast products. This study demonstrated that pro- and anti-inflammatory factors could be resolved and enriched in yeasts by suitable processing, with potential to improve specific activities.

1. Introduction

Not only is inflammation a primary pathology in many diseases, including inflammatory bowel disease (IBD), multiple sclerosis and rheumatoid arthritis, but pro-inflammatory status of the innate and adaptive immune systems also accompanies most chronic diseases, including obesity and cardiovascular disease (Berg & Scherer, 2005), neurodegeneration (Glass, Saijo, Winner, Marchetto, & Gage, 2010), insulin resistance (Bastard et al., 2006) and cancer (Porta et al., 2009). The initiation, propagation and resolution of inflammation is orchestrated by a multitude of cell types, effector species and biological pathways and successful suppression requires the appropriate biological target(s) of intervention.

In contrast, with pharmacologically-specific therapies, dietary factors are chemically heterogeneous and typically pleiotrophic in their biological targeting and can suppress inflammation by acting at a number of pathways to inhibit pro-inflammatory cytokine and cell expression, including COX-2, LOX, NF-κB, PPAR-γ, iNOS, among other pathways (Pan, Lai, & Ho, 2010; Recio, Andujar, & Rios, 2012). Evidence is growing for anti-inflammatory effects associated with the consumption of a wide range of dietary plants (Pan et al., 2010), long chain $n-3$ polyunsaturated fatty acids (He et al., 2009) and important micro-nutrients, including vitamin D (Querfeld, 2013) and anti-oxidant vitamins C and E. Furthermore, dietary interventions, using foods and extracts with an established safety profile, are of growing interest for preventing and ameliorating chronic disease (Pan et al., 2010).

Beta-glucan polysaccharides from cell walls of several types of fungi, including yeasts, are known to stimulate the innate immune system, including macrophages, T helper and NK cells (Bohn & BeMiller, 1995), and can prime leukocytes to produce an enhanced response against secondary microbial or mitogen challenge (Brown & Gordon, 2003). Benefits of β-glucan treatment have been

Abbreviations: IFN-γ, interferon gamma; IL-10, interleukin 10; LPS, lipopolysaccharide; PHA, phytohemeagglutinin.

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demonstrated in mice against secondary *Escherichia coli* (Seljelid, Rasmussen, Larm, & Hoffman, 1987) and *Staphylococcus aureus* infection (Pelizon, Kaneno, Soares, Meira, & Sartori, 2005). Furthermore, immune stimulatory activity of oral β-glucans has been demonstrated in mice (Tsukada et al., 2003) and livestock (Shen et al., 2009). For humans, oral intake of β-glucans produce consistent immune-stimulatory properties although there appear to be differences between soluble and insoluble forms of dietary β-glucans (Stier, Ebbeskotte, & Gruenwald, 2014).

However, in addition to β-glucans, autolysed forms of cultured yeasts release a complex array of compounds, including mannan-oligosaccharides and glyco-proteins, also derived from cell walls, peptides, amino acids, nucleotides, lipids and organic acids with various capacities for driving pro-inflammatory and less frequently anti-inflammatory responses (Catania et al., 2000). Yeast-derived nucleotides were also demonstrated to exert protection against secondary infection with *Streptococcus iniae* (Li, Lewis, & Gatlin, 2004).

Epicor is a supplement product marketed for its immune-modulatory activity, produced by anaerobic fermentation and autolysis of Baker's yeast in a proprietary medium (Embria Health Sciences: http://www.embriahealth.com, (Jensen, Hart, & Schauss, 2007)). Clinical trials conducted with Epicor support its immune-stimulation-mediated effects in lowering incidence and symptom severity of allergic rhinitis (Moyad et al., 2009) and colds and flu (Moyad et al., 2010) that reflected net stimulation of mucosal immunity (Jensen et al., 2008).

During the course of a systematic research program of bioactive peptide discovery (Bharadwaj et al., 2013), we recovered a glycopeptide-rich, β-glucan-depleted product from each of Brewer's (BR0263) and Baker's yeast (BA0263) substrates. The aim of this study was to investigate inflammation-regulation capacity of these products, in mitogen-stimulated human blood cells, using Epicor as a reference. Using solid-phase chromatographic fractionation, we have demonstrated that discrete pro- and anti-inflammatory components can be resolved from BR0263, BA0263 and Epicor. The chemical natures of anti-inflammatory factors in the enzyme-hydrolysed yeast products were also evaluated.

2. Materials and methods

2.1. General

Brewer's yeast was obtained from Lotus Foods Pty Ltd (Cheltenham, Vic, Australia), Baker's yeast from Lowan Whole Foods (Glendenning, NSW, Australia) and Epicor® from Vitamin Research Products (Carson City, NV, USA). Glutaminase was obtained from Daiwa Kasei K.K. (Shiga, Japan), Corolase PN-L from AB Enzymes GmbH (Darmstadt, Germany); Alcalase 2.4 L, Flavourzyme 1000 L and trypsin were from Novozymes, Bagsvaerd, Denmark. Triethanolamine (TEA), ethanol, lipopolysaccharide (LPS), phytohemeagglutinin (PHA) and hydrocortisone were obtained from Sigma Chemical Co. (St Louis, MO, USA). Interleukin 10 (IL-10) was from Bio Scientific Pty Ltd (NSW, Australia) and HPLC-grade acetonitrile was from Ajax Fine Chemical (NSW, Australia).

2.2. Enzyme hydrolysis and enrichment of bioactive yeast product by C18 reverse phase solid phase extraction

Brewer's and Baker's yeast were dispersed at 10% total protein (w/w) in 10 mM TEA containing 10% ethanol, pH 7.4. Enzyme hydrolysis using 5 enzymes, each at 0.5%, w/w, was conducted in sequence (glutaminase, Corolase PN-L, Alcalase, Flavourzyme and trypsin) and recovery of low mass products (BR0263, BA0263) after dialysis (6–8 kDa) was conducted as described in (Bharadwaj et al., 2013). The ion-exchange process described in Bharadwaj et al., 2013, was not applied for preparation of BR0263 and BA0263. A process blank, including all reagents and subjected to the same incubation and dialysis procedure was also prepared.

Hydrophobic molecular sub-fractions of BR0263 and BA0263 were prepared using C18 Reversed Phase solid phase extraction (Strata-X, 33 μm, Polymeric Reversed Phase cartridges, 500 mg/6 ml, Phenomenex, California, USA) using 100% acetonitrile for elution ('SPE' fraction) according to manufacturer instructions, as also described in (Bharadwaj et al., 2013). 'SPE' products were dried by evaporation under vacuum and stored at −20 °C.

2.3. Analysis of fibre and neutral non-starch polysaccharides (NNSP)

BR0263, BA0263 and Epicor products were finely milled before carbohydrate analysis. β-Glucans analysis was conducted using the optimised conditions described in the AOAC official method 995.16, (AOAC, 2000), which involves enzyme hydrolysis of 1,3- and 1,4-mixed linkage β-glucans with lichenase and β-glucosidase. However, in the absence of commercially available enzymes to cleave 1,6-linked β-glucans found in yeast cell walls, total β-glucan content was also determined indirectly by analysis of individual sugar components of the neutral non-starch polysaccharides (NNSP), whereby in the NNSP fraction, glucose release provides a measure of total β-glucan content, from which the specific 1,6-linked β-glucan content can be estimated by difference. The indirect method for total β-glucan analysis was based on the AOAC official method 994.13 (AOAC, 1999), involving hydrolysis of starch by treatment with potassium hydroxide and amylo-glucosidase. After ethanol-precipitation and washing of the NNSP fraction to remove any free glucose, the NNSP residue was treated with 1.2 M sulphuric acid (2 h) to hydrolyse the polysaccharides into component sugars before reducing (sodium borohydride) and acetylation of polyalcohols. Quantitative analysis was carried out by gas chromatography using allose as an internal standard and appropriate calibration profiles for individual sugars. Results represent the mean of duplicate analyses and coefficient of variation was better than 10%.

2.4. Analysis of metabolites by GC–MS

Approximately 10 mg of dried yeast products (BR0263, BA0263, Epicor and respective SPE sub-fractions) were added to Eppendorf tubes and dispersed in methanol (100%, 500 μl), internal standard ($^{13}C_6$-sorbitol/$^{13}C_5{}^{15}N$-Valine in water, 0.2 mg/ml, 2 μl). The sample mixture was vortexed for 30 s and then incubated for 15 min at 70 °C at 850 rpm before adding Milli-Q Water (500 μl), and centrifuging (13,000 rpm for 5 min). A 20 μl aliquot was transferred into a glass insert for TMS (trimethylsilyl) derivatisation and a 50 μl aliquot for TBS (tri-*tert*-butyldimethylsilyl) derivatisation prior to being dried *in vacuo*. Extracted yeast samples were placed in a snaplock bag with silica gel prior to derivatisation for GC–MS analysis as previously described (Jacobs, Lunde, Bacic, Tester, & Roessner, 2007).

For TMS derivatisation, the dried yeast products were re-dissolved in 10 μl of 30 mg/ml methoxyamine hydrochloride in pyridine and derivatised at 37 °C for 120 min with mixing at 500 rpm using the on-line derivatisation capability of the Gerstel autosampler. The samples were then treated for 30 min with 20 μl *N,O-bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) and 2.0 μl retention time standard mixture [0.029% (*v*/*v*) *n* dodecane, *n*-pentadecane, *n*-nonadecane, *n*-docosane, *n*-octacosane, *n*-dotriacontane, *n*-hexatriacontane dissolved in pyridine] with mixing at 500 rpm at 37 °C. Each derivatised sample was allowed to rest for 60 min prior to injection.