Research Article:
Microencapsulated Saccharomyces Cerevisiae Var. Boulardii and IgA Secretion From Intestinal Epithelia in Wistar Rats

Zohreh Farahnejad1, Donya Nikaein2, 3, Alireza Khosravi2, 3, Reza Rahmani4, Hassan Ghorbani-Choboghlo2*

1. Department of Medical Mycology, School of Medicine, AJA University of Medical Sciences, Tehran, Iran.
2. Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Department of Clinical Biochemistry, School of Medicine, Tehran University of medical science, Tehran, Iran.

*Corresponding Author:
Hassan Ghorbani-Choboghlo, PhD.
Address: Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
Phone: +98 (912) 7995285
E-mail: hghorbani67@ut.ac.ir

ABSTRACT

Background: Probiotics are live microorganisms with many health benefits for their host. The intestinal microbiota are the largest source of microbial variation and plays a significant role in host responses in health and disease. However, few studies have assessed the repercussions of probiotics regarding the morphology and immunology of the gastrointestinal tract in animal models. This study was designed to evaluate the effect of administering capsulated Saccharomyces species on gastrointestinal tract properties in rats.

Materials and Methods: In this study mice rats were feed with Saccharomyces Boulardii into two forms of capsulated and free. IgA was measured in duodenal and jejunal washings using ELISA assay according to the manufacturer’s instructions.

Results: Probiotic S. boulardii could increase IgA secretion from duodenum and jejunum in comparison with the control group, and this increase was significant in microencapsulated S.boulardii-treated group and in the duodenum of S. boulardii-treated group (P<0.05). Interestingly, IgA secretion was significantly higher in the intestines of rats treated with microencapsulated S.boulardii (P<0.05).

Conclusion: It can be concluded that S. boulardii is a potential probiotic yeast with immunostimulatory effects which can be used in the treatment of gastrointestinal disorders.
**Introduction**

Probiotics are live microorganisms with many benefits for their host [1]. Saccharomyces boulardii is a well-known probiotic used for the treatment of diarrhea [2]. S. boulardii could increase the secretion of IgA and other immune secretory components in the small intestine [3]. Thus, stimulation of the host’s mucosal immunity is a mechanism by which S. boulardii acts against intestinal pathogens [4-6].

Probiotics like S. boulardii will increase the nutritional value of food and protect food from degradation; however, to do this, they need to survive in food processing techniques and gastrointestinal tract [7]. Microencapsulation is a technique that protects the microorganisms from external influences [8]. Among products used for microencapsulation, sodium alginate is widely used in microencapsulation of probiotic bacteria in concentrations of (0.5-5%) [9]. This study was designed to evaluate the effect of S. boulardii microencapsulation on IgA secretion in a rat model.

**Materials and Methods**

**Animal model**

Male Wistar rats weighing 100-110g were obtained from the Department of Laboratory Animals, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. They were kept at 20°C under 12:12h light/dark cycle. Standard pellet food and water was provided for them ad libitum. The rats were divided into three groups, each containing 10 rats: MT group of rats receiving microencapsulated S. boulardii, the control (C) group receiving normal saline, and the T group receiving uncapsulated S. boulardii. Probiotics were gavaged to rats at the dosage of 2g/kg for 8 continuous weeks.

**Probiotic strain**

S. boulardii (ATCC 74068) was obtained from the Mycology Research Center. It was cultured on sabouraud dextrose broth (Merck, Germany) Supplemented with Chloramphenicol (0.05%) (SC) and incubated at 28°C, 180 rpm for 48h. The cells were centrifuged at 800g for 10 min and washed three times with sterile Phosphate-Buffered Saline (PBS) before use. The number of S. boulardii yeast cells were adjusted to 2x10^10CFU/ml with a hemocytometer before microencapsulation.

**Microencapsulation**

Extrusion technique was used for microencapsulation of S. boulardii as described previously [10]. In brief, the cells were suspended in (2%) sodium alginate solution. Then, this mixture was injected to 0.1M calcium chloride using a sterile insulin syringe. The formed droplets were isolated from suspension with centrifugation.

**IgA measurement**

After 8 weeks, the rats were euthanized, and their gastrointestinal tracts were isolated. Their intestinal mucosa was isolated, according to Lim et al. (1981) method with brief modifications [11]. In brief, intestinal content was washed out with cold PBS containing penicillin/streptomycin (100µg/mL) and centrifuged at 500g for 10 minutes. IgA was measured in duodenal and jejunal washings using Enzyme Linked Immunosorbent Assay ELISA assay method according to manufacturer’s instructions.

**Statistical analysis**

The obtained data were analyzed in SPSS V. 21. Analysis of Variance (ANOVA) was applied to compare statistical changes in different groups. Tamhane’s T2 post hoc test was used to compare data between groups. A P-value less than (0.05) was considered significant.

**Results**

Table 1 shows the results of IgA production by duodenal and jejunal epithelial cells. Probiotic S. boulardii increased

| Group | Intestinal Area | Mean±SD | Duodenum | Jejunum |
|-------|----------------|---------|-----------|---------|
| Control | 282±0.02a | 189±1.97a |
| MT | 460±3.47b | 540.3±2.14b |
| T | 340.7±0.92c | 242±0.57a |

*Different characters show significant differences

MT: Rats treated with Microencapsulates S. boulardii; T: Rats treated with uncapsulated S. boulardii
IgA secretion from duodenum and jejunum in comparison with the control group, and this increase was significant in the MT group and in the duodenum of T group (P<0.05). Interestingly, IgA secretion was significantly higher in the intestines of rats treated with microencapsulated S. boulardii (P<0.05).

Discussion

S. boulardii is a probiotic yeast mainly used in the treatment of gastrointestinal disorders. Enhancing the host immune system could be an important defense against infectious diseases of intestines, causing diarrhea [12]. In our study, S. boulardii increased the secretion of IgA from intestinal mucosa, which agreed with other studies [12-15]. However, this increase was higher in the duodenum of the T group and jejunum of the MT group. Nevertheless, this difference was not significant (P>0.05). The higher secretion of IgA in the jejunum of microencapsulated S. boulardii-treated rats could be the results of microencapsulation, which protected the yeast cells in the first parts of the intestinal tract and provided enough yeasts in jejunum that could affect IgA release by jejunal mucosa. Since intestinal infections are mostly initiated from jejunum, microencapsulation of S. boulardii could protect against intestinal disease as well as treatment of infectious disorders. It can be concluded that microencapsulation enhances the release of IgA by intestinal cells, and this could be an essential mechanism in the protection and treatment of intestinal illnesses.

Ethical Considerations

Compliance with ethical guidelines

The ethical issue of the present study was approved by Research Council of AJA University of Medical Sciences (Code: 1395.121)

Funding

This study was supported by vice Chancellor for Research of AJA University of Medical Sciences.

Authors' contributions

Designing: Ali Reza Khosravi, Zhoreh Farahnejad and Hassan Ghorbani Choboghlo; Laboratory experiments: Donya Nikaein, Hassan Ghorbani Choboghlo and Reza Rahmani; Manuscript writing, editing and revising: Donya Nikaein, Hassan Ghorbani Choboghlo and Reza Rahmani.

Conflicts of interest

Authors declared no conflict of interest

Acknowledgements

Authors would like to thank AJA University of Medical Sciences for their technical support and Mr Asad Balal for his excellent cooperation.

References

[1] Qamar A, Aboudola S, Warny M, Michetti P, Pothoulakis C, LaMont J. Saccharomyces boulardii stimulates intestinal immunoglobulin A immune response to Clostridium difficile toxin A in mice. Infection and Immunity. 2001; 69(4):2762-5. [DOI:10.1128/IAI.69.4.2762-2765.2001] [PMID] [PMCID]

[2] Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. PLOS One. 2012; 7(4):e34938. [DOI: 10.1371/journal.pone.0034938] [PMID] [PMCID]

[3] Buts JP, Bernasconi P, Vaerman JP, Dive C. Stimulation of secretary IgA and secretory component of immunoglobulins in small intestine of rats treated with Saccharomyces boulardii. Digestive Diseases and Sciences. 1990; 35(2):251-6. [DOI:10.1007/BF01567671] [PMID]

[4] Angolakis E. Weight gain by gut microbiota manipulation in productive animals. Microbial Pathogenesis. 2017; 106:162-70. [DOI:10.1016/j.micpath.2016.11.002]

[5] Cruchet S, Furnes R, Manry A, Hebel E, Palacios J, Medina F, et al. The use of probiotics in pediatric gastroenterology: A review of the literature and recommendations by Latin-American experts. Pediatric Drugs. 2015; 17(3):199-216. [DOI:10.1007/s40272-015-0124-6] [PMID] [PMCID]

[6] Elmer GW, Surawicz CM, McFarland LV. Biotherapeutic agents: A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. The Journal of the American Medical Association. 1996; 275(11):870-6. [DOI:10.1001/jama.275.11.870] [PMID]

[7] Dias DR, Botrel DA, De Barros Fernandes RV, Borges SV. Enzymatic immobilisation of lactobacillus acidophilus freely and microcapsulated in the intestine of rats treated with Saccharomyces boulardii. Microbial Pathogenesis. 2017; 106:162-70. [DOI:10.1016/j.micpath.2016.11.002]

[8] Lacroix C, Grattepanche F, Doleyres Y, Bergmaier D. Immobilised cell technologies for the dairy industry. In: Nedovic-Ronnie V, Willaert R, editors. Applications of Cell Immobilisation Biotechnology. Dordrecht: Springer; 2005. [DOI:10.1007/1-4020-3363-X_18]

[9] Shah NP, Ravula RR. Microencapsulation of probiotic bacteria and their survival in frozen fermented dairy desserts. Australian Journal of Dairy Technology. 2003; 55(3):139-44.

[10] Abdolhosseinizadeh E, Dehnavi AR, Pourjafar H, Homayouni A, Ansari F. The production of probiotics using lactic acid bacteria: The role of microencapsulation in the stability of the product. Carpathian Journal of Food Science & Technology. 2018; 10(3):72-80.

[11] Lim TS, Messilah N, Watson RR. Immune components of the intestinal mucosa of ageing and protein deficient mice. Immunology. 1981; 43(3):401-7. [PMID] [PMCID]
[12] Ghorbani-Choboghlo H, Zahraei-Salehi T, Ashrafi-Helan J, Yahyaraeyat R, Pourjafar H, Nikaein D, et al. Microencapsulation of Saccharomyces cerevisiae and its evaluation to protect in simulated gastric conditions. Iranian Journal of Microbiology. 2015; 7(6):338-42. [PMID] [PMCID]

[13] Szajewska H, Kołodziej M. Systematic review with meta-analysis: Saccharomyces boulardii in the prevention of antibiotic-associated diarrhoea. Alimentary Pharmacology & Therapeutics. 2015; 42(7):793-801. [DOI:10.1111/apt.13344] [PMID]

[14] Stier H, Bischoff SC. Influence of Saccharomyces boulardii CNCM I-745 on the gut-associated immune system. Clinical and Experimental Gastroenterology. 2016; 9:269-79. [DOI:10.2147/CEG.S11003] [PMID] [PMCID]

[15] Hudson LE, McDermott CD, Stewart TP, Hudson WH, Rios D, Fasken MB, et al. Characterization of the probiotic yeast Saccharomyces boulardii in the healthy mucosal immune system. PLOS One. 2016; 11(4):e0153351. [DOI:10.1371/journal.pone.0153351] [PMID] [PMCID]