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RESEARCH ARTICLE

Inhibitory effects of YCW and MOS from *Saccharomyces cerevisiae* on *Escherichia coli* and *Salmonella pullorum* adhesion to Caco-2 cells

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BACKGROUND: For many years, yeast cell walls (YCW) and mannan oligosaccharides (MOS) have been used as alternatives to antibiotics and health feed additives to enhance the growth performance and health of food animals. In the present study, the inhibitory effects of YCW and MOS on the adhesion of enteropathogenic bacteria to intestinal epithelial cells were tested.

METHODS: YCW and MOS were extracted from *Saccharomyces cerevis*iae (XM 0315), and the morphology of YCW and MOS bound to pathogenic bacteria was observed by scanning electron microscopy (SEM). Real-time fluorescent quantitative PCR was used to quantitatively analyze the effects of YCW and MOS on the adhesion of *Escherichia coli* (CVCC3367) and *Salmonella pullorum* (CVCC520) to Caco-2 cells.

RESULTS: The results showed that YCW inhibited *E. coli* and *S. pullorum* binding to Caco-2 cells by 95% and 74%, respectively, whereas MOS prevented *E. coli* and *S. pullorum* binding by 67% and 50%, respectively.

CONCLUSIONS: These data suggest that YCW has a stronger ability than MOS to inhibit pathogenic bacteria from adhering to Caco-2 cells *in vitro*.

Keywords YCW, MOS, *Escherichia coli*, *Salmonella pullorum*, Caco-2 cells

Introduction

For decades, antibiotics have been administered to livestock to promote their growth and control infectious diseases, including diarrhea, caused by pathogenic bacteria (Kogut, 2000; Fernandez et al., 2002; Ganner et al., 2010). However, this non-therapeutic overuse has provided selective pressure for the development of antibiotic resistance, which has rendered some antibiotics ineffective. The potential public health hazard of antibiotic resistance has led to a greater interest in the development and use of antibiotic alternatives that can maintain equivalent animal performance while protecting human health (Vieira et al., 2008; Tiago et al., 2012). Yeast cell walls (YCW) and yeast cell wall-derived mannan oligosaccharides (MOS) have been used as antibiotic alternative feed additives in livestock and poultry feed for many years (Becker et al., 2007; Ganner et al., 2013; Broadway et al., 2015). YCW and MOS have been shown to improve animal performance, feed efficiency, and gastrointestinal health (Shashidhara and Devegowda, 2003; Oyofo et al., 1989; Kogan and Kocher, 2007; Becker et al., 2007; Trevisi et al., 2012).

It is well known that some bacteria exert pathogenic effects by attaching to receptors on the epithelial surface (Rodrigues and Elimelech, 2009). Ofek et al. (1977) first showed that *E. coli* could colonize human mucosal cells in vitro, whereas in the presence of 5 mg/mL D-mannose, only 30% of the *E. coli* could adhere to the human mucosal cells. Since then, the adhesion of pathogenic bacteria to human mucosal cells has been extensively used to evaluate the infectivity of pathogenic bacteria and the effects of antibiotic alternatives (Sharon and Ofek, 2000; Ganan et al., 2009; Shoaf-Sweeney and Hutkins, 2009).

Several mechanisms have been proposed to explain the positive effects of YCW and MOS in animals. First, MOS is a

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high affinity ligand of type-I fimbriae, which are found on various pathogenic bacteria, including *E. coli* and *Salmonella* spp. Thus, MOS act through competitive binding to reduce pathogen attachment to host cells (Ofek et al., 1977; Ofek and Beachey, 1978; Jones et al., 1995; Spring et al., 2000; Bouckaert et al., 2006; Baurhoo et al., 2007; Rosen et al., 2008; Ganner et al., 2012; Ganner et al., 2013). So YCW and MOS present the binding ability to pathogens (Snellings et al., 1997; Sharon and Ofek, 2000). Moreover, YCW and MOS are selectively fermented by intestinal bacteria, which can lead to changes in the composition and/or activity of the gastrointestinal microbiota, and can increase the number of beneficial bacteria, such as lactobacilli and bifidobacteria (Sweeney Shoaf et al., 2008).

Although both YCW and MOS are promising antibiotic alternatives that bind to pathogens, whether there is a difference in the pathogen adhesion capacity of YCW and MOS is unknown. It is also not known whether extraction of MOS from YCW is required to maximize the beneficial effects against pathogenic bacteria. Therefore, in this study, the effects of YCW and MOS from *S. cerevisiae* on *E. coli* and *S. pullorum* adhesion to monolayers of enterocyte-like Caco-2 cells were investigated.

Materials and methods

Microorganisms and culture conditions

E. coli (CVCC3367) and *S. pullorum* (CVCC 520) were isolated from infected chicken intestine and purchased from China Veterinary Culture Collection Center (CVCC), respectively. *S. cerevisiae* XM 0315 was obtained from our own laboratory collection.

E. coli cells were grown in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, and 1% NaCl) for 14 h at 37°C statically. The cultivation conditions for *S. pullorum* were the same as those used for *E. coli* except that the culture was agitated at 100 rpm. *S. cerevisiae* XM 0315 was incubated in YPD broth (1% yeast extract, 2% peptone, and 2% glucose) with agitation (200 rpm) at 30°C for 48 h. *S. cerevisiae* cells were harvested by centrifugation (2700 *g*) for 30 min at 4°C, and washed three times. Finally, the cells were freeze-dried and stored until use.

Intestinal epithelial cells and culture conditions

Liquid nitrogen-frozen ATCC HTB-37 Caco-2 cells (purchased from American Type Culture Collection) were thawed at 37°C and inoculated into a 10-cm petri dish containing 7 mL of MEM supplemented with 10% heat inactivated fetal bovine serum (FBS; Gibco, USA), 1% nonessential amino acids (NEAA; Corning, USA), 1% penicillin/streptomycin (P/S; Corning) at 37°C with 5% CO_2 (Candela et al., 2005). After reaching 80%–100% confluence, the Caco-2 cells were digested with 3 mL of 0.05% trypsin-0.53 mM EDTA for 3 min at 37°C. Then, 3 mL of MEM containing 10% heat inactivated FBS was added to stop the enzymatic digestion. The cells were pelleted, resuspended in MEM, seeded into 24-well flat bottom plates containing cell culture medium without P/S, and incubated at 37°C with 5% CO_2. The culture medium was replaced every day until each well was filled with cells. Then, the cells were washed with warm phosphate buffered saline (PBS, pH 7.4) in preparation for the adhesion assay.

Preparation of YCW and MOS

Lyophilized yeast cells were used to prepare YCW and MOS based on a previously reported enzymatic method (Varelas et al., 2016), with modification. Briefly, 10 g of freeze-dried yeast were treated with 500 mg of lysozyme (40000 U/g; sunHY, Wuhan, China) dissolved in 50 mL of distilled water with mild agitation at room temperature for 2 h. Then, YCW was obtained by centrifugation (10000 rpm, 5 min) as a sediment, washed 5–6 times with distilled water, and freeze-dried until use.

Yeast MOS were extracted from YCW according to a previously reported method (Ganan et al., 2012), with modification. A YCW preparation was extracted with NaOH [1:5 (w/v) dry cell weight/1 M NaOH]. The extraction was conducted at 85°C for 3 h with mild agitation. Then, the mixture was centrifuged (3600 rpm, 30 min), and the supernatant was used to further extract MOS. Acetic acid was added to the supernatant to adjust the pH of the solution to 5.5. Then, the solution was centrifuged (2400 rpm, 10 min). The supernatant was harvested, dialyzed for 3–4 days, and freeze-dried for storage until use.

Scanning electron microscopy (SEM)

(i) Morphology of YCW and MOS. As the samples were freeze-dried powders, they could be observed directly by SEM after gold plating.

(ii) Adhesion of YCW and MOS to bacteria. Suspensions of *E. coli* and *S. pullorum* (OD_{600} = 5.0) were prepared for observation after washing three times in cold phosphate-buffered saline (PBS, pH 7.4). YCW and MOS were mixed with bacterial cells in a 1:1 ratio, respectively. The mixture was washed several times with distilled water at room temperature and then fixed with glutaraldehyde overnight at 4°C (Mirelman et al., 1980). After dehydration in a graded series of ethanol, the specimens were loaded into a small pouch made from filter paper with ultrathin sections inside. Then, the pouch was dried by a critical-point drying (CPD) method (Bray, 2000). After gold-plating, the ultrathin sections were analyzed with a Hitachi SU8010 scanning electron microscope (Hitachi, Japan) (Baharaeen et al., 1982; Bauer et al., 2001).

Anti-adhesion assay with RT-PCR

(i) Preparation of premixed solutions of YCW or MOS and bacteria. Bacterial cell suspensions were adjusted to OD_{600} = 0.2 with MEM medium containing 10% heat-inactivated FBS and 1% NEAA. Then, ultrasonic-treated sample solution (6 mg/mL) was added to the bacterial cell suspension at a 4:1 ratio (bacterial cell suspension:sample solution) and agitated magnetically to obtain a stable, well-dispersed solution.

(ii) Inhibition of bacteria attachment to Caco-2 cells by YCW and MOS. For the anti-adhesion assay, a culture of Caco-2 cells was prepared in 24-well flat bottom plates containing MEM medium without penicillin/streptomycin or other antibiotics, and was incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 1 h. Then, the premix solutions were added to the 24-wall plates and incubated at 37°C for 1 h. The wells were washed three times to remove unattached bacteria, including the negative and blank controls. Then, bacterial DNA was extracted from the wells (three parallel replicates were assessed at the same time) with the TIANamp Bacteria DNA Kit (TIANGEN, China) according to the manufacturer's instructions. Blank and negative controls were also assessed. The blank control consisted of YCW or MOS samples but without test bacteria, and the negative control consisted of test bacteria without YCW or MOS samples.

(iii) Quantification of adhered bacterial cells by real-time PCR. Bacterial cells (*E. coli* or *S. pullorum*) were quantified by real-time PCR using genus- or species-specific primers (listed in Table 1). The real-time PCR was performed in a Real-Time PCR System (BIO-RAD, USA) with SYBER Green I fluorophore to correlate the fluorescent signal with the amount of PCR product. Amplification was carried out in a 25-μL reaction containing 5 μL of bacterial DNA, 10 μM each primer, 6 μL of RNase-free ddH_2O, and 12.5 μL of 2× SuperReal PreMix Plus containing SYBER Green I (TIANGEN, China). The experimental programs was as follows: 1) a pre-denaturation step at 95°C for 15 min; 2) amplification, including 40 cycles of denaturation at 95°C for 10 s, annealing at 52°C for 20 s, and extension at 72°C for 30 s, with a transition time of 0.1°C/s, and a heating step at 95°C for 10 s; and 3) melting curve analysis, which was conducted at 65–95°C, with an increase of 0.1°C/s. As internal standards, we amplified dilutions of the bacteria (0.0625 ng/μL–2 ng/μL) in PBS (Candela et al., 2005). Linear regression was used to determine the relationship between the cycle threshold value (Ct) and the logarithm of the initial quantity of bacterial DNA. Finally, the concentration of DNA extracted from the bacteria attached to the Caco-2 cells was calculated. The number of bacteria bound to the Caco-2 cells was determined based on the relationship between bacterial DNA and CFU. The results are shown as bacterial CFU adhered to Caco-2 cells after removal of non-adherent bacteria. The anti-adhesion effects of YCW and MOS were determined by comparison to the bacterial CFU added.

Table 1 Genus- and species-specific primers used for quantification of bacterial cells in adhesion assays

Primer	Sequence	Specificity
*fim*Yf	5'-TCGTCATTCCATTACCTACC-3'	*Salmonella*
*fim*Yr	5'-AAACGTTGAAAAACTGAGGA-3'	*Salmonella*
27f	5'-AGAGTTTGATCCTGGCTCAG-3'	*Escherichia coli*
338r	5'-TGCTGCCTCCCGTAGGAGT-3'	*Escherichia coli*

Statistical analysis

The results are shown as the mean±standard deviation (SD) of three independent experiments. The significance of differences ($p < 0.05$) between samples was determined by using Student's *t*-test in SPSS 17.0.

Results

Cell morphology and adhesion observed by SEM

In this study, enzymatic hydrolysis was used to generate YCW extract instead of yeast autolysis. Then, MOS and protein components were obtained in a soluble form by fractionation via alkali extraction (Bychkov et al., 2010).

Ultrathin sections of the prepared YCW and MOS were gold plated and analyzed by SEM, and the surface morphologies of the samples were observed (Fig. 1). Figure 1a shows the structure of YCW, which was anxiolytic and rough. Figure 1B shows the structure of yeast MOS, which was characterized by amorphous shapes. SEM analysis also showed that the YCW and MOS used in this study could bind to both *E. coli* (Fig. 1 A_1 and B_1) and *S. pullorum* (Fig. 1 A_2 and B_2), as they adhered to these pathogenic bacteria.

Inhibition of *E. coli* and *S. pullorum* adhesion to Caco-2 cells

To quantitatively compare the abilities of YCW and MOS to inhibit the adherence of enteropathogenic bacteria to Caco-2 cells, a real-time quantitative PCR method was used to detect the number of bacteria adhered to the Caco-2 cells. The anti-adhesion results are quantitatively presented as histograms (Fig. 2). The ordinate was the CFU of adhered *E. coli* and *S. pullorum*. The lower the number of CFU, the fewer the bacteria adhered to Caco-2 cells. The ability of the YCW and MOS samples to inhibit the adhesion of bacteria to Caco-2 cells was confirmed. Figure 2-(1) shows the inhibitory effects of the samples on the adhesion of *E. coli* to Caco-2 cells. The number of *E. coli* adherent to Caco-2 cells (6.07×10^4 CFU) was lowest in the presence of YCW, and the inhibition ratio was 95%. The number of adherent *E. coli* cells was higher in the presence of MOS (4.40×10^5 CFU) than in the presence of YCW, and in the presence of MOS, the inhibition ratio was 67%.

The results also showed that *S. pullorum* adhesion was

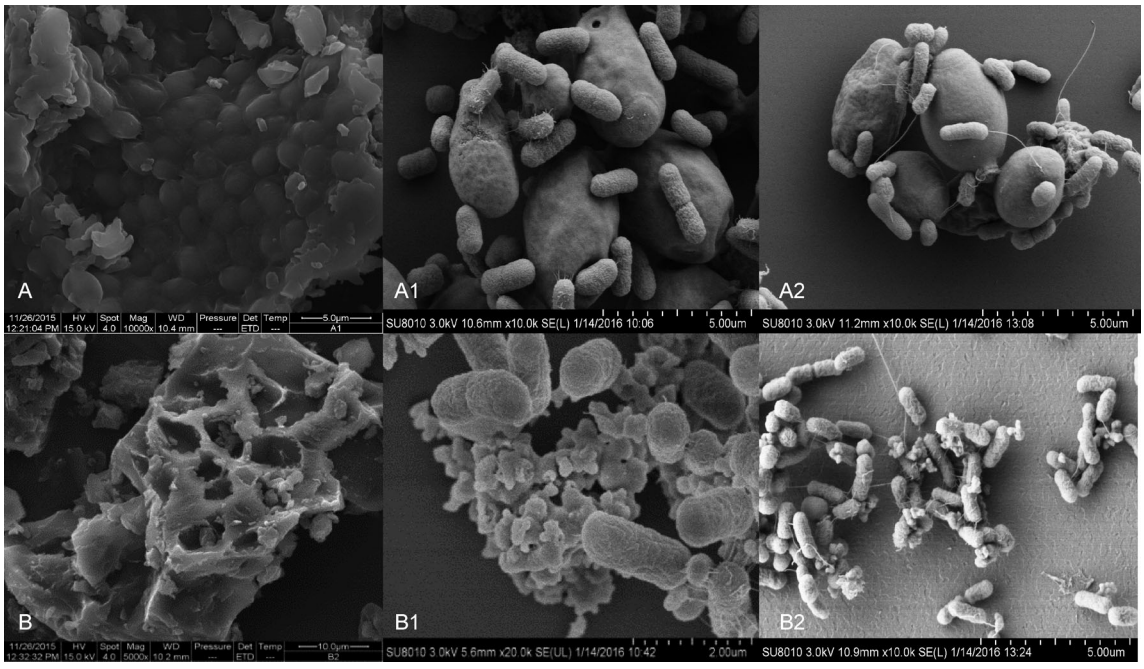

Figure 1 Morphology of YCW, MOS and adhesion assays from SEM. A: YCW; A1: The adhesion of YCW and E. coli(10.0k×); A2: The adhesion of YCW and Salmonella pullorum (10.0k×); B: yeast MOS; B1: The adhesive microstructure between yeast MOS and E. coli (20.0k×); B2:The adhesion of yeast MOS and Salmonella pullorum (10.0k×)

inhibited by the test samples [Fig. 2-(2)]. The YCW-treated *S. pullorum* samples had fewer CFU (~5 × 10^6 CFU) than *S. pullorum* incubated with yeast MOS, as the number of *S. pullorum* CFU was ~1 × 10^7. The inhibition ratios for YCW and MOS on *S. pullorum* were 74% and 50%, respectively.

It was obvious that the tendency to protect Caco-2 cells from invasion by *E. coli* and *S. pullorum* was similar. Based on the results, we could draw the following conclusions. MOS and YCW effectively inhibited *E. coli* and *S. pullorum* adhesion to Caco-2 cells. However, the capability of MOS to inhibit the adherence of enteropathogenic bacteria was not better than that of YCW, as YCW, which morphologically resembled intact cells, showed optimal inhibition against the adhesion of *E. coli* and *S. pullorum* to Caco-2 cells.

Discussion

In this study, we showed that adhesion of the enteropathogenic bacteria *E. coli* and *S. pullorum* to Caco-2 cells was significantly reduced by the addition of YCW and MOS from *S. cerevisiae*. In terms of effectiveness, YCW had a more significant effect on the adhesion of both *E. coli* and *S. pullorum* to Caco-2 cells, and it inhibited adhesion by ~95% and 74%, respectively, whereas MOS reduced adhesion by 67% and 50%, respectively (Fig. 2). To our knowledge, this is the first investigation of the anti-adhesive properties of YCW and MOS. Both YCW and MOS contain mannose, which can block the colonization of intestinal pathogens containing type 1 fimbriae through mannose-binding lectins (Rodrigues et al., 2009). Caco-2 cells are a clonal colonic cancer cell line that shows structural and functional characteristics of differentiated intestinal epithelial cells (Konkel et al., 1992). Therefore, Caco-2 cells have been widely used to quantitatively evaluate the adhesion capability of intestinal pathogenic bacteria *in vitro* (Oyofo et al., 1989; Vesterlund et al., 2005; Ganner et al., 2010). In addition, real-time PCR has been previously used to quantify adherent bacteria on monolayers of enterocyte-like Caco-2 cells in vitro, and the method was shown to be efficient, sensitive, and accurate (Candela et al., 2005).

In this study, YCW was isolated from *S. cerevisiae* after enzymatic lysis, and SEM analysis showed that structurally, the YCW extract consisted of intact cells (Fig. 1A). MOS was obtained as a soluble component after alkali treatment of YCW, and SEM analysis showed that it was amorphous (Fig. 1B). SEM also showed that YCW and MOS can bind to *E. coli* and *S. pullorum*. The three-dimensional structure of YCW may have made it easier to adhere to bacteria than it was for the soluble, amorphous MOS. Ganner et al. (2012) reported that protein-oligosaccharide interaction was mainly dependent on the three-dimensional structure rather than the total number of mannoses. Ofek et al. (2003) also postulated that, in addition to the receptor interaction, there may be hydrophobic and other non-specific interactions involved in bacterial adhesion. We observed that β-glucan from YCW can adhere to bacteria, although there was no specific lectin on its surface (data was not shown). Therefore, hydrophobic and

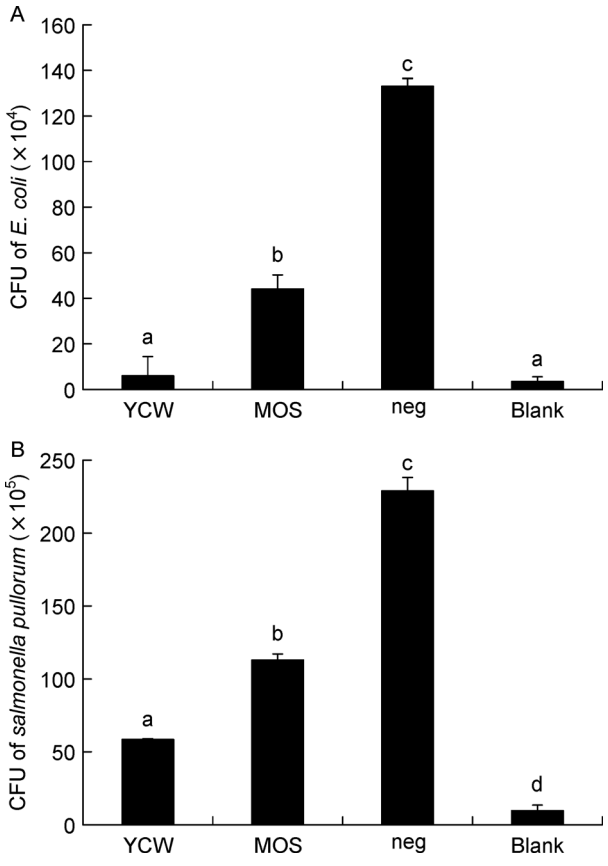

Figure 2 The amount of *E. coli* and *Salmonella pullorum* adhesion to Caco-2 cells after treatment of YCW and MOS. neg-negative control; blank–blank control. Results shown are average ±SD of three replicates. a,b,c,d–values labeled with different letters are significantly different ($p < 0.05$).

other non-specific interactions may play an important role in the adhesion process.

Based on our results, YCW more effectively inhibited the adherence of pathogenic bacteria to epithelial cells; therefore, in some applications, YCW can be used as a replacement for MOS. Further study is required to determine if YCW or MOS is a superior antibiotic substitute and to verify these results in vivo.

Conclusion

In summary, YCW, with intact cell structure, more effectively inhibited the adhesion of *E. coli* and *S. pullorum* to monolayers of enterocyte-like Caco-2 cells than did soluble, amorphous MOS *in vitro*.

Compliance with ethics guidelines

Xiaoqing Xu, Yu Qiao, Qing Peng, Long Gao and Bo Shi declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Baharaeen S, Vishniac H S (1982). A fixation method for visualization of yeast ultrastructure in the electron microscope. Mycopathologia, 77 (1): 19–22

Bauer C, Herzog V, Bauer M F (2001). Improved technique for electron microscope visualization of yeast membrane structure. Microsc Microanal, 7(6): 530–534

Baurhoo B, Letellier A, Zhao X, Ruiz-Feria C A (2007). Cecal populations of *lactobacilli* and *bifidobacteria* and *Escherichia coli* populations after in vivo *Escherichia coli* challenge in birds fed diets with purified lignin or mannanoligosaccharides. Poult Sci, 86(12): 2509–2516

Becker P M, Galletti S, Roubos-van den Hil P J, van Wikselaar P G (2007). Validation of growth as measurand for bacterial adhesion to food and feed ingredients. J Appl Microbiol, 103(6): 2686–2696

Bouckaert J, Mackenzie J, de Paz J L, Chipwaza B, Choudhury D, Zavialov A, Mannerstedt K, Anderson J, Piérard D, Wyns L, Seeberger P H, Oscarson S, De Greve H, Knight S D (2006). The affinity of the FimH fimbrial adhesin is receptor-driven and quasi-independent of *Escherichia coli* pathotypes. Mol Microbiol, 61(6): 1556–1568

Bray D (2000). Critical Point Drying of Biological Specimens for Scanning Electron Microscopy, Humana Press, 235–243

Broadway P R, Carroll J A, Sanchez N C (2015). Live yeast and yeast cell wall supplements enhance immune function and performance in food-producing livestock: A review (†,)(‡). Microorganisms, 3(3): 417–427

Bychkov A L, Korolev K G, Lomovsky O I (2010). Obtaining mannanoligosaccharide preparations by means of the mechanoenzymatic hydrolysis of yeast biomass. Appl Biochem Biotechnol, 162 (7): 2008–2014

Candela M, Seibold G, Vitali B, Lachenmaier S, Eikmanns B J, Brigidi P (2005). Real-time PCR quantification of bacterial adhesion to Caco-2 cells: competition between bifidobacteria and enteropathogens. Res Microbiol, 156(8): 887–895

Fernandez F, Hinton M, Van Gils B (2002). Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella Enteritidis* colonization. Avian Pathol, 31(1): 49–58

Ganan M, Carrascosa A V, de Pascual-Teresa S, Martinez-Rodriguez A J (2009). Inhibition by yeast-derived mannoproteins of adherence to and invasion of Caco-2 cells by *Campylobacter jejuni*. J Food Prot, 72(1): 55–59

Ganan M, Carrascosa A V, de Pascual-Teresa S, Martinez-Rodriguez A J (2012). Effect of mannoproteins on the growth, gastrointestinal viability, and adherence to Caco-2 cells of lactic acid bacteria. J Food Sci, 77(3): M176–M180

Ganner A, Stoiber C, Uhlik J T, Dohnal I, Schatzmayr G (2013). Quantitative evaluation of *E. coli* F4 and *Salmonella Typhimurium* binding capacity of yeast derivatives. AMB Express, 3(1): 62

Ganner A, Stoiber C, Wieder D, Schatzmayr G (2010). Quantitative in vitro assay to evaluate the capability of yeast cell wall fractions from *Trichosporon mycotoxinivorans* to selectively bind gram negative pathogens. J Microbiol Methods, 83(2): 168–174

Ganner A, Schatzmayr G (2012) . Capability of yeast derivatives to adhere enteropathogenic bacteria and to modulate cells of the innate

immune system. Appl Microbiol Biotechnol, 95(2): 289–97

Jones C H, Pinkner J S, Roth R, Heuser J, Nicholes A V, Abraham S N, Hultgren S J (1995) . FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the *Enterobacteriaceae*. Proc Natl Acad Sci U S A, 92: 2081–2085

Kogan G, Kocher A (2007). Role of yeast cell wall polysaccharides in pig nutrition and health protection. Livest Sci, 109(1-3): 161–165

Kogut M H (2000). Cytokines and prevention of infectious diseases in poultry: a review. Avian Pathol, 29: 395–404

Konkel M E, Corwin M D, Joens L A, Cieplak W (1992). Factors that influence the interaction of *Campylobacter jejuni* with cultured mammalian cells. J Med Microbiol, 37(1): 30–37

Mirelman D, Altmann G, Eshdat Y (1980). Screening of bacterial isolates for mannose-specific lectin activity by agglutination of yeasts. J Clin Microbiol, 11(4): 328–331

Ofek I, Beachey E H (1978). Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect Immun, 22(1): 247–254

Ofek I, Hasty D L, Sharon N (2003). Anti-adhesion therapy of bacterial diseases: prospects and problems. FEMS Immunol Med Microbiol, 38(3): 181–191 PMID:14522453

Ofek I, Mirelman D, Sharon N(1977). Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature, 265 (5595): 623–625

Oyofo B A, Droleskey R E, Norman J O, Mollenhauer H H, Ziprin R L, Corrier D E, DeLoach J R (1989). Inhibition by mannose of *in vitro* colonization of chicken small intestine by *Salmonella typhimurium*. Poult Sci, 68(10): 1351–1356

Rodrigues D F, Elimelech M (2009). Role of type 1 fimbriae and mannose in the development of *Escherichia coli* K12 biofilm: from initial cell adhesion to biofilm formation. Biofouling, 25(5): 401–411

Rosen D A, Pinkner J S, Walker J N, Elam J S, Jones J M, Hultgren S J (2008). Molecular variations in *Klebsiella pneumoniae* and *Escherichia coli* FimH affect function and pathogenesis in the urinary tract. Infect Immun, 76(7): 3346–3356

Sharon N, Ofek I (2000). Safe as mother's milk: carbohydrates as future anti-adhesion drugs for bacterial diseases. Glycoconj J, 17(7-9): 659–664

Shashidhara R G, Devegowda G (2003). Effect of dietary mannan oligosaccharide on broiler breeder production traits and immunity. Poult Sci, 82(8): 1319–1325

Shoaf-Sweeney K D, Hutkins R W (2009). Adherence, anti-adherence, and oligosaccharides preventing pathogens from sticking to the host. Adv Food Nutr Res, 55: 101–161

Spring P, Wenk C, Dawson K A, Newman K E (2000). The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. Poult Sci, 79(2): 205–211

Sweeney Shoaf K D, Hutkins R W (2008). Anti‐Adherence, and Oligosaccharides: Preventing Pathogens from Sticking to the Host. Adv Food Nutr Res, 55: 101–161

Tiago F C P, Martins F S, Souza E L, Pimenta P F, Araujo H R, Castro I M, Brandão R L, Nicoli J R (2012). Adhesion to the yeast cell surface as a mechanism for trapping pathogenic bacteria by *Saccharomyces* probiotics. J Med Microbiol, 61(Pt 9): 1194–1207

Trevisi P, Priori D, Gandolfi G, Colombo M, Coloretti F, Goossens T, Bosi P (2012). *In vitro* test on the ability of a yeast cell wall based product to inhibit the *Escherichia coli* F4ac adhesion on the brush border of porcine intestinal villi1. J Anim Sci, 90: 275

Varelas V, Liouni M, Calokerinos A C, Nerantzis E T (2016). An evaluation study of different methods for the production of -D-glucan from yeast biomass. Drug Test Anal, 8(1): 46–55

Vesterlund S, Paltta J, Karp M, Ouwehand A C (2005). Measurement of bacterial adhesion-in vitro evaluation of different methods. J Microbiol Methods, 60(2): 225–233

Vieira L Q, dos Santos L M, Neumann E, da Silva A P, Moura L N, Nicoli J R (2008). Probiotics protect mice against experimental infections. J Clin Gastroenterol, 42(Suppl 3 Pt 2): S168–S169