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Effect of fermented broth from lactic acid bacteria on pathogenic bacteria proliferation

S. Gutiérrez,* H. Martínez-Blanco,*† L. B. Rodríguez-Aparicio,*† and M. A. Ferrero*†[1]
*Departamento de Biología Molecular, Facultad de Veterinaria, and
†Instituto de Biología Molecular, Genómica y Proteómica (INBIOMIC), Universidad de León, 24071 León, Spain

ABSTRACT

In this study, the effect that 5 fermented broths of lactic acid bacteria (LAB) strains have on the viability or proliferation and adhesion of 7 potentially pathogenic microorganisms was tested. The fermented broth from *Lactococcus lactis* C660 had a growth inhibitory effect on *Escherichia coli* K92 that reached of 31%, 19% to *Pseudomonas fluorescens*, and 76% to *Staphylococcus epidermidis*. The growth of *Staph. epidermidis* was negatively affected to 90% by *Lc. lactis* 11454 broth, whereas the growth of *P. fluorescens* (25%) and both species of *Staphylococcus* (35% to *Staphylococcus aureus* and 76% to *Staph. epidermidis*) were inhibited when they were incubated in the presence of *Lactobacillus casei* 393 broth. Finally, the fermented broth of *Lactobacillus rhamnosus* showed an inhibitory effect on growth of *E. coli* K92, *Listeria innocua*, and *Staph. epidermidis* reached values of 12, 28, and 76%, respectively. *Staphylococcus epidermidis* was the most affected strain because the effect was detected from the early stages of growth and it was completely abolished. The results of bacterial adhesion revealed that broths from *Lc. lactis* strains, *Lactobacillus paracasei*, and *Lb. rhamnosus* caused a loss of *E. coli* K92 adhesion. *Bacillus cereus* showed a decreased of adhesion in the presence of the broths of *Lc. lactis* strains and *Lb. paracasei*. *Listeria innocua* adhesion inhibition was observed in the presence of *Lb. paracasei* broth, and the greatest inhibitory effect was registered when this pathogenic bacterium was incubated in presence of *Lc. lactis* 11454 broth. With respect to the 2 *Pseudomonas*, we observed a slight adhesion inhibition showed by *Lactobacillus rhamnosus* broth against *Pseudomonas putida*. These results confirm that the effect caused by the different LAB assayed is also broth- and species-specific and reveal that the broth from LAB tested can be used as functional bioactive compounds to regulate the adhesion and biofilm synthesis and ultimately lead to preventing food and clinical contamination and colonization of *E. coli* K92, *B. cereus*, and *Ls. innocua*.
Key words: lactic acid bacteria, *Lactobacillus*, *Lactococcus*, adhesion, probiotics

INTRODUCTION

Pathogenic bacteria or toxins produced by bacteria often enter the human body through food or drink, causing symptoms or illness with several mechanisms. *Staphylococcus aureus* and *Escherichia coli* belong to the normal flora of about 50% of people, although virulent strains, resistant to commonly used antibiotics, may cause sepsis and severe infections. Food-borne intoxication is caused by a heat-stable enterotoxin produced by *Staph. aureus* in consumed food, which produces nausea and vomiting (Nester, 2001). *Bacillus cereus* contaminates various types of final food products, such as cooked chilled meals, vegetables, meat, fish, milk, liquid egg, pastries, flavorings, oils, and fats (Wijnands et al., 2006; Baron et al., 2007). On the other hand, *Listeria monocytogenes* is a common bacterium in the environment and in animals, and it may be transferred to food and the human gastrointestinal tract via raw milk and contaminated dairy products. This organism may cause meningitis, sepsis, or abortion, but in practice only pregnant women and people with immune defects are in danger of catching the infection (Nester, 2001). *Staphylococcus epidermidis*, commensal coagulase-negative, may be transferred to the human gastrointestinal tract via breast milk because this microorganism belongs to skin microbiota (Iwase et al., 2010; Park et al., 2011).

Probiotics are defined as living microorganisms which act to keep the balance of intestinal microbiota after ingestion in sufficient numbers (Fuller, 1989). Lactic acid bacteria (**LAB**) are probiotic microorganisms known to produce antibacterial peptides and small proteins called bacteriocins, which enable them to compete against other bacteria in the environment. Lactic acid bacteria, such as *Lactobacillus* species, have protective effects against a variety of pathogenic infections in the

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[1]Corresponding author: ma.ferrero@unileon.es

gastrointestinal systems of humans and animals (Ahrné et al., 1998; Gill et al., 2001). These strains produce a wide range of antimicrobial agents and have been studied extensively with regard to their possible use in the field of probiotics. This bacteria may also be a useful alternative for the treatment and prevention of a variety of infectious diseases caused by oral, enteric, and urogenital pathogens (Shornikova et al., 1997; Caglar et al., 2006; Abad and Safdar, 2009).

Recently, probiotics have been studied principally for their potential as an alternative to antibiotics in the field of livestock production (Reid and Friendship, 2002; Reid, 2006). Commonly used probiotics such as *Bacillus*, *Lactobacillus*, *Bifidobacterium* species, and *Clostridium butyricum* have the potential to improve swine growth performance, to increase utilization of nutrients such as dietary starch, and to resist colonization by pathogens in the gastrointestinal tract of animals (Lee et al., 2001; Guo et al., 2006). Many studies suggest that LAB could protect sites against pathogenic bacterial invasion and colonization by preventing the attachment of these pathogens to sites. Lactic acid bacteria produce substances that inhibit the multiplication of pathogens by competing with other microorganisms for nutritional requirements. This might inhibit the multiplication of these agents by excreting substances, mainly H_2O_2, lactic acid, and bacteriocin-like substances (Andreu et al., 1995; Cadieux et al., 2002). Furthermore, the effect on growth is important when studying the relations established between different bacterial species. It is now known that only a very small fraction of bacteria are in the form of free-floating plankton. It is postulated that 99% of all bacterial cells exist as biofilms and only 1% live in a planktonic state (Ramadan et al., 2005; Sanclement et al., 2005). The development of biofilm communities is one of the main strategies for bacteria survival in a certain ecological niche. This state allows bacteria integrated into the biofilm to be protected from environmental fluctuation factors such as humidity, temperature, and pH and, in the case of infections antibacterial preparations applied to the host organism, lengthen the infection and also provide concentrated nutrients and waste disposal. In recent years interest has increased in eating healthy foods, mainly due to possible beneficial effects on the body.

In our laboratory, we studied different strains that may have probiotic potential (Monteagudo-Mera et al., 2011; Monteagudo-Mera et al., 2012). We then decided to focus on studying the competition that can exist between potentially probiotic bacterial strains and those that may be considered potentially pathogenic, both of which are common in the intestinal microbiota of the mammals. The former have been selected because of their antimicrobial activity and to achieve the displacement of potentially pathogenic strains from dairy products that could attached to the gastric epithelium. The latter were chosen because of their presence and their possible capacity to adhere to the gastrointestinal epithelium. Our aim was to establish the characteristics that inhibit or reduce the growth of potentially pathogenic strains and enhance the proliferation of probiotic to increase the added value of functional foods. The objective we propose in this paper is, therefore, to determine the effect that different fermented broths of LAB strains have on the viability or proliferation and adhesion of potentially pathogenic microorganisms.

MATERIALS AND METHODS

Strains and Culture Conditions

To carry out this work, we studied 5 strains of LAB, obtained from the bacterial culture collection of the Department of Hygiene and Food Technology, at the University of León (these were isolated from human feces or milk products) and from the American Type Culture Collection (**ATCC**, Manassas, VA), which were selected according to their probiotic and antimicrobial properties (Monteagudo-Mera et al., 2011, 2012). Meanwhile, 7 potentially pathogenic strains were used; these came from the ATCC, Spanish Collection of Type Culture (Paterna, Spain), and the Culture Collection of the Hannah Research Institute (Ayr, Scotland), which were selected based on their potential as food contaminants (Tables 1 and 2).

All LAB strains were grown in trypticase soy broth (**TSB**) containing 0.6% (wt/vol) yeast extract at 30°C for 24 h, with the exception of *Lactobacillus casei* ATCC 393, which was incubated for 36 h at the same temperature. Potentially pathogenic strains were cultured in the same medium, but at 37°C for 12 h, except *Pseudomonas fluorescens* and *Pseudomonas putida*, which were incubated at 30°C. When it was necessary, the strains were grown on trypticase soy agar (**TSA**) petri plates or TSA slant tubes under the same growth conditions as TSB with yeast extract medium. The strains were maintained in a mixture of TSB-glycerol (1:1) at −80°C until further use.

Determining the Effect of LAB on Pathogenic Bacterial Growth

To determine the potential inhibitory effect of LAB over pathogenic bacteria, both strain types were grown in TSA slant tubes at the appropriate temperature (see above) and time (Monteagudo-Mera et al., 2011), and when the optimal growth was reached, the cells were