

Evaluation of in vitro antagonism and protection against enteropathogenic experimental challenge of different strains of *Bifidobacterium*

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Abstract

This study was inspired by problems in the dairy industry and illustrates the importance of antimicrobial *Bifidobacterium*, against the germs of alteration. About forty-eight (48) strains of bifidobacteria isolated from different origin (breast-fed infant faeces, yoghurt (bifidus)), were isolated on MRS (in addition of 0.05% cystéine HCL et de 2 mg/l of nalidixic acid) medium, six strains of bifidobacteria belonging to the species (*Bifidobacterium longum*, *B. Brève*, *B. bifidum*) were isolated on MRS cystéine medium. In vitro antagonism test showed that our strains were able to produce antagonistic substances against various pathogenic microorganisms. We studied the antagonist power, *Bifidobacterium* against enteropathogenes (*S. aureus*, *Escherichia coli*, *P. aeruginosa*, *Salmonella. Sp*). by the agar diffusion method. The activity was completely destroyed by the action of proteolytic enzymes, indicating that the biologically active portion is proteinaceous. These properties suggest that inhibitory substance is considered like substance "Bacteriocin like, these results emphasize the importance of the antimicrobial activity of *Bifidobacterium* in the dairy industry. Additional tests were needed to determine the exact nature of the inhibitors.

Keywords: intestinal flora, antagonist activity, antimicrobial substances, Organic acids, bacteriocins like, enteropathogenes, inhibiting pathogens.

Introduction:

There is general agreement on the important role of the gastrointestinal (GI) microbiota in the health and wellbeing status of human and animals. The concept that certain micro-organisms, when supplied in sufficient quantities, confer direct benefits to the host is defined by the term 'probiotic', (Saadet *et al.*, 2012). They play an important role in human nutrition. In recent years there has been a significant increase in research on the characterization and verification potential health benefits associated with the use of probiotic and prebiotic, (Saadet *et al.*, 2012). It is generally accepted that probiotic food products should contain a minimal level of viable cells of 10^6 per gram or milliliter of product, although this value is relative since beneficial effect depends on the strain and targeted health benefit, (Reimann *et al.*, 2010). The potential mechanisms by which a probiotic agent might exert its protective or therapeutic effect include competition for nutrients or adhesion receptors, production of inhibitory metabolites or antimicrobial agents against pathogens, (Ariane *et al.*, 2010).

Bifidobacteria are anaerobic Gram positive bacilli belonging to the dominant gut microbiota in humans and animals. In recent years bifidobacteria have gained a lot of attention because their association with numerous health-promoting effects, even though some mechanisms of these beneficial effects remain unexplained, (Turroni *et al.*, 2009). Thus, various bifidobacterial strains are currently used as probiotics in functional food products, and selecting new probiotic strains is currently of great interest, (FAO/WHO). These strains must display several characteristics, one of which is that they must be of human origin. Therefore, in the perspective of either the understanding of the mechanisms of the beneficial effects of bifidobacteria or strain selection for probiotic uses, reliable enumeration and isolation of bifidobacteria from human feces are needed, (Ferraris *et al.*, 2010). These bacteria colonize the neonatal intestine from the first week after birth and inhabit the gastrointestinal tract throughout life where they contribute to human health and well-being, (Turroni *et al.*, 2009).

It is also known that the composition of the dominant species of the indigenous bifidobacteria varies with age, with *B. lactis*, *B. longum*, *B. breve* and *B. parvorum* found in children, which are replaced in the adulthood by *B. adolescentis*, *B. catenulatum*, *B. pseudocatenulatum* and *B. longum*, (Ariane *et al.*, 2010). Infection with enteric pathogens continues to be a health problem worldwide, especially in children. Intestinal epithelium provides the first line of defence of the organism, providing an efficient barrier against pathogens and macromolecules. The mucus layer and the resident gut microbiota protect the gut mucosa from adhesion and invasion of pathogenic microorganisms, (Candela *et al.*, 2010). In this respect, probiotics have been proposed for prevention and treatment of gastrointestinal tract (GIT) infections, (Rodríguez *et al.*, 2012). In recent years, Bifidobacteria have attracted considerable attention due to their overall beneficial effects on health, (Peter *et al.*, 2001); they play a significant role in maintaining the balance of intestinal microflora by correcting intestinal disorders and by fighting against diarrhoea and gastro-enteritis, (Hamma *et al.*, 2008).

The aim of this study was to identify these *Bifidobacterium*, isolated from different origin and to study their potential and antimicrobial activity against enteropathogenes by using different in vitro tests.

Material and methods

Strains origin

-The strains of *Bifidobacterium* used were derived from:

About 40 fresh fecal samples were obtained from newborn infants ((their ages less than 05 months), from several samples of commercial French yoghurt (Activia (bifidus)).

-Lyophilized *B.bifidum* ATCC 15696 (Bbf1), was obtained from the collection of Laval university, food science and nutrition (Québec, Canada, G1VOA6).

-Enteropathogenes strains: from the military hospital Collections Regional Oran provided; and from the institut Pasteur of Algeria, (Table 01). These pathogenic microorganisms were selected due to their role as pathogens for humans and their presence in the human gut. For the experiments of in vitro growth inhibition, (Arboleya *et al.*, 2011).