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Bacteriocin production by *Bifidobacterium* spp. A review

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ABSTRACT

Bacteriocins are ribosomally-synthesized antibacterial peptides. These compounds are produced by a broad variety of different bacteria belonging mainly to the genus *Bifidobacterium*, to which health promoting properties have frequently been attributed. However, despite the fact that the identification of *Bifidobacterium*-associated bacteriocins was first reported in 1980 and that they exhibit antimicrobial activity against pathogenic microorganisms such as *Listeria monocytogenes*, *Clostridium perfringens*, and *Escherichia coli*, relatively little information is still available about the antimicrobial compounds produced by strains of this genus. More detailed understanding of the action mechanisms of these antimicrobials could allow us to determine the extent to which their production contributes to the probiotic properties of specific bifidobacteria strains and, potentially, be of crucial significance for ultimate preservation of functional foods or pharmaceutical applications. Here we review what is already known about their structure, classification, mode of action, functionality, immunity, production and purification.

Contents

1. Introduction

Bifidobacteria are high GC, Gram-positive, non-spore-forming, non-motile and catalase-negative anaerobic bacteria belonging to the phylum of Actinobacteria (Ishibashi et al., 1997). They are able to ferment glucose to lactic and acetic acids via a metabolic pathway that is characterized by the presence of the enzyme fructose-6-phosphate phosphoketolase (F6PPK) (Ballongue, 2004; Gomes and Malcata, 1999). These microorganisms were first isolated by Tissier (1900), described as pleomorphic rods with different shapes, including curved, short and bifurcated Y shapes, and initially classified as *Bacillus bifidus communis*. Subsequently, they were renamed *Lactobacillus bifidus* before De Vries and Stouthamer (1967) suggested that they should be reclassified as a distinct genus (*Bifidobacterium*) because of the presence of F6PPK and the simultaneous absence of glucose-6-phosphatase dehydrogenase and aldolase, i.e. two enzymes present in lactobacilli (Ballongue, 2004; Cheikhyoussef et al., 2008; Ishibashi et al., 1997).

Bifidobacteria are an important group of human gut commensal bacteria, accounting for around 3–7% of the microbiota in adults and, according to some reports, up to 91% in newborns (Ballongue, 2004; Cheikhyoussef et al., 2009a). Some strains of *Bifidobacterium*

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possess traits that have resulted in them being employed as probiotics. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (FAO/WHO, 2001), probiotics are living microorganisms that, when ingested in sufficient quantities, exert health-promoting benefits to the host. Among the many probiotic traits that have been attributed to bifidobacteria are a) the induction of immunoglobulin production, b) improvement of food nutritional value by assimilation of substrates not metabolized by the host, c) anti-carcinogenic activity and d) folic acid synthesis (Bevilacqua et al., 2003; Cheikhyoussef et al., 2009a; Collado et al., 2005a; Gomes and Malcata, 1999; Touré et al., 2003). Interestingly for the purposes of this review, some bifidobacteria are also known to produce antimicrobials (Cheikhyoussef et al., 2009b; Gibson and Wang, 1994b; Gomes and Malcata, 1999; Ibrahim and Salameh, 2001) and, more specifically, bacteriocins (Anand et al., 1984, 1985; Cheikhyoussef et al., 2010; von Ah, 2006; Yildirim and Johnson, 1998; Yildirim et al., 1999).

Bacteriocins are ribosomally-synthesized antimicrobial peptides produced by bacteria that are active against other bacteria, either belonging to the same species (narrow spectrum) or even across genera (broad spectrum). Producing organisms are immune to their own bacteriocin(s), a property that is mediated by specific immunity proteins (Cotter et al., 2005b). Bacteriocin production takes place most frequently during the late exponential or early stationary phases of growth, is often influenced by quorum sensing and stress signaling (Klaenhammer, 1988; Kotelnikova and Gelfand, 2002; Riley and Chavan, 2007; Tagg et al., 1976), and is regarded as a probiotic trait (Dobson et al., 2012; O'Shea et al., 2012) contributing to the suppression of intestinal pathogens. In addition, the rise in demand for natural foods that do not contain chemical preservatives has increased the interest in their application as preservatives to ensure food quality and safety. Since the discovery of bacteriocins (Cascales et al., 2007; Cotter et al., 2005a), in-depth studies have been undertaken to get detailed information on their physicochemical properties, mechanisms of action and genetic determinants (Cotter et al., 2005a; Drider et al., 2006; Ennahar et al., 2000; Riley and Wertz, 2002; Tagg et al., 1976), all of which are of great significance for the ongoing attempts to commercialize them more extensively. A considerable part of research on bacteriocins has focused on the production and investigation of peptides from lactic acid bacteria (LAB) such as *Lactococcus* spp., *Leuconostoc* spp., *Enterococcus* spp., and *Pediococcus* spp., with a view to their potential application as natural preservatives of foods (Cheikhyoussef et al., 2009a; Deegan et al., 2006; Riley and Chavan, 2007). Despite the potential of bifidobacteria to suppress the growth of both Gram-negative and Gram-positive bacteria, their ability to produce bacteriocins has so far been underestimated, being their antimicrobial activity often ascribed to the inhibitory action of organic acids and the related pH decrease (Ballongue, 2004; Makras and De Vuyst, 2006; von Ah, 2006). However, exceptions exist.

Here we review the literature relating to bifidobacteria able to produce bacteriocins, with a focus on their distinctive features, factors influencing their production, purification, mechanisms of action and classification.

2. Antimicrobial compounds from *Bifidobacterium* spp.

Bifidobacteria have the capacity to synthesize organic acids and other antimicrobial compounds such as bacteriocins. Although some reports have suggested that the production of organic acids, via the heterofermentative pathways, is partially responsible for the inhibitory activity of bifidobacteria (Bruno and Shah, 2002; Ibrahim and Salameh, 2001), it is well accepted that at least some bifidobacteria also produce bacteriocins. In some cases, the antimicrobial activity was associated with the production of peptides, but the exact nature of the active substance was not determined (Anand et al., 1984, 1985; Bernet et al., 1993; Liévin et al., 2000; Meghrous et al., 1990); in other cases, the peptides involved were definitively identified.

Table 1 contains a list of known *Bifidobacterium*-associated bacteriocins and putative bacteriocins as well as their main characteristics. In general, it can be stated that research of *Bifidobacterium*-associated bacteriocins has been relatively unsatisfying and has provided more questions than answers. The following paragraphs provide information regarding a significant number of putative bacteriocins about which frustratingly little is known.

The first putative *Bifidobacterium*-associated bacteriocin found is bifidin produced by *Bifidobacterium bifidum* NCDC 1452. The antimicrobial activity of this strain was found to be the greatest when grown in skim milk, and from this medium it was extracted with methanol–acetone and partially purified by Sephadex G-15 chromatography. The purified product was refrigerated for 3 months or more without exhibiting any activity loss (Anand et al., 1984, 1985). Amino acid analysis of the peptide revealed high contents of phenylalanine and glutamic acid and, in less extent, threonine, aspartic acid, serine, glycine, proline, isoleucine and leucine. However, the study on bifidin did not progress since the mid-1980s.

A number of years later, Kang et al. (1989) described a *Bifidobacterium longum* strain that produced an uncharacterized antimicrobial, referred to as bifilong, that inhibited some Gram-negative and Gram-positive bacteria and was stable over a pH range of 2.5 to 5.0. Similarly, Meghrous et al. (1990) discovered thermoresistant proteinaceous compounds in the supernatant of *B. bifidum* cultures, which inhibited the growth of *Streptococcus*, *Lactococcus* and *Clostridium* spp. However, as the authors were specifically targeting antimicrobials able to inhibit Gram-negative bacteria, the active compounds were not isolated. Liévin et al. (2000) were successful in demonstrating the anti-*Salmonella typhimurium* activity of a highly lipophilic, low molecular weight (<3500 Da) compound produced by *Bifidobacterium* strains, which was precipitated with ammonium sulfate and partially purified by methanol–chloroform extraction and dialysis. However, once again, this compound was not further characterized. Following the same theme, Touré et al. (2003) isolated bifidobacteria strains from infants that displayed antagonistic activity against *Listeria monocytogenes*. Using methanol–acetone extraction, they purified the most hydrophilic proteinaceous antimicrobials, which were found to be resistant to high temperature (100 °C for 5 min) but sensitive to proteases. Saleh and El-Sayed (2004) provided a somewhat more detailed report on the production, in MRS broth with 0.05% L-cysteine. HCl, of putative bacteriocins, designated as bifilact Bb-12 and bifilong Bb-46, by *Bifidobacterium lactis* Bb-12 and *B. longum* Bb-46, respectively. These two bacteriocins were shown to exhibit strong activity against *Staphylococcus aureus*, *S. typhimurium*, *Bacillus cereus* and *Escherichia coli*. While the minimal inhibition concentrations (MICs) of partially purified bifilact Bb-12 and bifilong Bb-46 were found to be 40 and 20 mg/mL for *S. aureus* and 20 and 16 mg/mL for *E. coli*, respectively, one can expect that purified peptides, if obtained, would be even more active. Additional antimicrobials from six *Bifidobacterium* strains were found to exhibit broad inhibitory spectra against both Gram-negative and Gram-positive bacteria, namely *Clostridium difficile*, *Brochothrix thermosphacta*, *L. monocytogenes*, *S. aureus*, *Helicobacter pylori*, *S. typhimurium*, *Arcobacter butzleri*, and some pathogenic yeasts. These heat-stable compounds were sensitive to proteinases and resistant to pH in the range from 3 to 10 (Collado et al., 2005b), but were neither purified nor subject to further investigation. Finally, von Ah (2006) identified, recovered by methanol/acetone extraction and reversed-phase HPLC and partly characterized thermophilicin B67, a bacteriocin produced by *Bifidobacterium thermophilum* RBL67 that exhibited a narrow inhibition spectrum towards three *Listeria* strains and *Lactobacillus acidophilus*.

Ultimately, despite the many reports on *Bifidobacterium*-associated bacteriocins, bifidocin B from *B. bifidum* NCFB 1454 (Yildirim et al., 1999), bifidin I from *Bifidobacterium infantis* BCRC 14602 (partially sequenced) (Cheikhyoussef et al., 2010) and the lantibiotic bisin from *B. longum* DJO10A are the only bacteriocins that were in-depth