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Short Communication

Intestinal *Bacteroides* and *Parabacteroides* species producing antagonistic substances

Viviane Nakano*, Aline Ignacio, Miriam Rodriguez Fernandes, Marcia Harumi Fukugaiti and Mario J. Avila-Campos

Anaerobe Laboratory, Department of Microbiology, Institute of Biomedical Science, University of São Paulo, Brazil

ABSTRACT

Antagonistic substances produced by microorganisms are important to maintenance of the resident microbiota in different ecological niches. They also are used to prevent the exogenous bacterial colonization and invasion, and consequently the development of infectious diseases. In this study, the presence of bacteriocin-producing *Bacteroides* and *Parabacteroides* species isolates from human intestinal microbiota was determined by phenotypic assays. Sixty-three (55.2%) out of the 114 strains produced antagonic substances. Our results showed that *Bacteroides* and *Parabacteroides* species evaluated produced substances with hetero- or iso-antagonism effects. The iso-antagonistic effect was observed in *B. fragilis* (40.9%), *B. vulgatus* (50%) and *B. uniformis* (14.2%). The production of bacteriocins with both hetero- and iso-antagonic effects may suggest a defense against the invasion of exogenous microorganisms in the intestinal microbiota.

KEYWORDS: *Bacteroides*, *Parabacteroides*, bacteriocin, antagonistic effect

INTRODUCTION

The antagonistic effects are important in the maintenance of the resident microbiota in different ecological niches preventing the colonization and invasion of the exogenous bacteria, and it might be one of the mechanisms acting in the prevention of certain infectious diseases [1, 2]. The production of complex substances such as bacteriocins, enzymes, hydrogen peroxide, lactic acid, fatty acids and ammonia, as well as the presence of bacteriophages interfere in the bacterial growth [3].

Bacteriocins are proteins or peptides of different sizes, microbial targets, mode of action, and immune mechanisms, and are synthesized by ribosome of gram-negative and gram-positive bacteria [4, 5, 6] showing a bactericidal effect. Bacteriocins display broad and narrow spectra of activity against microorganisms, and it confers a selective advantage by killing other microbial competitor [7]. Its effects are commonly targeted on closely related species possessing specific receptor sites on cell surface [8].

These biological substances act against susceptible organisms, such as gram-positive bacteria by adsorption on specific receptors on the cell membrane, causing the loss of ribosome and dissolution or condensations of the nuclear material. On the other hand, in bacteriocin-producing gram-negative bacteria, these substances are attached to the outer membrane leading to cell disruption [1].

Bacteriocins produced by gram-positive bacteria are abundant and diverse. The differences among bacteriocins produced by gram-positive and gram-negative bacteria are: 1) gram-positive bacteria

*Corresponding author: Dr. V. Nakano,
Av. Prof. Lineu Prestes 1374, 05508-900,
São Paulo, SP, Brazil.
vivinkn@usp.br

are bacteriocin-specific and they are not necessarily lethal to the producing cell; and 2) gram-negative bacteria rely solely on host regulatory networks [9].

The production of different bacteriocin types, such as colicin, pyocin, klebicin, nisin, pesticins and others, are encoded either by chromosomal or plasmid genes, however, due to their structural similarities in the amino acid sequence and functions, they could have a common origin [1]. Most *Eubacteria* and *Archaebacteria* are able to produce these substances; most of the microorganisms produce at least one type of bacteriocin [8].

Escherichia coli and other closely related bacteria produce different bacteriocins called colicins which are active against *E. coli* and other members of the Family *Enterobacteriaceae* [10]. The production of colicins may serve as anti-competitors disabling the invasion of strains into an established microbial community. They may play a defensive role and act to prohibit the invasion of other bacterial strains or species into an occupied niche or to limit the advance of neighboring cells [4]. However, *E. coli* represents less than 0.5% of the bacterial population of human colon, whereas *Bacteroides* and *Parabacteroides* species represent about ~25% of the intestinal microbiota, despite these organisms being normally commensals in the gut microbiota, they can also be responsible for infections with morbidity and mortality [11].

It has been shown that bacteriocins produced by *Bacteroides* spp. and *Parabactreoides* spp. inhibit the RNA synthesis, but with no effect on DNA, protein, or ATP synthesis [1]. All species of the *Bacteroides* and *Parabacteroides* belonging to different origins, such as humans, animals and polluted water [5, 12] are able to produce bacteriocins.

In this study, the production of bacteriocin-like substances in *Bacteroides* and *Parabacteroides* species belonging to human intestinal microbiota was evaluated.

MATERIAL AND METHODS

Ninety eight *Bacteroides* strains (66 *B. fragilis*, 14 *B. vulgatus*, 7 *B. ovatus*, 7 *B. uniformis*, 2 *B. eggerthii*, 2 *B. thetaiotaomicron*, and 16 *Parabacteroides distasonis*) isolated from human intestinal microbiota were evaluated. Bacteria were isolated from April to December 2000, and identified by established method [13]. Strains were stored in skimmed milk at -80°C. The Ethic Committee of the Institute of Biomedical Sciences - USP, approved this study (Process number CEPSH-158). Bacteria were grown in brain heart infusion (BHI, Difco Laboratories, UK) supplemented with 0.5% yeast extract, under anaerobiosis (90% N_2, 10% CO_2) at 37ºC for 24 h.

Bacteriocin activity was determined by the double layer method [14]. Bacterial inocula were prepared in BHI by using a 0.5 McFarland scale (1.5×10^8 cfu/mL). Briefly, forty-eight-hour cultures were spotted simultaneously with a Steers' replicator onto the surface of BHI agar. After incubation (37°C 24 h), bacterial colonies were killed by exposure to chloroform for 30 min. Residual chloroform was allowed to evaporate, and dishes were overlaid with 5.0 mL of BHI soft agar (0.7%) inoculated with 0.2 mL of a 24-h BHI culture of an indicator strain. After 24 h of incubation in anaerobic conditions, the bacterial growth inhibition and presence of halos were observed and measured. Bacterial inhibition was scored positive if the zone was wider than 2 mm. Experiments were performed in duplicate. Bacteriocin activity was evaluated in all the 114 strains, and then, against themselves and five reference strains (*Bacteroides fragilis* ATCC 43858, *B. fragilis* GAI 97124, *Escherichia coli* pBR 322αH22, *E. coli* J53 pACYC 184 and *Actinomyces viscosus* ATCC 91014).

In addition, from inhibitory halos produced by bacteriocins, the presence of bacteriophages was also evaluated, placing small pieces of agar on the same susceptible bacteria.

RESULTS

Sixty-three (55.2%) out of 114 strains produced antagonistic substances. Most of *B. vulgatus*, *B. fragilis*, *B. ovatus* and *P. distasonis* produced bacteriocins (Table 1). The halo of inhibition sizes varied between 3 and 15 mm of diameter (Figure 1). A predominant hetero-antagonism (each strain against other different bacterial genus) was verified in *Bacteroides* and *Parabacteroides* species except to *B. uniformis*, but iso-antagonistic effect (each strain tested against the other ones of the same

Table 1. Antagonistic activity of species of *Bacteroides* and *Parabacteroides* isolated from intestinal microbiota.

Species (N°)	Antagonistic effect			
	Hetero		Iso	
	(N°)	(%)	(N°)	(%)
B. fragilis (66)	15	22.7	27	40.9
B. vulgatus (14)	2	14.2	7	50
B. ovatus (7)	4	57.1	0	-
B. uniformis (7)	0	-	1	14.2
B. thetaiotaomicron (2)	1	50	0	-
B. eggerthii (2)	1	50	0	-
P. distasonis (16)	5	31.2	0	-
Total (114)	28	24.5	35	30.7

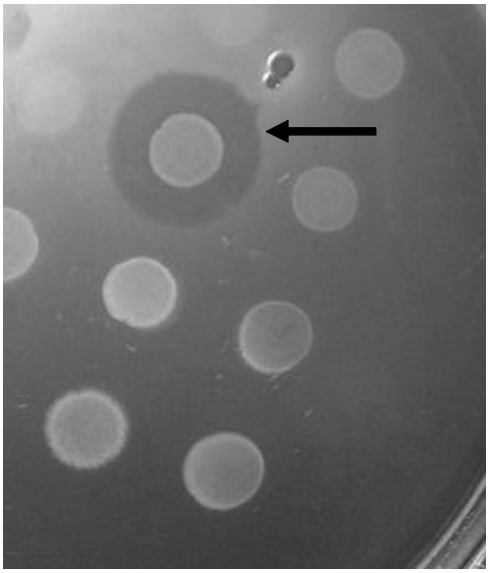

Figure 1. The arrow shows the inhibition of *B. vulgatus* produced by bacteriocin-producing *B. fragilis* against.

genus) was observed in *B. ovatus* (50%), *B. fragilis* (40.9%), and *B. uniformis* (14.2%) (Table 1). No strains produced auto-antagonism (each strain against itself). The presence of bacteriophages was not detected. Reference strains produced substances with iso-antagonism effect (*B. fragilis* GAI 97124), and hetero-antagonism effect (*B. fragilis* ATCC 43858, *E. coli* pBR 322αH22 and *E. coli* J53 pACYC 184). *A. viscosus* ATCC 91014 did not produce bacteriocin.

DISCUSSION

Peptides display an excellent broad-spectrum of antimicrobial activity, and they do not allow the development of resistance in target bacterial species. Bacteriocins are synthesized in ribosome and they are active against other microorganisms either of the same species (narrow spectrum) or of other genera (broad spectrum); however, the host' defense peptides and cell signaling mechanisms can also be involved.

Interestingly, bacteriocin-producing organisms display auto-immunity to this substance, and it is possible due a property mediated by specific immune proteins [15]. Antagonistic substances play a role in a microbial community by acting against bacterial competitors, colonization and invasion [2, 16]. On the other hand, bacteriocins produced by gram-positive bacteria appear to be involved in the bacterial quorum sensing, in bacterial consortia such as biofilms [16].

Studies in anaerobic bacteria, such as *Fusobacterium* spp., black-pigmented rods, *Bifidobacterium* spp., *Veillonella* spp. and *Bacteroides* species, have shown bacteriocin production and it has suggested a possible participation of these substances in the microbial regulation in human and animal intestinal ecosystems [5, 17, 18]. Wilhelm *et al.* [19] analyzed 84 intestinal anaerobic bacteria for their ability to produce bacteriocins against species of *Enterobacteriaceae*, gram-positive cocci, and

P. aeruginosa. Forty-nine (58%) out of 84 anaerobes inhibited the growth of at least one indicator bacterium. Species of *Bacteroides* and *Bifidobacterium* were considered the highest producers.

In this study, it was seen that a high percentage of intestinal *Bacteroides* species and *P. distasonis* (55.2%) were capable of producing bacteriocins, in accordance with Farias *et al.* [12] and Avelar *et al.* [5]. The bacterial species analyzed produced halos of inhibition between 3 and 15 mm diameter, and these halo sizes can suggest the production of more than one type of antagonistic substance, and possibly with different physicochemical and biological properties [18, 20].

In conclusion, most of the bacteriocin-producing *Bacteroides* and *Parabacteroides* species showed hetero- and iso-antagonistic effects, which could be important in the establishment and maintenance of this microbial group in the intestinal ecosystem. Besides, this unexplored bacterial characteristic may be important in this era of increasing resistance to several antibiotics.

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