Benedito Rodrigues da Silva Neto
(Organizador)

Pesquisa Científica e Tecnológica em Microbiologia

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APRESENTAÇÃO

A microbiologia é um vasto campo que inclui o estudo dos seres vivos microscópicos nos seus mais vaiados aspectos como morfologia, estrutura, fisiologia, reprodução, genética, taxonomia, interação com outros organismos e com o ambiente além de aplicações biotecnológicas. Como uma ciência básica a microbiologia utiliza células microbianas para analisar os processos fundamentais da vida, e como ciência aplicada ela é praticamente a linha de frente de avanços importantes na medicina, agricultura e na indústria.

De forma integrada e colaborativa a nossa proposta apoiada e certificada pela editora Atena é apresentar aqui a obra “Pesquisa científica e tecnológica em microbiologia” contendo trabalhos e pesquisas desenvolvidas em diversos institutos do território nacional contendo análises de processos biológicos embasados em células microbianas ou estudos científicos na fundamentação de atividades microbianas com capacidade de interferir nos processos de saúde/doença.

A microbiologia como ciência iniciou a cerca de 200 anos, entretanto os avanços na área molecular como a descoberta do DNA elevou a um novo nível os estudos desses seres microscópicos, além de abrir novas frentes de pesquisa e estudo, algumas das quais pretendemos demonstrar nesse primeiro volume da obra “Pesquisa científica e tecnológica em microbiologia”. Sabemos na atualidade que os microrganismos são encontrados em praticamente todos os lugares, e a falta de conhecimento que havia antes da invenção do microscópio hoje não é mais um problema no estudo, principalmente das enfermidades relacionadas aos agentes como bactérias, vírus, fungos e protozoários.

Acreditamos no potencial dessa obra em primeiro lugar pela qualidade dos trabalhos aqui apresentados, e em segundo pelo campo em potencial para futuras novas discussões, haja vista que enfrentamos a questão da resistência dos microrganismos à drogas, identificação de viroses emergentes, ou reemergentes, desenvolvimento de vacinas e principalmente a potencialização do desenvolvimento tecnológico no estudo e aplicações de microrganismos de interesse.

Temas ligados à pesquisa e tecnologia microbiana são, deste modo, discutidos aqui com a proposta de fundamentar o conhecimento de acadêmicos, mestres e todos aqueles que de alguma forma se interessam pela saúde em seus aspectos microbiológicos. Portanto a obra propõe uma teoria bem fundamentada nos resultados práticos obtidos em alguns campos da microbiologia, abrindo perspectivas futuras para os demais pesquisadores de outras subáreas da microbiologia.

Assim desejo a todos uma ótima leitura!

Benedito Rodrigues da Silva Neto

SUMÁRIO

CAPÍTULO 4

CARACTERIZAÇÃO DE UM PEPTÍDEO ANTAGONISTA PRODUZIDO POR *Bacteroides fragilis* ISOLADO DE PACIENTE COM INFECÇÃO INTRA-ABDOMINAL

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RESUMO: A microbiota intestinal indígena dos seres humanos é rica e diversificada. Como constituinte desta microbiota, o gênero *Bacteroides*, cujos membros são bastonetes Gram-negativos, estão associados a infecções intra-abdominais. *Bacteroides* podem sintetizar substâncias antagonistas, dando uma vantagem competitiva às amostras produtoras. O objetivo deste estudo foi avaliar a síntese de substâncias antagonistas por *Bacteroides* e *Parabacteroides* isolados de pacientes com infecções intra-abdominais. *Bacteroides fragilis* foi utilizado para extração, purificação e caracterização da substância antagonista. A atividade do extrato intracelular foi detectada com $(NH_4)_2SO_4$ nas concentrações de 30% (C30) e 50% (C50). C30 e C50 foram inativados por proteases e altas temperaturas. O extrato C50 foi submetido à cromatografia de troca iônica e as frações 1 a 4 apresentaram atividade antagônica. As frações foram aplicadas em cromatografia de gel filtração. As fracções 2 e 3 foram capazes de inibir a amostra reveladora. Estas frações foram submetidas à cromatografia de fase reversa e a fração 2C permaneceu ativa. A espectrometria de massas da fração 2C apresentou íons de 1300,00 Da. Os peptídeos e proteínas sequenciados descritos na base de dados BLASTP resultaram em 100% de identidade com uma proteína de secreção do tipo VII. A busca por similaridade no BANCO DE DADOS DE PEPTÍDEOS ANTIMICROBIANOS resultou em 42% de identidade com uma microcina. Os resultados indicam a produção de substâncias

antagonistas por *B. fragilis*. É plausível supor que eles desempenham um papel relevante nas relações interbacterianas em um ambiente complexo, como de uma infecção intra-abdominal.
PALAVRAS-CHAVE: *Bacteroides, Parabacteroides*, substância antagonista, infecção intra-abdominal.

CHARACTERIZATION OF AN ANTAGONIST PEPTIDE PRODUCED BY A *Bacteroides fragilis* ISOLATE OBTAINED FROM A PATIENT WITH INTRA-ABDOMINAL INFECTION

ABSTRACT: The indigenous intestinal microbiota of humans is rich and diverse. As a constituent of this microbiota, genus *Bacteroides*, whose members are Gram negative rods, obligate anaerobes and associated to intra-abdominal infections. *Bacteroides* can synthesize antagonistic substances, giving a competitive advantage to the producing samples. The objective of this study was to evaluate the synthesis of antagonistic substances by *Bacteroides* and *Parabacteroides* isolated from patients with intra-abdominal infections. *Bacteroides fragilis* was used for extraction, purification and characterization of the antagonistic substance. The activity of the intracellular extract was detected with $(NH_4)_2SO_4$ in concentrations of 30% (C30) and 50% (C50). C30 and C50 were inactivated by proteases and high temperatures. The C50 extract was subjected to ion exchange chromatography, and fractions 1 to 4 presented antagonistic activity. Fractions were applied in gel filtration chromatography. Fractions 2 and 3 were able to inhibit the developing sample. These fractions were submitted to reverse phase chromatography and fraction 2C, remained active. Mass spectrometry, from fraction 2C, presented ions of 1300.00 Da. The sequenced peptides and proteins described in the BLASTP database, resulted in 100% identity between two peptides. One of the sequenced peptides showed 100% identity to a type VII secretion protein. The search by similarity in the ANTIMICROBIAL PEPTIDE DATABASE resulted in 42% identity with a microcine. The results indicate the production of antagonistic substances by the *B. fragilis* strain. It is plausible to assume that they play a relevant role in interbacterial relationships in a complex environment such as intra-abdominal infection.
KEYWORDS: *Bacteroides*, *Parabacteroides*, antagonistic substance, intra-abdominal infection.

1 I INTRODUCTION

Bacteroides and *Parabacteroides* are two important genera that are part of the indigenous microflora of humans and other animals. This collection of microrganisms inhabiting the human body consists of complex microbial communities such as that present in the digestive tract, particularly in the oral cavity and intestines. As members of the gut microflora, *Bacteroides* and *Parabacteroides* offer several advantages to the

host, assisting in physiology and normal function of the gastrointestinal tract, helping in digesting complex polysaccharides, and also using nutritional resources, which aids in the intestinal colonization resistance by bacteria potentially harmful (Ishikawa *et al*., 2013). The various species that make up the microbial communities are involved in all forms of competition, whether by nutritional resources or space. To overcome their adversaries, microorganisms use all their resources, including secondary metabolites, enzymes and antibiotics. The competitions between bacteria, archaea, fungi and protozoa are often solved by the use of antimicrobial peptides (Wilson *et al*., 2015). Among the diseases associated with *Bacteroides* and *Parabacteroides*, there is intra-abdominal infection, its severity and high prevalence. After disruption of the intestinal wall, which may occur for different reasons (surgical wound, malignancies, appendicitis or trauma), the indigenous microbiota infect the peritoneal cavity normally sterile site. During the acute phase of infection (approximately 20 h), facultative anaerobes, especially *Escherichia coli* are established, promoting the fall of the redox potential of the environment. The atmosphere now low in oxygen, then allows bacteria obligate anaerobic, as representatives of *Bacteroides* and *Parabacteroides*, colonize and reproduce, passing to prevail during the chronic phase of infection (Wexler, 2016;. Nicoletti *et al*., 2008). *Bacteroides* and *Parabacteroides,* especially the *B. fragilis*, can express a wide variety of virulence skills such as synthetic enzymes, toxins secretion and production of antimicrobial substances, known as bacteriocins, which favors microrganism success in colonizing the host and aggression (Diniz *et al.,* 2000; Wexler, 2007; Papaparaskevas *et al*, 2011). With reference to antagonistic substances produced by anaerobic bacteria, in 1965, Beerens & Baron described for the first time, a bacteriocin synthesized by *Bacteroides*, but more detailed studies have not been conducted. Until 1983, only a few papers have been published, such as Booth *et al* (1977) who described a bacteriocin having a molecular mass greater than 300,000 Da, produced by *Bacteroides*, and the Mossie *et al.* (1979) have purified a bacteriocin produced by *B. fragilis* molecular weight between 13,000 and 19,000 Da. In 1981, Mossie *et al*. described the purification and characterization of another bacteriocin produced by *B. fragilis* and demonstrated in their ability to inteferir bacterial RNA synthesis by inhibiting RNA polymerase activity. In 1992, Farias *et al* reported the ability of producing bacteriocin-like substances for group *B. fragilis* isolates the oral cavity and marmosets intestine. Farias *et al.* (1994) showed the production of proteins with one or more bacteriocin-like activity for samples *B. fragilis* recovered primate species Callithrix. The extracellular extracts (S70) and intracellular (C70) showed activity against isolates of *B. fragilis* from callitrichids gut and against *Streptococcus sanguis* reference samples and Streptococcus pyogenes. The study of the cell surface molecules produced by *B. fragilis* has deserved prominence because they probably play an important role in colonization, persistence and communication with other microorganisms. To investigate the expression of antagonistic activity and characterize the substances involved in the phenomenon provide a better

understanding of microbial ecological relations, as well as the emergence of new perspectives regarding the use of bacteria producing antagonistic substances

2 I METODOLOGY

30 samples test of *Bacteroides* strains (11 *B. fragilis, 5 B. ovatus*, 1 *B. thetaiotaomicron*, 3 *B. uniformis*, 1 *B. vulgatus*, 6 *B. caccae*, 2 *B. capillosus*, 1 *B. eggherthii*) and 10 *Parabacteroides distasonis* strains isolated from patients with intra-abdominal infections (called test samples). As revealing, were used, in addition to the test samples, a panel of reference samples of bacteria with different types respiratory. Initially, the test samples were inoculated into brain heart infusion, pH 7.2, supplemented with 0.5% hemin, 0.1% menadione and 0.5% yeast extract (BHI-S). The cultivation was performed in the anaerobic chamber with 85% N2, 5% CO2 and 10% H2 at 37°C. After 48 hours, the samples were inoculated into Brain Heart Infusion Agar (BHIA) supplemented with 0.5% hemin, 0.1% menadione and 0.5% levedura extract (BHIA-S). The revealing samples were cultivated in liquid medium and then a 0.2 mL aliquot of the culture was inoculated into 3.5 ml of semisolid medium (0.7% agar). After incubation at 37ºC, the reading is performed by checking the presence of inhibition halos. To evaluate the presence of bacteriophages, fragment revealing the multiplication zone of inhibition was aseptically removed and it was macerated in saline solution pH 7.2 and then centrifuged at 23,400 g for 20 min. An aliquot of the supernatant was transferred to 3 ml of culture revealing sample. After incubation an aliquot was added to 3.5 mL of semisolid agar for revealing specific sample selected .The material was incubated and the presence of lysis zones is indicative of the presence of bacteriophages. The evaluation of the possible inhibition of fatty acids was carried out using S-BHIA with and without addition of 1% soluble starch. After the test, the zone of inhibition of proliferation of revealing sample was measured. (Apolonio *et al*., 2008). To evaluate interference of chloroform, the producer sample was inoculated in the spot on the surface of BHIA-S and, after incubation the revealing sample was inoculated around the producer sample spot, cross-shaped, without that the growth of producer sample was touched. The material was incubated and then evaluated the presence of inhibition halo. (Farias *et al*, 1992). The production H_2O_2 was investigated by incorporation of catalase 0.03% w / v in BHI-S. The presence of the revealing sample multiplication inhibition zone only in medium without addition of catalase was considered indicative that the antagonist activity is due to the presence of H_2O_2 (Hamada & Ooshima, 1975).

EXTRACTION OF INTRACELLULAR FRACTION- The cell pellet was washed twice with 0.01M Tris-HCl buffer, pH 8.0, by centrifugation at 16,200 g for 30 min. After this procedure, the pellet was sonicated at 50 W in an ice bath for 30 cycles of 40 s each, at 40 s intervals. The resulting sonicated fraction was again centrifuged

and the supernatant was collected, subjected to ammonium sulfate precipitation and dialysed to obtain the intracellular fractions, called C-30, C-50 and C-80.

CHARACTERIZATION OF ACTIVE FRACTIONS- pH- The extracts were diluted 1: 1 (v / v) in the universal buffer and the solution was then incubated at 37 ° C at 15 and 30 min, 1, 2, 3, 4, 5 and 6 h intervals , 1, 2, 3, 6, 10, 20 and 30 days. As a control, the activity of the buffer and the diluted fraction in 0.01 M Tris-HCl, pH 8.0, at the ratio of 1: 1 (v / v), incubated in the same condition as the test tubes was checked. TEMPERATURE- Aliquots of fractions were subjected to heat treatments: -86 ° C (freezer), -20 ° C (freezer), 4 ° C (Refrigerator), 25 ° C (room temperature), 37 ° C (oven), 50 ° C, 60 ° C, 80 ° C and 100 ° C (water bath) and 121 ° C (autoclave) , 1, 2, 4 and 8 h, 1, 2, 8, 20, 30 days, 6 and 12 months. As a control, the diluted extract was used without any heat treatment. PROTEOLYTIC ENZYMES- Protein K, α-chymotrypsin, trypsin, papain, and pepsin were used. The tests were performed by diluting aliquots of the active protein extracts in the enzyme solutions in a ratio of 1: 1 (v / v). The material was incubated, then, the preservation of the antagonistic activity was evaluated and the results expressed in UA / mL. As a control, the enzymatic solutions and extracts diluted 1: 1 (v / v) were used. ORGANIC SOLVENTS- The solvents used were acetone, acetonitrile, iso-propyl alcohol, butanol, ethanol, hexane and methanol 10% aqueous solutions and 50% of the organic solvents were prepared and then sterilized by filtration. The extracts were diluted 1: 1 (v / v) in these solutions and then incubated at 25 ° C for 1 h. Controls were performed using the aqueous solutions of the organic solvents and the extract diluted 1: 1 (v / v).

PURIFICATION OF ACTIVE FRACTIONS BY CHROMATOGRAPHY- Initially, fast protein liquid chromatography (FPLC) was used on Mono-Q ™ 5/50 GL Tricorn ™ column in a linear gradient of 0-100%. Fractions from ion exchange chromatography with antagonistic activity were subjected to gel filtration on a Superose 12 HR 10/30 column. The active fractions obtained from the above purification step were subjected to high performance liquid chromatography (HPLC on C8 column (4.6 mm in diameter x 25 cm in length). The eluted fractions were monitored using wavelength 280 and 220 nm, concentrated by lyophilization and diluted in sterile Milli-Q® water.

SDS-PAGE- One part of the gel was stained by silver and the other was washed to remove SDS and possible microbial contaminants and then routed to reveal in situ activity. The reading was performed by observing the presence of lines inhibiting the multiplication of the developing sample. The molecular mass of each active band was estimated by comparison with pre-stained protein standard (Farias *et al*., 1994)

TRIPTIC DIGESTION- The gel was rehydrated with trypsin solution for 40 min in an ice bath. The extraction was carried out with 5% formic acid and 50% acetonitrile. The sample was concentrated to about 10 µL and sent for analysis in mass spectrometry. As a negative control, a sample-free gel fragment and as a positive control, bovine serum albumin solution (1 mg / mL) were used, both of which were treated in parallel with the sample.

MASS SPECTROMETRY- The fractions and the material were lyophilized and dissolved in α-CHCA (α-cyano-4-hydroxycinnamic) and super DHB (2,5-dihydroxybenzoic acid) matrix, at the ratio of 1 1 (v / v). The molecular mass was determined using MALDI-TOF / TOF AutoflexTM® III. For data analysis, the Flex Analysis 2.4 program was used.

DETERMINATION OF PRIMARY SEQUENCE OF ANTAGONIST SUBSTANCE- The identity of the peptides was verified by AutoflexTM® III MALDI-TOF / TOF Homology analysis between the primary sequence obtained and those of other substances was carried out using the Basic Local Alignment Search Tool - National Center for Biotechnology Information NCBI-BLAST

3 I RESULTS

Considering *Bacteroides* samples, heterantagonism was detected in 18 (60.0%) of the 30 samples tested, five (16.7%) of which, all *B. fragilis*, also expressed isoantagonism. The occurrence of autoantagonism was not detected. It was observed heteroantagonism expression by 29 (72.5%) and isoantagonism 5 (12.5%) of the 40 samples tested. Antagonist activity was observed only against phylogenetically related bacteria. Clinical samples used as indicator, *B. fragilis*, *B.ovatus*, B. *uniformis*, B. *vulgatus*, *B.caccae and B. capillosus* showed heteroantagonistic activity to at least three of test samples. The sample *B. fragilis* D 111.4 was the one who expressed antagonism more often exhibited the broadest spectrum of activity and generated more visible.

FACTORS INTERFERENCE- BACTERIOPHAGES- lysis zones due to the presence of bacteriophages were not observed in the test performed. FATTY- The addition of starch to the growth medium did not prevent expression of antagonism, indicating that fatty acids and other acids were not responsible for antagonism. CHLOROFORM- The results of testing for antagonist activity research with or without addition of chloroform were similar, indicating that this substance is not responsible for the inhibition of revealing sample. HYDROGEN PEROXIDE- The addition of catalase to the culture medium did not inhibit the antagonism of expression, showing that the production of H2O2 is not responsible for the activity.

PROTEIN EXTRACTION - ANTAGONIST ACTIVITY TEST OF THE FRACTIONS OBTAINED- The intracellular protein fractions obtained from the sample *B. fragilis* D 111.4, precipitated with 30% $(NH_4)_2SO_4$ (C30) and 50% (C50) showed antagonistic activity against *B. caccae* D 79.3 and *B.ovatus* D 54.1. Thus, C30 and C50 were selected for subsequent steps in the study.

PURIFICATION OF C30 AND C50 FRACTIONS- The application of the extract C30 in ion exchange chromatography did not generate active fractions. With regard to the C50 extract, 50 fractions were generated, among which fractions 1 to 4, referring to a single peak and presented antagonistic activity. Fractions 1 and 4,

from ion exchange chromatography, constituted pool 1, which, applied in gel filtration chromatography, generated 26 fractions. Of these, fractions 2 and 3 were able to inhibit the sample. The chromatogram resulting from the gel filtration chromatography is shown in Figure 4. Pool 2, consisting of fractions 2 and 3 from the gel filtration chromatography, was subjected to reverse phase chromatography. Fifty fractions were generated, the fraction 2C being active against the developer sample.

SDS PAGE- Inhibition of the developmental microrganism *B. ovatus* D 54.1 was verified by all the extracts tested, in a region corresponding to the band with molecular mass <10 kDa.

MOLECULAR MASS OF ANTAGONIST SUBSTANCE- In the fraction analyzed by chromatography reverse phase, the ions of approximately 1300 Da generated a more intense signal. In the fraction analyzed by trypsin digestion, the fragments were analyzed and sequenced. Trypsin digestion allowed good fragmentation of the peptide.

ANALYSIS OF AMINO ACID SEQUENCES- The search performed by similarity in the BLASTP database showed 100% identity with a type VII secretory protein. The sequence was VTANRNQWG. The search performed by similarity between the sequenced fragments and proteins described in the Antimicrobial Peptide Database, from fragmentation obtained with trypsin digestion, resulted in 42% identity with a microcine of Streptomyces. The sequence found was GMAAFKSIFGGMSWY.

4 I DISCUSSION

The most frequently isolated anaerobic of these infectious processes is *B. fragilis*, involved in the development of the intra-abdominal abscesses that occur in the peritoneal cavity. While intra-abdominal infections are typically polymicrobial and are generally associated with the indigenous microbiota, *B. fragilis* is one of the few bacteria known to be capable of inducing abscess formation as the sole infecting organism in experimental animal models (Cao *et al*., 2014). In this study, only *Bacteroides* exhibited antagonistic activity under the experimental conditions employed. In the group, 43.33% of the samples tested showed heteroantagonism only and 16.67% expressed heteroantagonism and isoantagonism, exclusively, *B. fragilis*. A similar result was reported by Avelar *et al.* (1999) found that 57% of *B. fragilis* samples, including clinical specimens, were bacteriocin-producing. Autoantigen activity was not observed. Several reports describe that samples producing antagonistic substances synthesize an immunity protein for their protection (Coyne *et al*., 2016; Lobo *et al*., 2016). However, in a dense and diversified microbiota environment, the bacteriocin produced not only exerts its antagonistic effect, but can also trigger the production of bacteriocins by neighboring cells, “setting the competitor on the counterattack.” In this study, the researchers observed that bacteriocins induce the

expression of inhibitory substances by neighboring samples and that the potency of a bacteriocin and its induction ability go together. In a competition environment between antagonistic samples, the most "toxic" competitor will prevail (Majeed *et al.,* 2013). Protein extracts C30 and C50 were tested for characterization. Regarding the evaluation of thermotolerance, loss of antibacterial activity was observed by exposure to temperatures above 50 ° C. Similar results were reported by Farias *et al.* (1994), which also characterized a bacteriocin of *B. fragilis* that lost its activity when the extract was treated with temperatures above 60 ° C. The C30 and C50 antagonist activity was preserved at lower temperatures, such as -20 ° C and -86 ° C, for up to 12 months, the last time the test was performed. At 37 ° C and 25 ° C, its activity was maintained for 7 days.

For the purification of the antagonist substance present in the C50 intracellular protein extract, was subjected to ion exchange chromatography. Fractions 1 to 4 exhibited antagonistic activity. Subsequent to ion exchange chromatography, the active fractions were subjected to filtration chromatography. The active fractions originated (2 and 3) were located at a peak at the beginning of the Chromatogram, indicating particles with larger molecular weights. In the next purification step, the reverse phase liquid chromatography on a C8 column (HPLC) was used, and 50 fractions were collected. One of them, fraction 2C, remained active against the revealing sample. The result of the mass spectrometry experiment, from fraction 2C, presented ions of approximately 1300 Da, which generated a more intense signal. An antagonist substance with molecular mass less than 10 kDa was also detected in the in situ development experiment performed after SDS-PAGE. The search performed by similarity between one of the sequenced fragments (VTANRNQWG) and proteins described in the BLASTP database, from fragmentation obtained on reverse phase chromatography, resulted in 100% identity with a type VII secretion protein. The search performed by similarity with proteins described in the Antimicrobial Peptide Database, from fragmentation obtained with trypsin digestion (GMAAFKSIFGGMSWY), resulted in 42% identity with a Streptomyces microcine. *Bacteroides* secrete antibacterial proteins (BSAPs), which act on phylogenetically related samples. Bacteria evolved a remarkable variety of sophisticated nanomachines to export various pathogenicity factors through the bacterial cell envelope. Because bacteria use different mechanisms to compete in microbial communities and the intestinal microbiota is an extremely dense ecosystem, cell-cell contact, substances presents as a very prevalent antagonistic mechanism in the gut (Coyne *et al*., 2016). Relatively little is known about the ecology of the human intestinal microbiota and the combination of factors regulating the composition of an intestinal microbial community. The competition for bacterial interference is beginning to be studied and appreciated as an important contribution to understanding the dynamics of intestinal bacterial populations. In summary, the data demonstrate the need to continue studies on the production of antagonistic proteins by *B. fragilis*, as well as the analysis of extraction

methods that allow the optimization of the purification protocol. Future studies are desirable to allow a better understanding of the influence of these proteins on the ecological relationships that occur in the intestinal tract of humans and their potential of application in the inhibition of samples associated with the etiopathogenesis of intra-abdominal infections.

5 I CONCLUSIONS

The expression of antagonistic activity was detected only for the genus *Bacteroides*. Hetero-antagonistic activity was observed only against phylogenetically related samples and isoantagonistic activity was detected only for *B. fragilis*. No sample expressed self-starvation. The exclusion of factors that could interfere in the interpretation of phenotypic test results reinforces the hypothesis that the expressed antagonism is due to the production of proteinaceous substance (s), possibly bacteriocin (s). Intracellular extract of *B. fragilis* D111.4 sample with 30% (C30) and 50% (C50) saturated ammonium sulfate shows antagonistic activity against *B. ovatus* and *B. caccae*, respectively. C30 and C50 presented low stability when subjected to heat treatments, they were active against different pH values and several organic solvents and their protein nature was confirmed by inactivation by proteases. Data obtained by SDS-PAGE indicates that the C50-detected antagonist substance has molecular mass less than 10 kDa and therefore is a peptide. Analysis by mass spectrometry, from fraction 2C obtained from reversed phase chromatography, revealed ions of approximately 1300 Da, which generated a more intense signal. The search performed by similarity with proteins described in the BLASTP database resulted in 100% identity of a fragment (VTANRNQWG) with a type VII secretion protein. The search performed by protein similarity described in the Antimicrobial Peptide Database resulted in 42% identity of a fragment (GMAAFKSIFGGMSWY) with a microcine of *Streptomyces.*

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