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Evaluation of antimicrobial activity of the endophytic actinomycete R18(6) against multiresistant Gram-negative bacteria

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ABSTRACT

Endophytic actinomycetes are promising sources of antimicrobial substances. This study evaluates the activity of metabolites produced by the endophytic actinomycete R18(6) against Gram-negative bacteria multiresistant to antimicrobials. R18(6) isolate was grown in submerged cultures under different conditions: carbon source, temperature, pH and incubation time to optimize antimicrobials production. The actinomycete grown in base medium supplemented with 1% glucose, pH 6.5 and incubation at 30 ºC for 96 h with shaking at 100 rpm, exhibited the highest activity against the used Gram-negative bacteria. Minimum inhibitory concentration (MIC) of the crude extract produced by the microorganism varied between 1/32 and 1/256. It had bactericide or bacteriostatic activity, depending on the Gram-negative organism. The active extract was stable at high temperatures, and unstable in medium containing proteolytic enzymes. Micromorphology of R18(6) was investigated by optical and scan microscopy, revealing that it was morphologically similar to the genus *Streptomyces*.

Key words: endophytic actinomycetes, *Streptomyces*, multiresistant Gram-negative bacteria, extract.

INTRODUCTION

Actinomycetes are Gram-positive bacteria that produce over 60% of bacterial metabolites, known by the year 2000 (Sosio et al. 2000). A significant portion of these organisms' genome (between 5% and 10%) is used in the production of these metabolites (Baltz 2008), among which antimicrobials stand out. Since 1940, when the first antimicrobial agent was isolated from the genus *Streptomyces*, actinomycetes have become the main sources of active metabolites.

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These bacteria play an important role in the rhizosphere. They have considerable influence in plant growth, protecting roots from pathogenic microorganisms and environmental factors (Hasegawa et al. 2006). Some species establish a specific relationship with plant tissues, and are called endophytic actinomycetes (Crawford et al. 1993). The benefit that this relationship represents to plants may result from the production of secondary compounds by these microorganisms. These metabolites may be phytohormones, antibiotics, and siderophores, which act directly on the plant metabolism or affect pathogenic agents by antibiosis or competition (Hasegawa et al. 2006).

The economic relevance of the genus *Streptomyces* lies in the production of active metabolites. The production of secondary metabolites that have considerable biological activity places the genus in an important position, amidst other genera of actinomycetes (Bérdy 2005, Bibb 2005). For example, around 80% of the antimicrobials currently known are produced by *Streptomyces* species (Prabavathy et al. 2006).

Despite the wide variety of antimicrobials available, the indiscriminate use of these compounds promotes the emergence of resistant microorganisms. Resistance to antimicrobials is a complex problem, which depends on several factors such as the microorganisms themselves, the medicament used, and the environment (Monroe and Polk 2000).

In this scenario, the present study evaluates the potential of the endophytic actinomycete R18(6) to produce active metabolites against multiresistant Gram-negative bacteria isolated in clinical and natural environments. Additionally, growth conditions were optimized for the production of active metabolites by the bacterium, which were characterized based on stability against proteolytic enzymes and temperature variation.

MATERIALS AND METHODS

The Actinomycete

The endophytic actinomycete R18(6) analyzed, was isolated from the roots of the tomato plant, *Lycopersicon esculentum* (Oliveira et al. 2010). The sample was recovered in dishes containing casein starch agar (CSA) (1% starch, 0.12% casein, 0.2% NaCl, 0.2% KNO_3, 0.2% K_2HPO_4, 0.005% $MgSO_4$, 0.001% $FeSO_4$, 0.002% $CaCO_3$, 0.6% bacteriological agar) and incubated at 30 ºC for 10 days.

Gram-Negative Isolates

Twenty-two Gram-negative bacteria (Table I), stored in glycerol 20%, were recovered in trypticase soy agar (TSA) at 35 ºC for 24 h and used to assess the antimicrobial activity of the extract produced by R18(6).

Susceptibility Profile of Bacterial Isolates to Antimicrobials

The Gram-negative bacteria used were analyzed to develop an antibiogram using the Kirby-Bauer method (CLSI 2009). Inhibition halos were interpreted following the standard method M100-S22 (CLSI 2012). The antimicrobials used (n = 20) belonged to the classes most commonly prescribed in treatments against Gram-negative bacteria: Amoxicillin/clavulanate (AMC 10 μg), ampicillin (AMP 10 μg), aztreonam (ATM 30 μg), ceftazidime (CAZ 30 μg), cephalothin (CFL 30 μg), cefoxitin (CFO 30 μg), ciprofloxacin (CIP 5 μg), chloramphenicol (CLO 30 μg), cefpodoxime (CPD 10 μg), ceftriaxone (CRO 30 μg), cefotaxime (CTX 30 μg), ertapenem (ETP 10 μg), streptomycin (EST 10 μg), gentamicin (GEN 10 μg), imipenem (IMP 10 μg), meropenem (MER 10 μg), nitrofurantoin (NIT 300 μg), norfloxacin (NOR 10 μg), sulfamethoxazole/trimethoprim (SUT 25 μg), tetracycline (TET 30 μg). Multiresistance of Gram-negative bacteria was defined as the resistance to, at least, three different classes of antimicrobials.

Optimization of Growth Conditions

The influence of the parameters in different growth culture conditions was assessed in order to enhance the production of active metabolites by R18(6) during growth. Four carbon sources were used (starch, saccharose, glycerol, and glucose) in 1% base medium (0.03% casein, 0.2% NaCl, 0.2% K_2HPO_4, 0.005% $MgSO_4$, 0.002% $CaCO_3$, 0.2% KNO_3). After the determination of the optimal carbon source for the production by R18(6) of a crude extract that exhibited the best antimicrobial activity, the influence of incubation temperature was evaluated. Five temperatures were used (30 ºC, 35 ºC, 40 ºC, and 45 ºC). After, ideal pH was

established using pH values of 4.0, 4.5, 5.0, 6.0, 6.5, 7.0, and 8.0, pH values of the growth medium were adjusted with the buffers McIllvaine (pH 4.0 and 5.0), acetic acid and acetate (pH 4.5), potassium phosphate and sodium hydroxide (pH 6.0, 7.0, and 8.0), and sodium phosphate (pH 6.5) (Assumpção and Morita 1968). Incubation times for the production of active R18(6) metabolite extract were also assessed every 24 h during the 10-day incubation period.

Production of the Crude Extract in Submerged Culture

Initially, a pre-inoculum was prepared transferring a loopful of R18(6), previously grown in a CSA dish to two 250-mL flasks containing 50 mL base medium supplemented with 1% glucose. Contents were then incubated at 30 ºC for 48 h with constant shaking (100 rpm). Next, three mL of this pre-inoculum were transferred to new flasks containing the same culture medium. The growth conditions for R18(6) were the same as those used to prepare the pre-inoculum. During the 10-day incubation period, a 100-µL aliquot was retrieved every 24 h, centrifuged (10 min; 13,000 rpm), and used in the antibiosis assay by the agar well diffusion method.

Antibiosis Assay by the Agar Well Diffusion Method

The antibiosis assay was adapted from Devillers et al. (1989). A bacterial suspension was adjusted to 0.5 McFarland standard (10^8 CFU/mL) and inoculated into Petri dish containing Mueller-Hinton agar. After seeding, wells were made on agar, using cylinders measuring 9 mm in diameter at identical distances across one another, and filled with 100 µL of the extract previously centrifuged for 10 min at 13,000 rpm. Dishes were incubated at 4 ºC for 16 h, for the extract to diffuse in the culture medium. Subsequently, incubation at 35 ºC for 24 h ensued, for bacterial growth. Antimicrobial activity was measured based on inhibition halos. All assays were carried out in duplicate. The results were analyzed using the analysis of variance (ANOVA) and the Tukey test for comparison of multiple means at 95% significance.

Growth Curve of R18(6)

A growth curve was constructed to evaluate growth time of R18(6) and correlate it with the production phase of the active metabolite extract. R18(6) was grown in submerged culture for ten days under the conditions described above as optimal for the production of active metabolite extract against Gram-negative isolates. The growth medium in one flask was filtered through a 0.45-µm porosity cellulose membrane, every 24 h during the 10-day growth period. Dry weight of cell mass was established on the difference between the final weight of the membrane, after filtration, and the initial weight.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) was determined using the micro-dilution method in broth, according to the standard method (M7-A7 (CSLI 2006). Incubation took place at 35 ºC for 24 h and 48 h. Seventeen Gram-negative isolates (Table I) were used. MIC was defined as the lowest concentration of the R18(6) crude extract able to inhibit bacterial growth.

Effect of Different Treatments on the Activity of R18(6) Extract

The active metabolites present in the R18(6) extract were partially characterized based on the stability of proteolytic enzymes and different incubation temperatures. The stability against enzymes was evaluated treating the crude extract with 2% (final concentration) of trypsin (25 mg/mL), papain (100 mg/mL), proteinase K (50 mg/mL), lysozyme (50 mg/mL), and pepsin (100 mg/mL) solutions. All reactions were carried out in microtubes. Incubation conditions were 37 ºC for 1 h.

TABLE I
Gram-negative bacteria used in this study.

Environmental isolates[1]	Clinical isolates[2]
Citrobacter koseri 3CC04*	*Citrobacter freundii* 17*
Citrobacter koseri 3EC04	*Enterobacter cloacae* 49*
Citrobacter freundii 1CC09*	*Enterobacter cloacae* 62*
Pantoea agglomerans 3AE03	*Escherichia coli* 70*
Enterobacter intermedius 1CC07	*Klebsiella oxytoca* 90*
Escherichia coli 1DE14*	*Klebsiella pneumonia* 15*
Escherichia coli 4EC05	*Klebsiella pneumoniae* 222*
Klebsiella pneumonia 1CE02*	*Morganella morganii* 162*
Klebsiella oxytoca 2BS08	*Proteus mirabilis* 102*
Proteus mirabilis 3DC01*	*Pseudomonas aeruginosa* 54*
	Pseudomonas aeruginosa 59*
	Pseudomonas aeruginosa 230*

[1]Samples isolated from the waters of Diluvio stream, Porto Alegre, RS, Brazil (Oliveira et al. 2012). [2]Samples provided by Dr. Ana Lucia Souza Antunes, Laboratory of Analysis, Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, RS, Brazil.
*Isolates used in the MIC assay.

Thermostability was analyzed at 30 ºC, 40 ºC, 50 ºC, 60 ºC, 70 ºC, 80 ºC, 90 ºC, and 100 ºC in a water bath. Incubation times chosen for the assays at the temperatures ranging from 30 ºC to 90 ºC were 15 min and 30 min. Stability at 100 ºC was evaluated for 3 min, 5 min, 10 min, 15 min, and 30 min. Antimicrobial activity of the active metabolites after treatment was evaluated using the well diffusion method against the bacterium *Klebsiella pneumonia* 222. The crude extract was the positive control. The tests were conducted in duplicate, the inhibition halos were measured and the residual activity (RA) of metabolites was calculated using the equation adapted from Oliveira (2004):

$$RA\ (\%) = (H_T - 9)/(H_C - 9) \times 100$$

Where,
H_T = Mean inhibition halo of crude extract after treatment (mm)
H_C = Mean inhibition halo of the control (mm)
9 = diameter of the well (mm)

Morphological Characterization of R18(6)

The micro-morphology of R18(6) was characterized by microscopy. The attributes of the aerial mycelium and hyphae were observed in an optical microscope. The morphology of hyphae and the surface of spores was assessed by scanning electron microscopy (SEM) (Williams et al. 1989). Microcultive was carried out in CSA for ten days and four days, for optical microscopy and SEM analysis, respectively, at 30 ºC.

RESULTS

Of the Gram-negative isolates used, 64% were multiresistant to the antimicrobials used in the assay (Table SI – Supplementary Material). Based on these findings, one multiresistant isolate from each species was chosen for the assays to determine optimal growth conditions for the production of active metabolites by R18(6) (Table SI).

The extract produced with the growth of R18(6) in the presence of glucose as a carbon source was active against 89% of the Gram-negative bacteria, among them the exception was *E. intermedius*1CC07. In turn, the extract obtained with glycerol was active against four isolates: *E. intermedius* 1CC07, *K. oxytoca* 90, *C. freundii* 17, and *K. pneumoniae* 222 with inhibition halos of

15 mm, 16 mm, 18 mm, and 16 mm, respectively. The use of saccharose as a carbon source produced metabolites that were effective against *C. freundii* 17 and *K. pneumoniae* 222, with inhibition halos of 13 mm and 17 mm. The active metabolite extract produced using starch was active against one isolate only: *K. oxytoca* 90 (15 mm).

The metabolite extract produced at 30 ºC was active against 8 of the 9 Gram-negative bacteria (89%) with significant differences from the other isolates when grown at 35 ºC (Table II). At 40 ºC and 45 ºC, no cell mass growth was observed, and extracts did not exhibit antimicrobial activity. The extract produced at 25 ºC was active only against *E. cloacae* 62, with inhibition halo of 12.5 mm.

TABLE II
Results of the optimization of the production of active R18(6) metabolites using glucose as carbon source (incubation temperatures of 30 ºC and 35 ºC).

Gram-negative bacteria	Mean inhibition halos (mm)	
	30 ºC	35 ºC
E. intermedius 1CC07	16.0	13.0
C. koseri 3CC04	17.0	12.0
K. oxytoca 90	17.5	13.0
E. coli 70	16.0	0.0
E. cloacae 62	16.0	0.0
M. morganii 162	14.5	0.0
P. aeruginosa 54	0.0	0.0
C. freundii 17	17.3	0.0
K. pneumoniae 222	19.2	14.0

The medium buffered at pH 6.5 was used in the other assays, since the metabolite extract produced under this condition was active against 100% of Gram-negative bacteria, compared with pH values of 6.0, 7.0, and 8.0 (data not shown). In turn, R18(6) did not grow at pH 4.0, 4.5, and 5.0.

The metabolic activity of the extracts, was incubated for 72 h, 96 h, 120 h, and 168 h and did not vary significantly when the Tukey test was applied (Table III). However, when cells were grown for 96 h, the extract was active against the highest number of Gram-negative isolates. The Gram-negative bacteria were not inhibited when the actinomycete was incubated for 24 h, 48 h, 144 h, and 192 h, though cell mass increased. Incubation times of 216 h produced metabolites that were active against *C. freundii* 17 and after 240 h of growth, the metabolites were active against three isolates: *E. intermedius* 1CC07, *P. aeruginosa* 54, *K. pneumoniae* 222.

The growth curve showed that the R18(6) reached steady-state growth with incubation time of 72 h and longer.

In the MIC assay, the metabolite extract concentration was 1/32, 1/64, and 1/128 after 24 h of incubation for *K. pneumoniae* 15, *C. freundii* 17, and *E. cloacae* 49, respectively. When incubated for 48 h, *E. cloacae* 49 grew in all dilutions used, while isolates *C. freundii* 17 and *K. pneumoniae*15 did not grow at the 1/32 dilution. The isolates *K. oxytoca*, *C. koseri*, *E. coli*, *P. mirabilis*, and *P. aeruginosa* grew in all dilutions of R18(6) metabolite extract (Table IV).

The enzyme activity assay showed that R18(6) metabolite extract retained 94%, 82%, and 77% of the antimicrobial activity after treatment with trypsin, papain and lysozyme, and proteinaseK, respectively. The statistical analysis revealed that treatments with the enzymes differed significantly. Treatment with pepsin completely inhibited antimicrobial activity of R18(6) metabolite extract.

The residual activity of the extract treated at the temperature range 30 ºC – 90 ºC varied between 47% and 88%. After an incubation at 80 ºC for 15 min, activity decreased. At 90ºC for 30 min, residual activity dropped to 47%. When treated at 100ºC for 3 min, the extract retained 82% of activity, which dropped to 0% after 30 min of incubation.

Optical microscopy and SEM of the micro-growth of R18(6) showed the presence of aerial mycelia and spiral terminal chains of spores with septate hyphae and smooth spores. These characteristics may indicate that the microorganism may be from the *Streptomyces* genus.

TABLE III
Results of the optimization of the production of active R18(6) metabolites using 1% glucose as carbon source and different incubation times.

Gram-negative bacteria		Incubation time / mean inhibition halos (mm)			
		72 h[a,c]	96 h[a]	120 h[a,b]	168 h[a,c]
E. intermedius	1CC07	0.0	0.0	0.0	12.8
C. koseri	3CC04	12.3	13.5	0.0	14.5
K. oxytoca	90	12.8	14.0	13.3	0.0
E. coli	70	15.5	15.3	0.0	13.5
E. cloacae	62	12.3	14.5	13.3	13.5
M. morganii	162	13.8	13.5	0.0	0.0
P. aeruginosa	54	0.0	13.8	12.0	17.0
C. freundii	17	0.0	17.5	13.5	0.0
K. pneumoniae	222	15.8	21.0	15.0	16.5

Identical letters show no statistically significant differences between treatments (Tukey test, $p>0.05$).

TABLE IV
Results of the minimum inhibitory concentration (MIC) of the crude extract produced by the endophytic actinomycete R18(6) against environmental and clinical samples after 24 h of incubation.

Gram-negative bacteria	Metabolite extract concentration										
	Extract	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024
Environmental Samples											
K. pneumoniae 1CE02	+	+	+	+	+	+	+	+	+	+	+
C. koseri 3CC04	+	+	+	+	+	+	+	+	+	+	+
E.coli 1DE14	+	+	+	+	+	+	+	+	+	+	+
C. freundii 1CC09	+	+	+	+	+	+	+	+	+	+	+
P. mirabilis 3DC01	+	+	+	+	+	+	+	+	+	+	+
Clinical Samples											
K. pneumoniae 15	-	-	-	-	-	-	+	+	+	+	+
K. pneumoniae 222	+	+	+	+	+	+	+	+	+	+	+
K. oxytoca 90	+	+	+	+	+	+	+	+	+	+	+
E.coli 70	+	+	+	+	+	+	+	+	+	+	+
E. cloacae 62	+	+	+	+	+	+	+	+	+	+	+
E. cloacae 49	-	-	-	-	-	-	-	-	+	+	+
C. freundii 17	-	-	-	-	-	-	-	+	+	+	+
P. mirabilis 102	+	+	+	+	+	+	+	+	+	+	+
M. morganii 162	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa 54	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa 59	+	+	+	+	+	+	+	+	+	+	+
P. aeruginosa 230	+										

+ : positive cell growth, - : negative cell growth.

DISCUSSION

The susceptibility profiles of Gram-negative bacteria showed that 64% of isolates were resistant to, at least, three different classes of antimicrobials. The multiresistance presented by these isolates may be associated with resistance mechanisms such as: enzyme inactivation and efflux pumps, among others (Sundsfjord et al. 2004, Weber and Piddock 2003). Gram-negative bacteria, mainly

Escherichia coli, *Klebsiella* sp., *Enterobacter* sp., and *Pseudomonas* sp., are often reported to be multiresistant microorganisms (Pagès et al. 2008).

The extract produced by R18(6) during the growth in a medium containing glucose as a carbon source stood out, compared to other carbon sources used. This was probably due to the antibiotic activity against the largest number of Gram-negative bacteria. These results show that the synthesis of active compounds by microorganisms is not steady and can be adjusted by modifying the culture conditions (Al-Zahrani 2007). Previous studies analyzed the influence of different carbon sources, and discovered that a glucose medium favors the production of active metabolites (Gupte and Kulkarni 2002, Sujatha et al. 2005, Al-Zahrani 2007).

Confirming the findings in the literature (Elleuch et al. 2010, Sujatha et al. 2005, Selvin et al. 2009), 30 ºC was the ideal temperature to cultivate R18(6) and to optimize the production of active metabolites since the extract produced, exhibited the highest antimicrobial activity against Gram-negative bacteria.

pH is one of the most important parameters of culture media, as it directly influences activity of several enzymes that plays an essential role in the metabolism of microorganisms (Guimarães et al. 2004). This relationship was observed in the present study, since the extract produced at pH 6.5 was active against 100% of Gram-negative bacteria.

Incubation time of 96 h of R18(6) afforded the production of the extract with the highest antibiotic activity. Banga et al. (2008) and Sujatha et al. (2005) analyzed the production of active metabolites by isolates of *Streptomyces* sp., observing that the maximum amount of antibiotics was produced by cultures grown for 96 h.

The growth curve of R18(6) revealed that steady-state growth started with 72 h of incubation. The fact that the 96 h incubation time was considered the best is related to the fact that the metabolite was active against the highest number of Gram-negative bacteria. This result allows suggesting that the active metabolite is a product of secondary metabolism. According to Bibb (2005), the limited nutrient availability during steady-state growth promotes the formation of secondary metabolites that are required for microorganism survival.

The analysis of the MIC showed that the extract dilutions 1/128 and 1/256 exerted bacteriostatic activity against *E. cloacae* 49. The same finding was observed for isolates *C. freundii* 17 and *K. pneumonia* 15, challenged with the 1/32 and 1/64 extract dilutions. The comparison between MICs of isolates of the same species revealed differences in inhibition power of the R18(6) extract. This may be due to the differences in susceptibility profiles and resistance mechanisms. The isolates *K. oxytoca*, *C. koseri*, *E. coli*, *P. mirabilis*, and *P. aeruginosa* were not inhibited in this assay, indicating that the extract may have lost its inhibitory power, due to dilution, or because it was not purified.

The extract exhibited residual activity after treatment with trypsin, papain, lysozyme, and proteinase K, but not with pepsin. These enzymes are endopeptidases that cleave peptide bonds that are distant from terminal carboxyl and amine groups (Bender and Kézdy 1965, Chipman and Sharon 1969, Ebeling et al. 1974). Pepsin is a proteinase responsible for the cleaveage of peptide bonds formed by the aromatic amino acids tyrosine and phenylalanine (Fruton et al. 1961, Knowles 1970). The evaluation of stability against proteolytic enzymes showed that the active metabolite has peptide bonds that, when hydrolyzed, lead to the loss of activity. Pei et al. (2013) evaluated the stability of the bacteriocine produced by *Lactobacillus paracasei* CICC 20241 against several enzymes (proteinase K, pepsin, papain, trypsin, and chimiotrypsin). The authors observed that activity of the metabolite was thoroughly inhibited by the

five enzymes, showing that a peptidic portion is responsible for the activity.

The residual activity of R18(6) extract after incubation at 80ºC for 15 min was 88%. This result suggests that the extract may be exposed to temperatures of up to 80 ºC, without losing much of its activity. In turn, the activity of the extract heated to 100 ºC started decrease with 3 min of incubation, dropping progressively until complete inactivation, with 30 min of incubation. This result indicates that the chemical structure of active compounds present in the crude extract does not tolerate higher temperatures for long periods of time. Thermal stability of protein molecules may be associated with intrinsic factors concerning their primary and secondary structures such as for example, the presence of disulfide bonds, for instance, which favor the stabilization of the molecule (Gomes et al. 2007, Pei et al. 2013).

The micromorphology of R18(6) was characterized. It indicated that the actinomycete belongs to the genus *Streptomyces,* since it presents an aerial mycelium with a spiral terminal spore chains, apart from septate hyphae and smooth spores, which are typical of the genus (Williams et al. 1989).

The findings reported here show that the endophytic actinomycete R18(6) is a potential candidate for the production of active metabolites against multiresistant Gram-negative bacteria. However, more studies are necessary, with regard to the purification and characterization of the components of R18(6) extract that exhibit antibiotic activity.

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RESUMO

Os actinomicetos endofíticos são promissoras fontes de compostos antimicrobianos. Este estudo avaliou a atividade dos metabólitos produzidos pelo actinomiceto endofítico R18(6) contra bactérias Gram-negativas multirresistentes a antimicrobianos. O isolado R18(6) foi cultivado em culturas submersas sob diferentes condições: fonte de carbono, temperatura, pH e tempo de incubação para otimização da produção do composto. O actinomiceto cultivado em meio base suplementado com 1% de glicose, pH 6.5 e incubação a 30 ºC sob agitação constante de 100 rpm durante 96 h, mostrou a melhor atividade contra as bactérias Gram-negativas testadas. A concentração inibitória mínima produzida pelo microorganismo variou entre 1/32 e 1/256. Revelou atividade bactericida ou bacteriostática de acordo com o isolado Gram-negativo. O extrato ativo mostrou-se estável em altas temperaturas e instável em meio contendo enzimas proteolíticas. A micromorfologia R18(6) foi avaliada, sob microscopia óptica e de varredura revelando que era morfologicamente similar ao gênero *Streptomyces*.

Palavras-chave: actinomicetos endofíticos, *Streptomyces*, bactérias Gram-negativas multirresistentes, extrato.

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SUPPLEMENTARY MATERIAL

TABLE SI - Susceptibility profiles of Gram-negative bacteria isolated from environmental and clinical samples challenged with the antimicrobials chosen for the assay. Dishes were incubated at 35 ºC for 24 h.