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ARTICLE

Extracellular extract of *Bacillus* sp. halotolerant bacterium is cytotoxic to 786.0 human renal adenocarcinoma cell line

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ABSTRACT: (Extracellular extract of *Bacillus* sp. halotolerant bacterium is cytotoxic to 786.0 human renal adenocarcinoma cell line). Halotolerant microorganims are considered as an encouraging source to obtain potentially promising bioproducts in several industrial sectors. The potential for biotechnological applications presented by these microorganims highlights the importance of research aiming to find biologically active biomolecules. In this context, this study aimed to analyze the *in vitro* cytotoxicity of the extracellular extract from *Bacillus* sp. halotolerant bacterium in 786.0 human adenocarcinoma cell line. The bacterium was isolated from a saline habitat in Brazil (05º 03.947'S; 37º 16.318'W) using BHI media at 37ºC, biochemical and morphological aspects were verified and the extracellular extract was obtained by immersion of the culture medium in the solvent ethyl acetate after a 24h incubation period. The cytotoxicity was verified by MTT assay. Cells were cultured for 24h ($5x10^3$ and $1x10^4$ cells/well) in different extract concentrations. Biochemical and morphological analyses classified the species as halotolerant, supporting up to 13% of NaCl. Two extract concentrations, 75 and 100 µg/mL, showed significant cytotoxic effects against 786.0 cell line.
Key words: anticancer, bioproduct**,** cytotoxicity, microorganism.

RESUMO: (Extrato extracelular de bactéria halotolerante Bacillus sp. é citotóxica para linhagem celular 786.0 de adenocarcinoma renal humano). Micro-organismos halotolerantes são considerados como uma fonte promissora para a obtenção de bioprodutos potencialmente promissores em diversos setores industriais. O potencial de aplicações biotecnológicas referente a esses micro-organismos evidencia a importância de pesquisas destinadas à busca de biomoléculas biologicamente ativas. Nesse contexto, esse estudo teve como objetivo analisar in vitro a citotoxicidade de extrato extracelular de bactéria halotolerante Bacillus sp. em linhagem celular de adenocarcinoma humano 786.0. A bactéria foi isolada de habitat salino no Brasil (05º 03,947'S; 37º 16,318'O) usando meios BHI a 37 ºC, aspectos bioquímicos e morfológicos foram verificados e o extrato extracelular foi obtido por imersão do meio de cultura no solvente acetato de etila após um período de 24h de incubação. A citotoxicidade foi verificada pelo ensaio de MTT. Células foram cultivadas por 24h ($5x10^3$ e $1x10^4$ células/poço) em diferentes concentrações do extrato. Análises bioquímicas e morfológicas classificaram a espécie como halotolerante, suportando até 13% de NaCl. Duas concentrações do extrato, 75 e 100 µg/mL, mostraram efeitos citotóxicos significativos frente à linhagem celular 786.0.
Palavras-chave: Anticâncer, bioproduto**,** citotoxidade, micro-organismo.

INTRODUCTION

The halotolerant microorganisms are those that can grow in the presence or absence of high concentrations of salt. Many halophiles and halotolerant organisms can grow in a wide range of NaCl concentration, with requirement or tolerance to some salts, depending on the nutritional factors and the environment (Dassarma & Arora 2002). These microorganisms are classified according to the ability to grow in NaCl concentrations ranges, and can then be classified as moderate halotolerant microorganisms (0-15% NaCl) and extreme halotolerant (0-30% NaCl) (Barbosa 2005).

The halotolerant microorganisms are considered as a potential source of secondary metabolites of interest in several industrial sectors, such as food, pharmaceutical, cosmetic and chemical (Soria 2004). The major secondary metabolites are produced because of some external factor such as infection, nutritional change, competition and predation and may exert beneficial or toxics effects on the human body and other animals (Silva *et al.* 2014, Chin *et al.* 2006, Pessoa *et al.* 2006). These metabolites of pharmaceutical interest are widely isolated from terrestrial microorganisms and have provided important contributions to the development of antimicrobial agents

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(penicillin, cephalosporins, aminoglycosides, tetracycline, polyketides, streptomycin, neomycin and polyene candicine), immunosuppressants (cyclosporine and rapamycin), reducing cholesterol (lovastatin and mevastatin), anthelmintic (ivermectin), anti-diabetic (acarbose) and antitumor agents (pentostatin, peplomycin, epirubicin and actinomycin D) (Newman *et al.* 2003, Butler 2005, Sneader 2005, Demain 2006, Thomas *et al.* 2011).

Some of the applications of bioproducts isolated from this type of microorganism that can be highlighted are the bacteriorhodopsins because of their photochromic properties. They are used in the elaboration of optical materials, holography and special light modulators. The organic solutes, which have the function of stabilizing biomolecules and cells, act as salts antagonists and stress protectors; the biopolymers, such as the exopolysaccharides, liposomes, polyhydroxyalkanoates; the enzymes, here we mention hydrolases and isomerases; the halocines that protect against myocardial infarction; the carotenoids (β-carotene); and the long chain polyunsaturated fatty acids, used as supplements in the human diet (Soria 2004).

Therefore, the research field of natural products produced by microorganisms must be expanded significantly as it increased discovery of new microorganisms and their bioproducts (Newman & Cragg 2012). In this context, this pioneering study on the biotechnological potential of soil microbiota of saline from São Camilo (Mossoró, Rio Grande do Norte, Brazil), aimed to analyze *in vitro* cytotoxic activity of bacterium Bacillus sp. extracellular extract on human renal adenocarcinoma (786.0) and human embryonic kidney (HEK-293) cell.

MATERIAL AND METHODS

Bacteria collection and isolation

It was collected 100 g of saline soil of São Camilo (05°03.947' S; 37°16.318' W), Mossoró, Rio Grande do Norte, Brazil, in sterile plastic bags and adopting aseptic techniques. For isolation, the samples were suspended in flask with 90 mL of enrichment solution (2% $Na_4P_2O_7$) and stirred in a shaker for 30 minutes, followed by equal period to decant. Subsequently, 10 mL solution was resuspended in 90 mL dilution solution (2.25 g NaCl, 0.1 g KCl, 0.04 g $CaCl_2$; 0.05 g $NaHCO_2$, to distilled water 1000 mL), and remained for 10 minutes to decant.

Next the dilution solution was submitted to a serial dilution from 10^{-1} to 10^{-5}. Aliquots of 1 mL were seeded in Petri dishes containing PDA medium (potato dextrose agar) with different concentrations of NaCl (5%, 15% and 30%) and incubated at 37 °C for 24 h until the appearance of small colonies. Streaks in test tubes containing BHI (Brain Heart Infusion - HIMEDIA™) were performed to ensure the purity of the colonies, which was confirmed by Gram staining and light microscopy analysis.

Preservation of bacterial strain

To ensure genetic stability of bacterial strain isolated, two preservation methods were used: the preservation method in distilled water and inclined tube. To preserve in distilled water, 1 mL aliquots of distilled water were placed into microtubes accurately identified and sterilized at 121 °C for 15 minutes. Then three disks (8 mm diameter) of media containing bacterial colonies were transferred to microcentrifuge tubes with the aid of sterile straw. For the preservation method in inclined tubes, BHI medium tube was streaked with inoculating loop. Samples from both methods were stored at 4 °C.

Morphological and biochemical study

Subsequently to bacteria isolation it was performed macroscopic and microscopic description of the colonies. For the macroscopic description, it was observed, in Petri dishes containing BHI agar, colonies of bacteria with characteristics such as pigmentation and appearance. The microscopic characterization was performed with the aid of an optical microscope (Leica DMLS) in smear slides containing bacterial sample stained by Gram method. Images of slides were also taken using the camera Samsung PL120 Zoom Lens.

Biochemical tests were performed according to Mc Faddin (2003) methodology with some modification. Briefly, it was added to the support medium 5% (w/v) NaCl for each test. Then, DNAse, catalase, urease, idol, gelatin, esculin, nitrate, citrate tests were performed. It was also observed the growth of the colonies on different substrates (maltose, galactose, glucose, sucrose and lactose), in different concentrations of NaCl (5% to 30%) at different pH values (5, 7, 9) and temperature (4, 28, 37 and 45 ºC). All tests were conducted using test tubes and petri dishes under the conditions established by the methodology (adapted from Deshmukh 2003)

Bacteria scale growth and extracellular extract

Bacterial colonies grown in liquid BHI medium and stored in the refrigerator were reactivated in test tubes containing 10 mL BHI broth and then incubated at 37 ºC for 24 hours. To obtain the extracellular extract it was used the methodology described by Thomas *et al.* (2011) with some modifications.

Thereafter the turbid test tubes, containing bacterial colonies, were stirred manually and an inoculum of 500 µL was suspended in 2 erlemayer flasks of 125 mL each containing 50 mL nutrient broth (beef extract 3%, 5% peptone, NaCl 5%), and then incubated at 37 °C for 24 hours in orbital Shaker at 150 rpm. After this period, the nutrient broth containing the bacterial colonies was centrifuged at 2000 rpm for 15 min, the precipitate discarded and the supernatant suspended in organic solvent ethyl acetate (EtOAc) 1:1 (v/v).

The final solution was then evaporated with vacuum (BT 351 Rotary Evaporator, BIOTHEC) and lyophilized (Freeze dryer LS 3000, Terroni). The lyophilized extract was then diluted in distilled water containing 0.1% dimethyl sulfoxide (DMSO) and filtered in 0.22 µm syringe filters (K18-230 Kasvi). At this point, the extract was reserved for cytotoxicity bioassay.

Cell lines

In this work, two cell lines were used, the 786.0 (human renal adenocarcinoma) and HEK-293 (human embryonic kidney cells), kindly donated from the Laboratory of Natural Polymers, Universidade Federal do Rio Grande do Norte (UFRN). The lines 786.0 and HEK-293 were maintained in T25 culture flasks (Corning®) with RPMI-1640 (Roswell Park Memorial Institute) and DMEM culture (Dulbecco's Modified Eagle Medium), supplemented with 10% FBS (Fetal Bovine Serum) and 1% antibiotics (100 IU peniciline/100 µg/mL streptomycin) and incubated at 37 °C with an atmosphere of 5% CO_2. Cell monolayers were disrupted with trypsin-EDTA for passing cells. Every day cell growth was monitored in an inverted microscope.

MTT Assay

For the cytotoxicity bioassay, the Mosmann (1983) method was used with some modifications. The cell line 786.0 was cultured in 96-well, 100 µL/well (5 x 10^3) containing RPMI with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, incubated in a CO_2 incubator (LABOVEN™) with 5% CO_2 for 12 hours at 37 °C for cell adhesion. Subsequently, the medium was removed and replaced by the medium without supplementation, then the plates were incubated for additional 24 hours.

Plate wells were set as negative control contained only the culture medium and the same concentration of DMSO in the extract, and other wells set as treatment contained extracellular extract at concentrations of 10, 25, 50, 75 and 100 µg/mL. After the period of 24 h of incubation, the medium was removed and 100 µL serum-free medium containing 0.5 mg/mL MTT (3 - (4,5-24 hours dimethylthiazol-2-iol) -2 diphenyltetrazolium bromide, Anresco™) was added to each well and incubated for 4 hours. Then, the solution was removed and 100 µL of ethanol was added to the wells to solubilize the formazan.

The plates were shaken for 10 minutes and the absorbance was recorded in a spectrophotometer (Epoch BIOTEK™) at 570 nm. To verify cytotoxicity on the cell line of human embryonic kidney HEK-293 the same methodology described above was used. DMEM was used instead of RPMI-1640 and cell concentration of $1x10^5$. The bioassay was performed in triplicate.

Statistical Analysis

Data were analyzed by one-way ANOVA followed by Tukey test (GraphPad Prism 5.0 program®). The level of significance was $p < 0.05$.

RESULTS

Isolation, morphology and biochemistry

It was isolated a species belonging to the genus *Bacillus* sp. from the saline habitats, classified as moderate halotolerant bacteria because of its development from 0% to 13% salt concentration NaCl (w/v).

This classification is given by the distribution needs of salt concentration and the ability of the microorganism to grow in the salt concentration ranges. The need and/or tolerance of bacteria to the NaCl concentration for its development is what guides the classification.

Table 1. Morphological and biochemical characteristics of halotolerant bacterial isolate.

Growth pH 5	+	Catalase	+
Growth pH 7	+	DNAse	-
Growth pH 9	-	Lactose	-
Growth T ≤ 4ºC	-	Maltose	o
Growth T 28º C	+	Glucose	-
Growth T 37ºC*	+	Galactose	-
Growth T 45ºC	+	Sacarose	o/f
Idol	-	Oxidase	-
Motility	+	Gelatin	-
Pigmentation	Cream	Esculin	+
Gram	+	Nitrate	-
[NaCl] 0 to 13%	+	Citrate	+
[NaCl] 14 to 30%	-	Urea	-
Habitat	Saline soil	Methyl red	+
Aspect	Gelatinous	VP	-
Shape	Short bacillus		

Abbreviations: +, positive to the test; -, negative to the test; o, oxidized; o/f, oxidative/fermentation; VP, Voges-Proskauer; T, temperature;*, optimum temperature range for the growth of isolated bacteria.

The species was characterized by presenting homogeneous cream pigmentation, a gelatinous appearance with rounded edges. The shape of the colonies, which was verified by the method of Gram staining showed that the bacterium is short bacillus and classified as Gram +.

Concerning the growth, the bacteria showed a development in a temperature range from 28 °C to 45 °C, showing an extreme resistance to high temperatures. However, at low temperatures of around 4 °C, there was no colony formation. Tests showed that the optimum growth temperature was around 37 °C.

The bacteria grew in pH 5 and 7, there is no development in the pH 9. Biochemical tests validated for the target species of bacteria showed the bacterial ability to grow on substrates such as maltose and sucrose, and no preferential growth in the exclusive presence of glucose. The specimen has morphological and biochemical characteristics, which helped to categorize the genus *Bacillus* sp. (Table 1).

MTT bioassay

The extracellular extract of halotolerant species of bacteria belonging to the genus *Bacillus* sp. obtained by incorporation with the solvent EtOAc showed cytotoxic effect (measured by MTT bioassay) against human renal carcinoma line (786.0) at concentrations of 75 (61%; p=0.001) and 100 µg/mL (59%; p=0.001), after 24 hours of treatment (Fig. 1).

Tests with the extracellular extract of halotolerant bacteria on kidney HEK-293 cell line showed the same nephrotoxic effect when analysed by MTT *in vitro* bioassay

for a period of 24 hours of treatment. The concentrations of 50 µg/mL (59%; p=0.001), 75 µg/mL (57%; p=0.001) and 100 µg/mL (52%;p=0.001) were responsible for this effect (Fig. 2).

DISCUSSION

The interest in the diversity of microorganisms that need hypersaline media to survive is growing thanks to studies of the adaptation mechanisms to conditions of high salt concentration. As a result of the natural conditions and those promoted by man, the extremophile environments (hypersaline, in this case) are increasing, which provide bioprospection of halotolerant microorganisms (González-Hernándes & Peña 2002).

In this study, one strain halotolerant bacteria belonging to the genus *Bacillus* sp was isolated from saline land. It presents specific characteristcs (Table 1, Table 2) like high adaptive capacity and osmoadaptation. These are vital parameters for this type of microorganisms (Barbosa 2005) because of its lack of growth in the presence of glucose as substrate. Another possible explanation for this growth deficiency is the well-known fact that the substrate used, depending on its concentration, can induce inhibition of the microorganisms development (Hiss 2001).

It is noteworthy that these microorganisms present two main mechanisms that allow survival against osmotic stress brought about by high salt concentrations. The first mechanism called salt-in, cells maintains a high concentration of intracellular solute, equivalent to external concentration, which require intracellular proteins adaptations. The second strategy is called compatible-solute, in which the cells maintain low concentrations of salts in the cytoplasm, and the osmotic pressure of the medium balanced by compatible solutes without adjustments in the intracellular system (González-Hernándes & Peña 2002, Flores *et al.* 2010).

Table 2. Classification of Bacteria regarding the need and / or tolerance to NaCl.

Bacteria	NaCl concentration
Not halophilic	Up to about 1%
Marine	1 to 3%
Lightly halophilic	2 to 5%
Moderately halophilic	5 to 20%
Extremely halophilic	20 to 30%
Moderate halotolerant	0 to 15%
Extreme halotolerant	0 to 30%

Source: Adapted from Dassarma & Arora (2002), González-Hernández & Peña (2002) and Barbosa (2005).

Concerning the cytotoxic potential of extracellular extracts obtained from halotolerant bacteria on human renal carcinoma cells, there was a clear anti-proliferative activity of 61% and 59% at concentrations of 100 and 75 µg/mL, respectively (Fig. 1).

This cytotoxicity is understood as the ability of a substance to interfere significantly in cellular integrity and/or trigger diverse mechanisms that cause drastic changes in cell growth, causing some cell death pathway (Oliveira 2009).

ISO 10993-5 standards establish a qualitative classification for cytotoxicity of chemical complexes tested *in vitro* on four ranges of cytotoxicity. A severely cytotoxic substance is classified based on effect on cell viability above 50% of the total cell concentration, moderately cytotoxic effect when cell viability between 50% and 79% is present, slightly cytotoxic with values between 80% and 89%, and not cytotoxic when cell viability is greater than or equal to 90% (ISO 1999, Oliveira 2009). The ef-

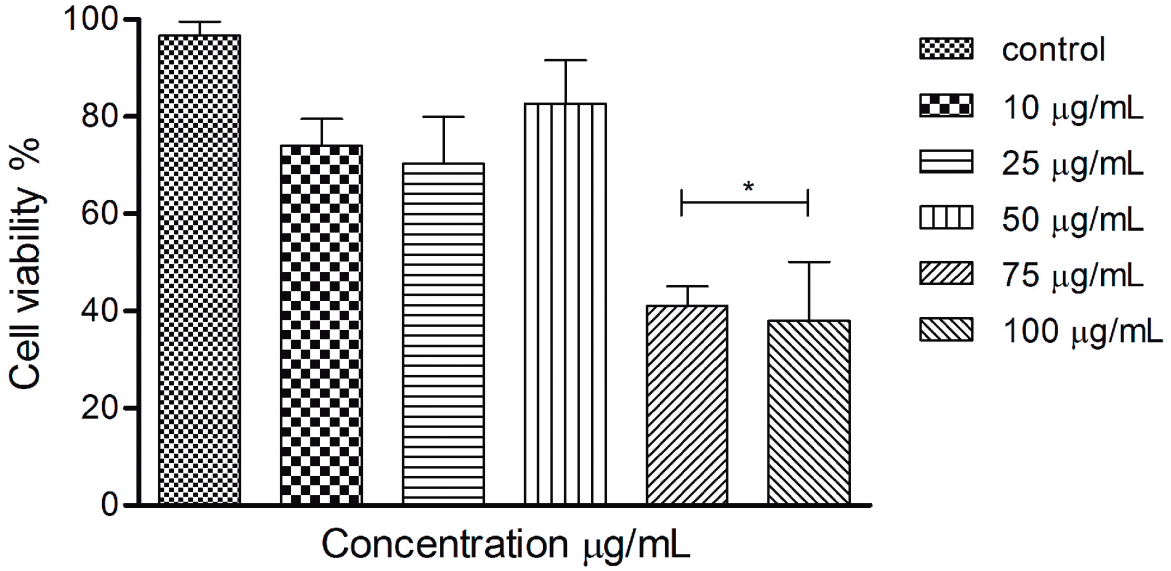

Figure 1. Cytotoxic effect of extracellular extracts obtained from halotolerant bacterium *Bacillus* sp. on lineage 786.0, determined by MTT assay. The concentrations of 75 and 100 µg/mL of extract resulted in a cytotoxic effect on human renal adenocarcinoma cells for a period of 24 hours of treatment. Control culture medium containing 0.1% DMSO without FBS. ANOVA followed by Tukey * p <0.05.

Table 3. Classification of extracellular extract cytotoxicity on cell line 786.0 in accordance to ISO 10993-5 (ISO 1999).

Cytotoxicity	Range viability %	[µg/mL]
not cytotoxic	> 90	Control
slightly cytotoxic	80 to 89	-
moderately cytotoxic	50 to 79	25 and 50
severely cytotoxic	< 50	75 and 100

Source: Adapted from ISO 10993-5 (ISO 1999).

fect of ethyl acetate fraction of the extract was classified as severely cytotoxic at concentrations of 75 µg/mL and 100 µg/mL in an incubation period of 24 hours (Table 3).

Bacteria of the genus *Bacillus* are known to produce various chemical compounds with complex peptide characteristics, which are gaining prominence as new classes of bioactive products used in the cancer prevention and treatment (Jeong *et al.* 2007, Chan *et al.* 2012, Baeriswyl & Heinis 2013, Bernardes *et al.* 2013). Thus, it is believed that the composition of the extract in question may present non-specific chemical characteristics of peptides.

However, *in vitro* studies related to the biological activities, more specifically the anticancer potential, of bioproducts isolated from the same genus are still scarce. Even so, it is shown by *in vitro* analyzes that secondary metabolites extracted from *Bacillus* feature a high cytotoxic activity on colon and leukemia cancer cells. In addition to this effect, many compounds showed mechanisms of programmed cell death characteristic of apoptosis (Jeong *et al.* 2008, Chan *et al.* 2012).

Also noteworthy is the presence of flavonoids and steroids extracted from microorganisms *Aspergillus* sp. and *Bacillus* sp isolated from a variety of habitats, by the method of extraction with the organic solvent ethyl acetate incorporation. The metabolite extracted from these microorganisms showed anti-proliferative effect with characteristics of apoptosis on cell lines HeLa and MCF-7 (Thomas *et al.* 2011).

El-Sersy and colleagues (2012) isolated the *Bacillus subtilis* species from sea water which yielded a byproduct (ε-poly-L-lysine (ε-PL)) with a potent anticancer activity against lineages of leukemia, liver and colorectal cancer. According to the study, this bioproduct was isolated pioneeringly at the marine bacterium *Bacillus subtilis*.

In the study performed by Chen *et al.* (2013) it was isolated a substance from the species *Bacillus amyloliquefaciens*, an exopolysaccharide, which led to anticancer action in lineages of human gastric cancer (MC-4 and GSC-7901). This was the first time that this substance was isolated from the genus *Bacillus*.

Multiple bioassays demonstrated a severe cytotoxic effect of prodigiosin (2-methil-3-6-fentil metoxiprodiginina) a pigment which has been isolated from different bacteria (Arthaud *et al.* 2012, Espona-Fiedler *et al.* 2012, Hsieh *et al.* 2012, Lu *et al.* 2012). This substance is in preclinical studies by Pharmaceutical Company AIDA against pancreatic cancer, and presents different action mechanisms, such as cell cycle interference by inhibiting topoisomerase I and II, inducing intracellular acidification processes (Hsieh *et al.* 2012).

The bioassay MTT, although used as a cytotoxicity assay, it does not specifically distinguish cell death pathway. In viable cells, fully functioning, active transport systems which are also present in the early apoptotic cell death occurs. Unlike what happens in necrosis, that there is no such functionality (Mousinho 2010). Whether the mechanism of tumor cell death in kidneys observed in this study is that of apoptosis, there is necessarily an advantage over necrosis. Apoptosis has genetically controlled events that does not cause damage to neighboring tissue

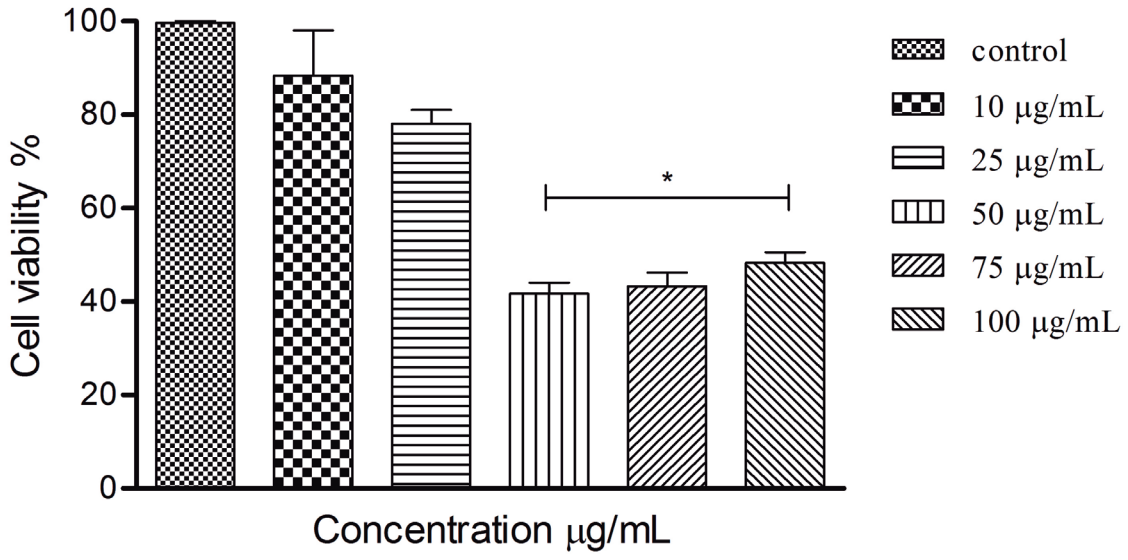

Figure 2. Cytotoxic effect of extracellular extract obtained from halotolerant bacterium *Bacillus* sp. on the cell line of embryonic human kidney HEK-293 determined by the MTT method (incubation time of 24 hours). Control culture medium containing 0.1% DMSO without FBS. The concentrations of 50, 75 and 100 µg/mL showed statistically significant effect. ANOVA followed by Tukey * $p < 0.05$.

mostly, a characteristic that will benefit the treatment of patients with kidney cancer.

Apoptosis is the type of cell death involved in most substances with anticancer activity, thus it is the most exploited as anticancer treatments. However, cell type, morphology, genetics, microenvironment, mutation presented by cells, anticancer concentration and time of exposure to the anti-tumor compound, are important factors that define the type of induction of cell death involved in this activity (Araújo 2013).

These new compounds with anticancer activity should elicit different action mechanisms in the body in order to be able to act specifically on whether inhibition of tubulin polymerization (acting on DNA) in enzymatic blockade processes, or cellular microtubules (Brandão *et al.* 2010).

In the case of *in vitro* tests, in order to verify an antiproliferative effect in tumor cells, it is a scientific requirement to determine the effect of test substance in non-tumor cells and normal cells. This test, MTT, allows the analysis of the selectivity of the test substance by tumor cells, validates the continuing pharmacodynamic studies involved in the anticancer effect, associating the degree of toxic effect on normal cells with potential action against tumor cells (Mousinho 2010, Araújo 2013).

During the process of optimization of bioactive compounds studies, the potency of its activity is the main parameter to be observed. However, to avoid the failure of a new chemical entity pharmacokinetics should be considered. Therefore, the ADME (absorption, distribution, metabolism, excretion) should be analyzed. However, its study occurs mostly *in vivo* experiments, still in the preclinical phase (Montanari & Bolzani 2001).

The extracellular extract of halotolerant bacteria presented significant nephrotoxic effects on the cell line HEK-293 in a treatment period of 24 hours in this study. Also it presented interference on cell viability of 52% at the concentration of 100 µg/mL, 57% at a concentration of 75 µg/mL and 59% at a concentration of 50 µg/mL, with no dose-dependent response (Fig. 2).

Mostly, substances with anticancer activity have cellular mechanisms and / or molecular targets that are also shared in non-tumor cells, so it is expected some effect of toxicity (Mousinho 2010).

Thus, when dealing with an extract formed by a chemical complex, which did not have an isolation of a specific class of chemical compound, it is interesting to verify the effect-benefit in relation to the concentration of compound that may be responsible for severe anticancer effect and its respective toxic effect on normal cells (Montanari & Bolzani 2001).

Therefore, the study of the pharmacophore group present on the chemical's complex matrix is necessary to determine the essential and unessential structures needed to ensure the preservation of biological activity and thereby enhance its cytotoxic effect on tumor cells and consequently reduce its toxic effect in non-tumor cells (Montanari & Bolzani 2001).

From microorganisms, especially bacteria, many bioactive chemical compounds have been isolated. This shows the great potential to achieve sustainable bioproducts with several applications for biotechnological processes, particularly involved in triggering the active effect with novel mechanisms of action to combat cancer. These bioproducts can be largely isolated from microorganisms and studied *in vitro* screening programs potentiating the antitumor activity data (Ortega-Morales *et al.* 2007, Das *et al.* 2008, Hayashida-Soiza *et al.* 2008, Kwakye-Awuah *et al.* 2008 Barreiro & Bolzani 2009, Demain & Vaishnav 2011, Nascimento *et al.* 2012, Newman & Cragg 2012).

Studies with halotolerant bacteria demonstrated encouraging results in the production of bioproducts with specific characteristics superior to chemical products used for heavy industries. Thus, these microorganisms are a source of valuable molecules.

It is important to remember that the thermostable DNA polymerase belonging to the PCR processwas obtained from a extremophyle bacteria. This increases the relevance of extracting secondary metabolites from extreme bacteria, as in the case of halotolerant from the genus *Bacillus*, in order to apply in biopharmaceutical processes (Austin 1989, Mcgenity *et al.* 2000, Santos *et al.* 2001, Adrio & Demain 2006, Dodia *et al.* 2006, Essghaier *et al.* 2009).

It is also presented nephrotoxic effect on human embryonic kidney line of HEK-293 under the same experimental conditions, indicating that the extract, probably has peptide, flavonoid and/or steroids (Newman & Cragg 2012) features presented in the extracellular extract of the bacterium and showed cytotoxic activity in both strains. This shows the great potential of obtaining bioproducts with anticancer *in vitro* biological activity.

Thus it is concluded that the extract obtained from the extracellular halotolerant species of bacteria belonging to the genus *Bacillus* sp. isolated from saline in Rio Grande do Norte, Brazil, showed cytotoxic activity *in vitro* classified as severe at concentrations of 75 and 100 µg/mL on the human lineage of renal adenocarcinoma (786.0).

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