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POTENTIAL USE OF COD ISOLATED BACTERIA (GADUS SP.) FOR TREATMENT OF SALINE RESIDUES AND EFFLUENTS

***[1]Thayli Ramires Araujo, [2]Guilherme Pereira Schoeler, [3]Roger Vasques Marques, [1]Flávia Liége Schutz Voloski, [4]Eduarda Hallal Duval, [2]William Terra Neves, [2]Luciara Bilhalva Corrêa and [1]Érico Kunde Corrêa**

[1]Departament of Agroindustrial Science and Technology – Postgraduate Program in Food Science and Technology – UFPel
[2]Engeneering Center – Ceng
[3]Federal Institute of Education, Science and Technology of Rio Grande do Sul – IFRS
[4]Veterinary College – UFPel

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ABSTRACT

The legal responsibility of the destination and treatment of waste and industrial effluents is due to the generator, the appropriate treatment and installation for disposal have become a problem, since industrial production is increasingly generating complex waste. Residues with concentrations of salt and organic matter from the chemical, agri-food, leather and oil industries negatively affect conventional treatments, inhibiting enzymatic activity of microorganisms. The search for viable technologies for the treatment of this waste, such halophilic bacteria are those that need a saline concentration in the medium to develop and can be an alternative for the treatment of wastes and effluents with high levels of salt. The objective of this study was to evaluate the growth potential of isolated bacteria from dry salted cod (Gadus sp.) in salinity, considering future applications in the treatment of saline residues and effluents. The samples were divided for microbial growth according to different temperature, oxygenation and pH conditions to obtain isolates submitted to biochemical tests. From the 4 fermented isolates, resistance of the different saline concentrations was observed, classifying them as at least moderate halophilic bacteria, allowing their use in future studies as indicators in treatments with saline residues and effluents.

****Corresponding author: Thayli Ramires Araujo,***

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INTRODUCTION

Effluents and solid waste from industrial, domestic, hospital, commercial, agricultural, utility and public cleaning activities, including sludge from treatment and generated by equipment, require technical and economically viable solutions Godecke *et al*., (2012); Abnt, (2004), that when poorly managed from collection to final disposal can degrade the environment resulting in severe impacts on the availability of resources (Simonetto and Lobler, 2014).

Currently, about 11 billion tons of solid waste are generated in the world each year, industrialization has introduced a series of products to meet the demand and population needs and consequently, wastes that can not be degraded in the environment, where about 120 billion tons of natural resources are used per year to meet the demands of growing production and consumption. Among the countries that stand out for this generation, China and the countries of Asia and the Pacific, hold about 50% of the world generation, showing a scenario of increase of this residue profile in developing countries in the coming years (Song *et al*., 2015).

The current society has caused a series of changes in the ecosystems, mainly for the search of new natural resources in order to establish high consumption patterns in face of the population growth, resulting in inadequate generation and accumulation of waste and effluents, boosting soil, air and water contamination (Morais *et al*., 2016). Law No. 12,305 / 2010, which establishes the National Solid Waste Policy (NSWP), which establishes the principles and guidelines for the integrated management and the management of solid waste, in this sense mining, energy, timber and agribusiness industries, even though they bring economic benefits for the development of a country entail many risks for the environment. Also according to NSWP, the destination of industrial waste is the responsibility of the generator, having the obligation for the appropriate treatment and final destination of the waste. However, many companies lack economic capacity and end up not properly managing their waste (Ipea, 2012; Garcia *et al*., 2015). Appropriate treatment for each type of effluent and waste is a central problem, since modern industrial production is increasingly generating more products with more pollutants such as plastics, fertilizers and pesticides, adding transport costs, adequacy and treatment, as well as greater environmental and social risks (Neves & Mendonça, 2016). One of the most important problems to be faced in the issue of environmental pollution is the generation of saline wastes that are derived from industrial processes, with salt being a key element for several sectors such as the chemical industry, in the alkaline chlorides sector; agrifood, for food preservation; leather, in the process of tanning; and oil, in the refinement stage. All these industries generate large quantities of waste water and sludge with high concentrations of sodium chloride (NaCl) and organic matter (Lefebvre & Moletta, 2006). The industrial treatments for the generated residues can be classified as physical, chemical and biological, and may occur in aerobic and anaerobic processes capable of receiving higher amounts of organic load per volume unit (Freire *et al*., 20000).

Conventional treatments are adversely affected by the presence of salinity and high organic load, and just as the sludge with large amounts of salts must be treated to be disposed of safely (Bódalo *et al*., 2007). In fact, sodium chloride concentrations in both wastewater and sludge make it impossible for the microbial activity in the medium to suit the conventional treatments Ye *et al*., (2009), as a consequence there is a low removal of the demand for chemical and biological oxygen and an increase in suspended solids (Abou-elela *et al*., 2010).
Saline stress can affect bacterial cells, causing loss of water by elevation of the solute in the extracellular environment and the toxicity of salt chloride in the cell interior affecting ionic balance and enzymatic activities (Li *et al*., 2014; Oliveira *et al*., 2016). On the other hand, the soil disposal of saline sludge generated in the treatment of effluents can affect the microbial activity of the soil and its toxicity affects plants and animals (Andrade *et al*., 2016; Khan *et al*., 2016). There are several classifications for haloophilic bacteria. However, one of the most widely used is the one classified according to the saline concentration in the medium, being classified as slightly halofilic (1-3% NaCl), moderately halophilic (3-15% NaCl) and extremely halophilic (above 15 % NaCl) (Piñar *et al*., 2014). Therefore, the development of new technologies for the treatment and recovery of waste and effluents can contribute to the desired sustainability of different economic activities (Marques *et al*., 2016). Thus, the objective of this study was to evaluate the growth potential of isolated bacteria of salted dry cod in saline environment, considering future applications.

MATERIAL AND METHODS

The isolates were extracted from a sample of dried salted cod (Gadus sp.), purchased at the local market in Pelotas, analyzed at the Waste Laboratory of the Engineering Center of Federal University of Pelotas (FUPel). Initially 25g of sample was weighed and homogenized with 225mL of lactose broth with pH adjusted to the desired ranges (4.5, 7.0 and 9.5) in the experiment. An aliquot was transferred to test tubes containing 9mL of 0.1% sterile peptone water to form the first dilution, the tubes were vortexed for 10 seconds. From the tubes, a 1mL aliquot was transferred to the plates following the surface exhaustion techniques on Blood agar (aerobiose) and Plate Count Agar (PCA) in overcoat (microaerobiosis), after drying the inocula, were incubated Inverted in greenhouse at 35 ° C, 45 ° C and 60 ° C for 48h, the experiment was performed in duplicate. After the incubation period, the morphology and color of the colonies were evaluated, repeating the isolation process until obtaining pure colonies, starting the biochemical, morphological and in vitro culture tests.

Biochemical and morphological testing

Biochemical and morphological tests were applied to the isolates according to the Compendium of Methods for Microbiological Examination, following the tests of Citrate, Indole, MR - VP, Catalase, Oxidation and Gram stain.

In vitro culture

Of the eleven isolates obtained, four were selected for in vitro culture, each isolate was transferred with a sterile handle to test tubes containing 10 mL Brain Heart Infusion (BHI) and sodium chloride (NaCl) at different concentrations (0.5%, 2%, 5%, 7.5%, 10%) incubated overnight in an greenhouse at 35 ° C for 10 hours in order to remove the strains from the latency state. Subsequently, 10 mL of each tube were transferred to the different concentrations of BHI salt with the respective inoculum to Erlenmeyers containing 190 mL of sterile BHI broth with the same concentrations of NaCl and incubated at 35 ° C for 5 hours in an incubator with orbital shaking, Shaker (Lactea®), at 100 rpm, initiating the fermentation process. At each hour of the fermentation 1 ml aliquots of each erlenmeyer were removed and transferred to tubes containing 9 mL of 0.1% peptone water and vortexed. For the Standard Plate Count (SPC) the surface exhaustion technique was used with the 3 largest consecutive dilutions, transferring 0.1 mL of each tube to a plate containing Plate Count Agar (PCA) medium incubated at 35 ° for 24 hours.

Experimental design

The in vitro culture process followed a randomized design with three replicates in a two - factorial arrangement, followed by the fermentation time in hours (0; 1; 2; 3; 4; 5) and the second the NaCl concentration (0.5; 2, 5, 7.5, 10%), the data obtained were analyzed by Variance Analysis at 95% confidence. Occurring statistically meaningfulness, the data were evaluated by Linear Regression with adjustments to polynomial models, according to equation 1.

$$y = y0 + ax \quad \text{.....(1)}$$

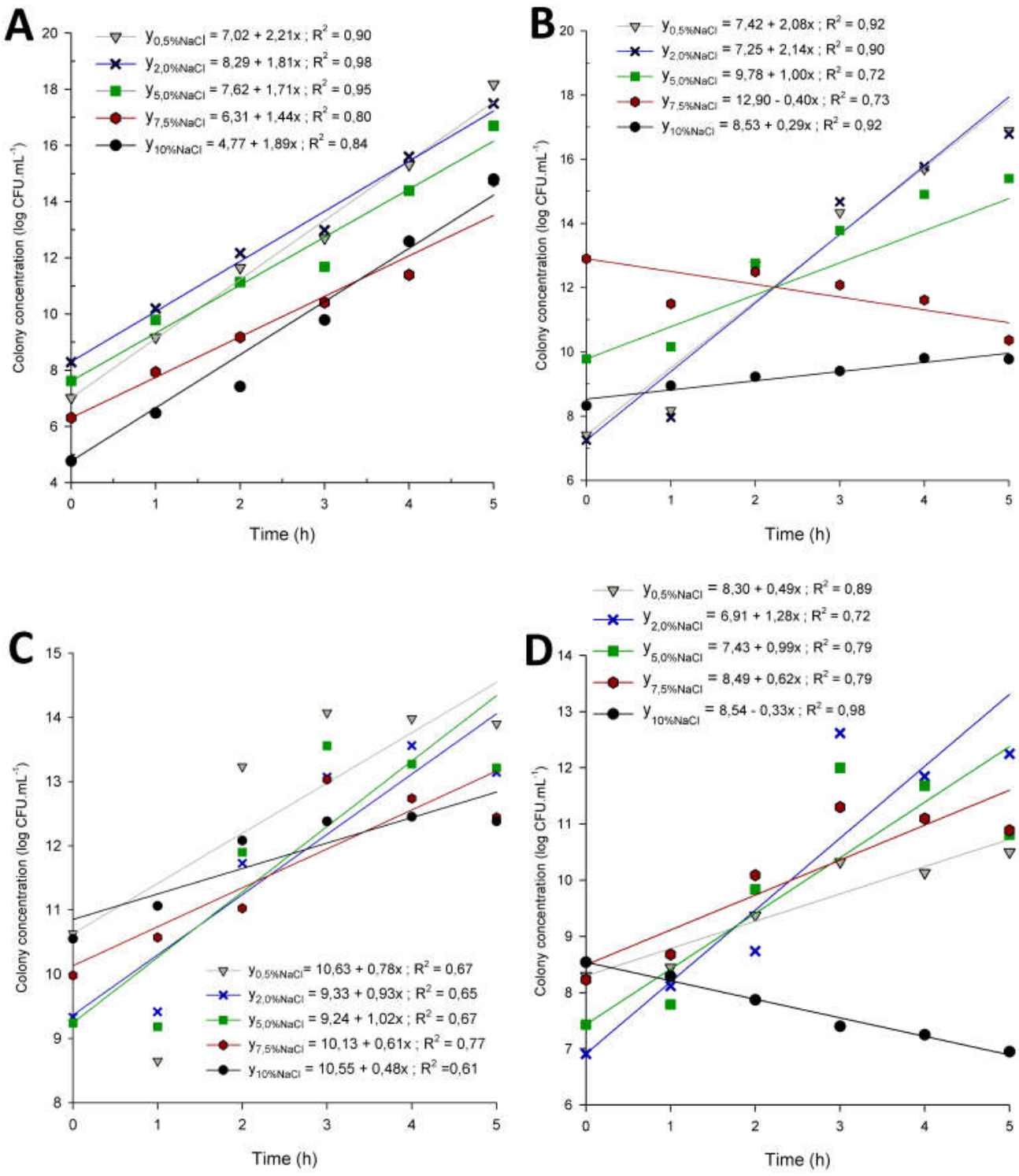

Figure 1. Growth profile of strains A, B, C and D in in vitro culture exposed to five different salt concentrations

Where "y" is the colony concentration (log. CFU.mL-1), "y0" and "a" are constants of the model and "x" is the time process in hours.

RESULTS

Of the 11 isolates incubated under different oxygen, pH and temperature conditions, four of them (A, B, C and D) presented better results in the growth analyzed in plates, both with temperature of 45 ° C, isolate A in pH 9,5 by microaerobiosis, isolated B at pH 9,5 by aerobiose, isolated C at pH 7 and isolated D at pH 4,5 both by microaerobiosis, all of these were applied to the fermentation process. However, it was observed that there was no growth in the isolates incubated at 60 ° C regardless of the pH and oxygen conditions.

By Gram staining test, isolates A and C in microaerobiosis are characterized as gram positive bacilli and gram positive cocci isolated D, while in aerobiose isolate B was identified as Gram negative bacillus. Table 1 presents the biochemical tests applied to the four selected isolates.

Table 1. Results of isolated biochemical tests A, B, C and D

Sample	Isolated	Citrate	Indole	MR	VP	Catalase	Oxidase
Cod	A	+	-	-	+	+	+
	B	+	-	+	+	-	+
	C	+	+	-	-	-	-
	D	+	-	+	+	+	+

Biochemical tests were able to investigate the chemical activities of each isolate selected for in vitro culture. The C isolate was negative for the catalase, oxidase, methyl red and Voges-Proskauer tests, whereas for the indole test the C isolate was the only one with the positive result. In the citrate test, all the isolates were positive. Isolate B, as well as isolate D, showed positive activity for the catalase, oxidase, methyl red tests. From the in vitro culture of the selected isolates, data were obtained for the construction of the growth charts in the different saline conditions proposed (0.5, 2.5, 5, 7.5, 10%) during the 5-hour period represented by Figure 1.

For strain A, comparing the development at different saline concentrations showed the same growth trend in all NaCl concentrations. At the beginning of the fermentation process (x = 0) presented cell concentrations of 7.02; 8.29; 7.62; 6.31 and 4.77 log CFU.mL-¹ respectively to 0.5; 2; 5; 7.5; 10% sodium chloride in the medium. For the maximum study period (x = 5), was observed 17.57 log CFU.mL-¹ in saline concentration of 0.5%, however in the maximum salt concentration, 10%, this strain had maximum growth of 14.22 log CFU.mL-¹ in 5 hours, presenting significant salinity tolerance of the medium during the fermentation process. Strain B showed initial cell concentrations (x = 0) of 7.42; 7.25; 9.28; 12.9; 8.53 log CFU.mL-1. In the presence of 0.5% and 2% NaCl in the medium, isolate B showed the same growth trend, reaching the end of the fermentation process (x = 5) at 17.95 log CFU.mL-1 at 2% of saline concentration. However, for 7.5% the growth line decreased during the 5 hours of fermentation analyzed, from 12.90 log CFU.mL-1 to 10.9 CFU.mL-1, the lowest value of cell concentration for this isolate at the end of the process was at the concentration of 10% salt in the medium with 9.98 CFU.mL-1 (x = 5). For strain C, growth occurred throughout all the fermentation process, at the end of the process (x = 5) the growth in saline concentration of 0.5% reached 14.53 CFU.mL-1 and at the concentration of 10% salt was 12.90 CFU.mL-1. For the initial cellular concentration, 5% resulted in 9.24 log CFU.mL-1, lower value when compared to the other concentrations, in counterpoint, during the period the strain had a better development reaching 14.34 log CFU.mL- 1. For strain D, there was growth at all concentrations except for 10% NaCl, where since the initial period (x = 0) there was a decline until the end of the fermentation period (x = 5), obtaining a minimum growth value of 6.89 CFU.mL-¹. For the 2% saline concentration, this isolate reached the maximum value of 13.31 log CFU.mL-1 for x = 5, with the same growth tendency for 0.5% and 7.5%, respectively, 11.35 and 11 , 59 log CFU.mL-1 at the end of the process (x = 5).

DISCUSSION

In the study, both isolates A and C presented the same morphological characteristics as the same growth rate before the different saline concentrations in which they were exposed in the in vitro culture. Evaluated the growth of isolates with similar characteristics to A and C for 24 hours in saline concentrations of up to 15%, the exponential growth phase was observed during this period, however the pH range considered optimal according to the author was established in the range of 9-10, a characteristic that resembles isolated A in the present study (Dodia *et al*., 2006). This behavior evaluated in A and C, indicate that these isolates are classified as having at least moderate halophilic bacteria, since the development of these two isolates occurred in up to 10% NaCl in the fermentation process (Piñar *et al*., 2014). Although isolates A and C have the same growth tendency and Gram staining has characterized as gram positive bacilli, also found by Muñoz *et al*., (2012); Lee *et al*., (2010), they differ in the chemical activities, represented by the biochemical tests, which were shown in table 1. The C for the tests of indole, oxidase, Voges-Proskauer and catalase showed the presence of enzymes which end up degrading more complex compounds into simpler compounds. The developmental relationship with the pH of the medium divides the halophilic microorganisms into neutrophils and alkaliphiles, with the ideal growth range of 7.2 and 9.5, respectively, according to the isolates presented that were submitted to fermentation, the pH range Of the colony forming units (CFU), isolates A and C, were adjusted to pH 9.5 by classifying these as alkaliphilic halophils (Sallto *et al*., 2012). As for the isolated D, the concentration of 10% salt inhibited the growth of the isolate, this fact can be explained by the effect of the presence of NaCl in the cellular respiration negatively affecting the energy production process from the organic matter present in the medium. With the increase of the electrical conductivity, the saline concentration is higher, it may have impeded the cellular respiration of the microorganism altering its growth over time, as well as for non-halophilic microorganisms can cause plasmolysis and cellular disruption because it can not effect osmoregulation (Yuan, 2007). For isolate B, the decline phase occurred at the 7.5% salt concentration and the overnight period was sufficient for the bacteria to develop reaching the stationary phase before the microbial growth period by aeration and agitation, and yes The opposite occurred at the 10% saline concentration, the isolate did not leave the lag phase of adaptation to the medium during the 5 hours proposed for growth. With the increase of the osmotic pressure there is an upward growth in the microbial activity, however this process occurs slowly precisely by the adaptational medium (WONG *et al*., 2008).

Conclusion

By means of the tests carried out, it was concluded that 4 isolates were able to resist and develop in the saline medium, also in acid pH as basic, classifying them as at least moderate halophilic bacteria, allowing their use in future studies as indicators in treatments with residues and saline effluents before banned by the absence of microorganism adapted to the saline environment.

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