

Evaluation of the Biotechnological Potential of Isolated Bacteria of the Soil in the Degradation of Residues of Skin Swine

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Abstract

The degradation of industrial residues is of great importance for the maintenance of the environment. Therefore, the objective of this study is to verify the proteases production for bacteria of the genus isolated *Bacillus* of soil. The samples of skin residue were collected in industries of the area west of Santa Catarina, using residue of skin rude swine and residue of skin processed swine. The verification of the biotechnological potential consisted of the use of these residues in middle of culture and inoculation of the respective species of bacteria. The results were more expressive in the samples of skin processed swine, where it showed a degradation average around 43.55% (p/p). While, in the samples of residue of skin rude swine, an average of degradation of 20.60% (p/p) happened. In this way, the residue of skin processed swine is more sensitive than the catalytic activity of the bacterial enzymes.

Keywords

Bacteria, *Bacillus*, Skin Swine, Degradation, Bioremediation

1. Introduction

The area West of Santa Catarina has a great potential in the industrialization of foods of animal origin. Great part of the industries here installed finds environmental problems, due to the generation of solid residues [1].

The inadequate disposition of residues in the soil and in the superficial waters is recognized as one of the serious problems of the present time. The effects of the rejects releasing in the nature are incalculable and they relapse mainly in the shortage, in the contamination of the water, and in the increase of the associated diseases of

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the environmental pollution. Among the techniques for treatment and final disposition of solid residues of industries, they are among the ones that present a smaller cost of the bioremediation of those residues through the action of microorganisms, as bacteria and fungi, present in the soil [2]-[4].

The bioremediation is the technique that consists of the application of biodegradable processes in the treatment of residues to recover and to regenerate atmospheres (mainly water and soil) that suffered negative impacts, maintaining the biological balance in ecosystem. Also it is called environmental biotechnology, and for using, in controlled way, microbiologic processes that usually happen in the nature to remove pollutant. Specifically, she acts through the introduction of additional biological processes for the decomposition of the residues that favor and they increase the speed of the natural process of degradation [5].

The bioremediation is a methodology, well accepted by the public and is a very efficient way in the recovery of atmospheres [6], because it doesn't affect the ecological balance. Bacteria, yeasts and filamentous fungi are agent's effective transformers, because they have ability in degrading a wide diversity of organic substances, commonly found in the residues generated by the industries [4].

The bacteria are easily found in the environment and they carry out several functions in the biosphere. Many of them aid in the production of several types of foods, and others cause important pathologies, which attack the human beings. Some of them have a great potential in generating enzymes that degrade the organic matter, and therefore, it uses this material type as substratum. This degradation is very important for the environment, because the elimination of these residues won't cause any damage for future generations [7] [8].

The bacteria that aid in the degradation and in the production of foods have been used for a long time in the industry of foods, which facilitate production of the foods in a significant way, as well as the human being's life. It is important that every year there are new discovered species of bacteria that influence the provision industry, bringing new benefits to the human being [9].

This work seeks, therefore, to isolate and to select lineages of microorganisms of the soil, with potentiality to degrade residues of provision industries, seeking an application in the bioremediation process.

2. Materials and Methods

2.1. Sampling

The residues of skin swine, so much processed and rude, used in this study were given up by nutritious companies of the area of Concordia (SC). 1 kg of residue of skin rude swine and 1 kg of residue of skin processed swine were collected.

2.2. Isolated Bacteria of the Soil

The experiments were accomplished at the laboratory of biochemistry of the IFC-Concordia. Soil samples, of the located native forest in the city of Concordia (SC), they were collected with assist of flasks and sterile spatulas. A total of 5 g of soil was added to 100 ml of sterile water in an erlenmeyer. The solution was agitated resulting in a homogeneous suspension vigorously. Of this suspension brackets of 1 ml were removed and transferred for 2 test tubes half containing BHI (Merck, Darmstadt, Germany). The broth was incubated to 37°C for 24 h, after this period, a bracket of 100 µl was removed and with assist of a loop of Drigalski, took place the dispersal of the suspension on half TSA (Merck, Darmstadt, Germany). The plates were incubated to 37°C for 24 h, and proceeded the coloration of Gram.

2.3. Identification of *Bacillus* spp.

The identification of *Bacillus* spp. it was accomplished in agreement with the methodology recommended by [10], in a total of 8 tests (tests of manitol fermentation, glucose, lecitinase production, reaction of the catalase, reduction of nitrate, test VP (Voges-Proskauer) modified, decomposition of the tyrosine, mobility test).

2.4. Analysis of the Samples

For accomplishment of the tests with the residue of skin processed swine and rude, were weighed three samples for each bacterium type. For each sample, they were weighed 5 g of residue (previously dry) and transferred for erlenmeyers of 250 ml and mixed with 100 ml of distilled water. All of them were taken for autoclave to 120°C for 15 min. After, the residue was used as middle of culture. 100 µl of a saline solution were inoculated contain-

ing 10^6 CFU/ml of each bacterium approximately in the respective erlenmeyer of the middle of culture. The experiment was accomplished in triplicate for each bacterium. After having inoculated the bacteria, the samples were incubated in at 37°C for 72 h. In an interval of every 24 hours, the samples were agitated to increase the degradation potential. At the end of the incubation period, the samples were filtered in paper filter (previously dry and weighted) and put in drying to 50°C for 24 h. In the end of this time, the samples were weighed for the calculation of income of the degradation. The experiment was repeated three times independently, each time in triplicate.

2.5. Calculation of the Income

The evaluation of the income of the degradation of the samples was accomplished starting from the difference of initial mass of the residue in relation to the final mass of the residue. Starting from the mass difference among the samples the income percentage was made calculations in that each bacterium has to degrade the skin residue. The calculation is summarized in

$$R = \left(\frac{\text{MSI} - \text{MSF}}{\text{MSI}} \right) \times 100 \quad (1)$$

where R refers to the degradation of the residue in percentage (enzymatic activity); MSI is the mass of the sample initial dry; MSF refers to mass of the sample final dry. Data were analyzed using the statistical software Stats D + (version 1.0) by descriptive statistical method.

3. Results

The results of the degradation of the industrial residues for action of bacterial enzymes are described below (Table 1 and Table 2).

The average of the income of the degradation of the skin processed swine was of 42.12 (*B. licheniformis*) to 44.83% (*B. cereus*), while the medium income varied of 19.73 (*B. cereus*) to 21.16% (*B. subtilis*) for the skin rude swine. Being like this, with base in the found results, it can be checked that the degradation of the residues of skin processed swine was more effective, when this residue suffers technological processes. Besides, these results demonstrate that the proteases production for species of *Bacillus* is similar, because the variation among the income averages was very close.

4. Discussion

The difference in the averages of the income is harnessed to the process type that the raw material is submitted. One of the stages of this process is the heating at high temperatures, causing the break of chemical connections among the components of the residue. This cleavage facilitates the action of bacterial enzymes with the capacity of hydrolysis proteins. The hydrolysis of proteins for heating reduces the size of the same ones, and they expose the residues of amino acids for the action of bacterial proteases [11].

Table 1. Income (in%) of the degradation of the residue of skin processed swine by bacteria of the gender *Bacillus*.

Bacteria	Mass of the sample initial dry (MSI) g*	Mass of the sample final dry (MSF) g*	Income (%)
<i>B. cereus</i>	5.02	2.67	46.81
	5.04	2.82	44.05
	5.02	2.83	43.63
<i>B. licheniformis</i>	5.03	2.96	41.15
	5.01	2.90	42.12
	5.06	2.88	43.08
<i>B. subtilis</i>	5.02	2.71	46.02
	5.03	2.85	43.34
	5.05	2.94	41.78
	5.04	2.82	44.05

*Each value is the average of three determinations in three alternate days.

Table 2. Income (in%) of the degradation of the residue of skin rude swine by bacteria of the gender *Bacillus*.

Bacteria	Mass of the sample initial dry (MSI) g*	Mass of the sample final dry (MSF) g*	Income (%)
<i>B. cereus</i>	5.05	4.03	19.80
	5.03	4.12	18.09
	5.07	3.99	21.30
<i>B. licheniformis</i>	5.03	4.06	19.28
	5.00	3.87	22.60
	5.08	4.02	20.87
<i>B. subtilis</i>	4.99	3.84	23.05
	5.06	4.04	20.16
	5.03	4.01	20.28
	5.05	4.03	19.80

*Each value is the average of three determinations in three alternate days.

The income of approximately 45% for the residue of skin processed swine presents a relevant importance for the subject of the discard of industrial residues. A solution for this problem could be related with the fact that the companies should count with tanks digesters, and in these equipments they add bacterial cultures of the gender *Bacillus*. This digestion is in a slow way, but it presents the great advantage of being clean in environment terms. Added to the fact of this process to be slow, there is the subject that the tanks would need a considered physical space, because the accumulation of residues in the tanks should consider the capacity of biodigestion of these bacteria; in other words, of the speed of proteases production. The advantage of this technique is directly related to the cost of implantation, which requires only the starter colonies and due to this, based on what we know about bacterial metabolism, the microorganisms would multiply rapidly reaching the maximum capacity of digestion in a very short period of time. For the market, the use of techniques that help to control the environmental pollution becomes extremely expensive at first. Therefore, industries still demonstrate a certain reluctance to utilize resources for the implantation of environmental monitoring programs. Unjustly so, because this type of degradation process coincides with the current market condition in which a low-cost technique can be used and yet still have a most satisfactory performance.

In this way, there are few industries that get to destine these residues in a correct way. The option for use of coming enzymes of microorganisms is done since 1950, and among these, the most important are bacteria and mushrooms. The bacteria that accomplish degradation of organic matter belong, mainly, to the goods *Bacillus*, *Pseudomonas*, *Corynebacterium*, *Aeromonas*, *Mycobacterium*, *Burkholderia*, and so on [12] [13] and several fungi of the goods *Cunninghamella*, *Phanerochaete*, *Fusarium*, *Candida*, *Trametes*, *Penicillium*, *Pleorotus*, *Aspergillus*, *Chrysosporium*, *Bjerkandera*, and so on [13].

Some species of *Bacillus* already have a history of safe use in the industry, besides in the production of addictive of food [14]. In another study, it was demonstrated that the activity emulsified proteins produced by the species of *B. licheniformis* [15]. Therefore, the process of degradation of these residues can also be harnessed to the production of other bacterial metabolites, such as glycoproteins and lipoproteins, which would facilitate the action of the proteases.

Nonetheless, the issue of caring and preservation of the environment is beyond a shadow of a doubt the one factor that concerns our entire planet, and the discovery of new techniques for the treatment of industrial residues is of addition importance for the survival of the planet.

Therefore, one of the humanity's great challenges is the subject of the preservation of the environment. So, this work looked for an alternative for the degradation of coming residues of industries that process skin swine. The obtained income is extremely promising, opening the possibility of this methodology to be used in pilot scale in the industries as an attempt of reducing the environmental impact caused by these, when they spill in their environment residues.

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