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Bacterial Isolation ability to Metabolize Organic Matter

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Abstract

Contaminated rivers represent an interesting source of microorganisms capable of degrading different substrates, considering candidates for isolation and purification processes used in contaminated water. The strategy of bioremediation technologies is the use of different metabolic pathways and increased degradation of native processes to eliminate or reduced the contaminating substances. The isolates were monitored in medium contained the different substrates (carbohydrates, proteins and glycerol). Nineteen bacterial isolates from the three monitored water bodies were obtained, the percentage of capacity to assimilate strains with different carbohydrates varied depending on the compound, 100% of the strains degraded dextrose and sucrose, degraded 86% starch, 66% casein and none of the isolates showed hydrolysis of gelatin and lipase production. Results that support combined efforts being made in the search for technologies more for clean water treatment, as well as the conservation of this resource.

Keywords: Bacterial isolates, water pollution, organic matter, degradation.

Introduction

Water pollution is an environmental problem, the waters are accompanied by organic matter, nutrients and trace amounts of metals. The physicochemical treatments allow partial removal of the organic load, however the costs are high, therefore it is advisable the use of other processes as the biologic[1-3].

For the selection of microorganisms may influence the reduction of organic matter in the effluent is necessary to analyze the metabolic capacity of different organic substrates.

Contaminated rivers represent an interesting source of microorganisms capable of degrading different substrates, considering candidates for isolation and purification processes used in contaminated water. Currently has been put emphasis on environmental biotechnology and sustainable development, including biological techniques can be effectively applied in the remediation of water contaminated by organic pollutants[4]. The strategy of bioremediation technologies is the use of different metabolic pathways and increased degradation of native processes to eliminate or reduced the contaminating substances. Because most of natural products and synthetic compounds, some are degraded by bacteria, regardless of their molecular weight and structural complexity, microorganisms have become key factor in the bioremediation and play an important role in water treatment systems[5,6]. The objective was to evaluate the ability to hydrolyze organic matter by bacterial isolates from water bodies.

Material and Methods

A single sample of 500 mL in each of the areas of study took: San Baltazar lagoon (19.006133,-98.213258), university campus lake (19.00454,-98.205405) and Atoyac river (19.015769,-98.244876), three are located in the city of Puebla, Mexico (figure-1).

The samples were transported at room temperature to the laboratory for processing, 10 vials were placed each containing 2 mL of water collected and carried out decimal dilutions 10^{-1} to 10^{-7}, by streaking 50 mL in nutrient agar plates and incubated at 30° C for 48 hours. Quantitated and selected colonies with different visible characteristics of each sample were stored on nutrient agar.

The isolates were grown on agar in duplicate to evaluate the assimilation of carbon (Carbon Assimilation Medium-CAM). Carbohydrates are used dextrose, sucrose and starch. Composition of CAM per liter: 500 mL of agar solution, medium basal mineral 500 mL (10 g carbohydrate, 5 g NaCl, 1 g K_2HPO_4, 0.1 g $MgSO_4$ 7 H_2O and 1g $(NH_4)_2$ SO_4 pH 6.5-7.0. The agar solution sterilized by autoclaving for 15 minutes at 121° C and the basal mineral medium was sterilized by filtration (Millipore membrane 0.8 µm). From stock culture of each of the isolates were plated on each of the mediums with their respective carbohydrate are incubated at 30^0C for 24 hours, and the reading is performed by the presence or absence of growth.

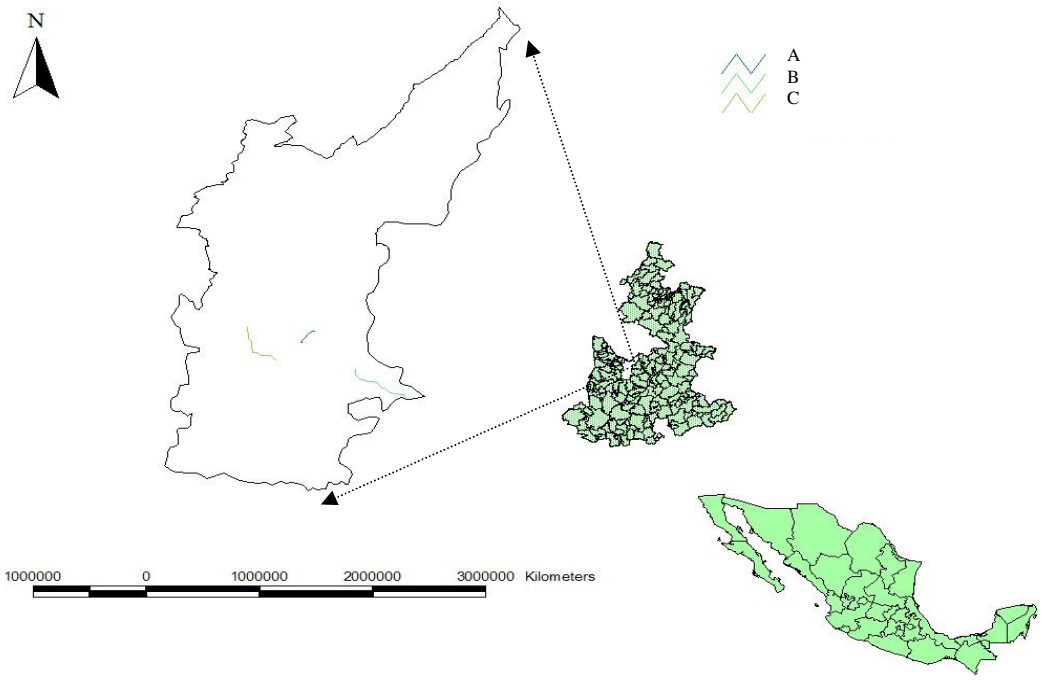


Figure-1
Geographical location in Puebla-Mexico in the three areas of water sampling for bacterial isolation (A) San Baltazar lagoon, (B) river Atoyac, (C) university campus lake

To evaluate the hydrolysis of casein bacterial strains were grown in a medium containing casein and consisting of two fractions, (1) TSA (250 mL of distilled water with 1.5% agar), (2) skim milk (10 g in 250 mL of distilled water). Each fraction was sterilized separately at 115^0C for 30 minutes. The strains were grown by a thick central estria and incubated at 30^0C for 5 days, reading the test was conducted by observing the appearance of a clear halo around the bacterial growth, when the bacterium is able to hydrolyze casein.

For the hydrolysis of gelatin was used nutrient agar with 0.4% gelatin, pH 7.2, sterilized by autoclaving 20 minutes at 115^0C. The strains were inoculated at 30^0C for 2 to 14 days and the reading of the test was performed with a solution of mercuric chloride (15 g mercuric chloride, 20 mL concentrated hydrochloric acid and 100 mL distilled water). The plates were dipped in 10 mL of reagent, Non-hydrolyzed gelatin to form an opaque white precipitate reagent and the hydrolyzed gelatin as a clear zone appears around the estria.

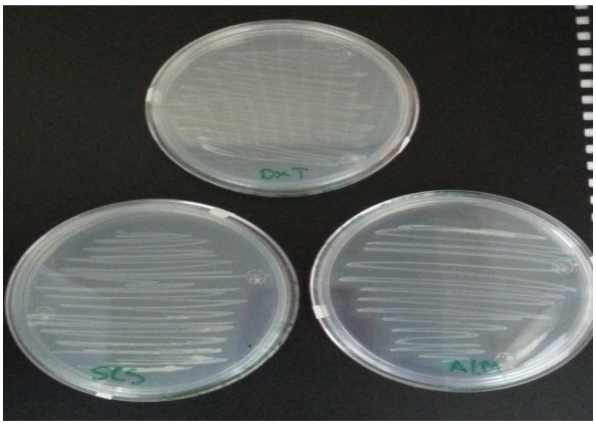
To evaluate the production of lipase-glycerol agar medium was used: 10 g peptone, 5 g sodium chloride, 0.1 g calcium chloride, 1 L distilled water, 10 mL glycerol and 15 g agar, pH 7.0. The plates were striated and incubated at 30^0C for 7 days, when the microorganism is capable of hydrolyzing glycerol appears in the culture medium a precipitate around the bacterial growth due to the combination of Ca_2+ and the fatty acids released by the hydrolysis.

Results and Discussion

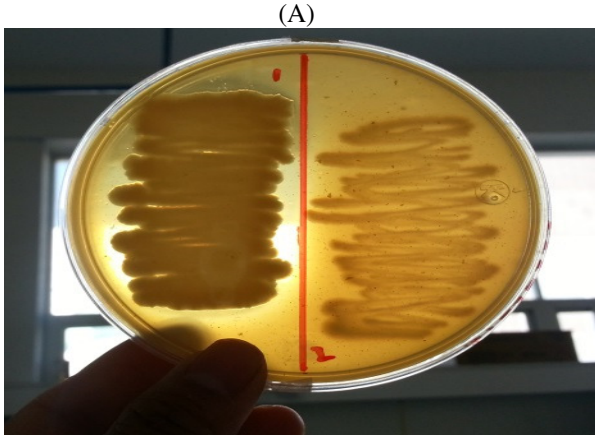
Nineteen bacterial isolates from the three monitored water bodies were obtained; the results show that the composition of the bacteria in morphology and response is varied gram stain which gives the possibility to find bacteria with different physiological, biochemical and metabolic characteristics. The percentage of capacity to assimilate strains with different carbohydrates varied depending on the compound, 100% of the strains degraded dextrose and sucrose, degraded 86% starch, 66% casein and none of the isolates showed hydrolysis of gelatin and lipase production (figure-2).

Discussion: Nineteen bacterial isolates from water bodies of the city of Puebla were obtained. San Baltazar lagoon all isolates show ability to degrade dextrose and sucrose, however, the degradation of starch (polysaccharide) showed different behavior. For the production of proteases all isolates having the ability to degrade casein, however none gelatin degradation, and the degradation of fats was negative in all isolates. Atoyac river isolates presented degradability of carbohydrates. The percentages indicate that most of the strains tested showed versatility in the use of carbon sources for growth and therefore for removal of these compounds from the medium in which they are present. The results are in close relation to the place of done these bacteria were isolated; as different levels of contamination may influence the bacteria present characteristics to adapt to the use of a wide range of carbohydrates. Although the degradation of pollutants in nature is often the result of the activity of a

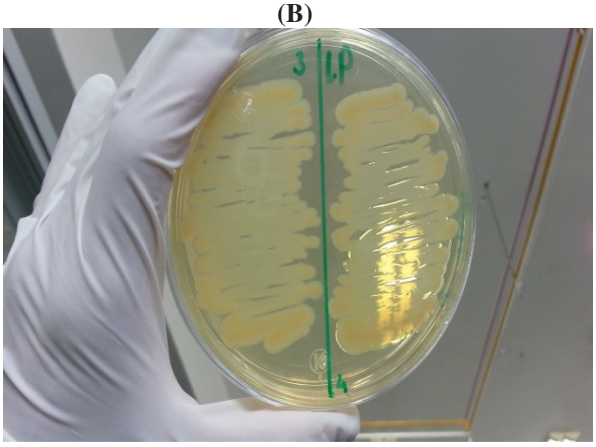
microbial consortium rather than a single organism, the potential degrader consortium depends on the potential that microorganisms present individually in their interaction with specific pollutants[7-10], for this reason have isolated autochthonous capacity to remove organic matter is a pathway for future use[11].



(A)



(B)



(C)

Figure-2

Cultures of the isolates in the presence of different substrates metabolized are presented. (A) testing hydrolysis of carbohydrates (dextrose, sucrose and starch), (B) comparison between isolated 1 (positive), 2 (negative) casein degradation, (C) isolated lipase negative substrate

Conclusion

The isolation bacteria in water bodies allow having 19 autochthonous bacterial isolates with versatility in its interaction with organic contaminants from water, such as carbohydrates, proteins and lipids. Highlights include strains that can be used independently or in bacterial consortiums for reducing organic matter in domestic wastewater by incorporation into water treatment systems, results that support combined efforts being made in the search for technologies more for clean water treatment, as well as the conservation of this resource.

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