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Influence of Bacterial Microbiota on the Organic Matter Content of Shrimp Pond Soil

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ABSTRACT

Shrimp activity is associated with the impact of bacterial communities. Therefore, this research aimed to evaluate the influence of the bacterial microbiota on the organic matter content of the soil of the shrimp lagoon in La Segua-Ecuador. Starting from a descriptive approach, the field research method and documentary review were used. In total, 25 soil samples were collected in 5 quadrants of 100 m^2. The bacterial DNA was extracted by using the Powersoil® kit and the identification of the strains was carried out with the 16SrDNA gene. The organic matter content was determined by Walkley-Black titration. The genus Bacillus was predominant in the bacterial strains; moreover, individuals of the genera Exiguobacterium, Acinetobacter, Prolinoborus, Arthrobacter Planococcus were identified with more than 99% homology for all cases. It was concluded that the organic matter content is suitable for shrimp farming.

Keywords: molecular analysis, shrimp farming, organic water content, bacterial identification, soil.

INTRODUCTION

Understanding how agricultural practices impact soil microbiota is an important issue towards a more sustainable agriculture. Recent studies on deforested lands have shown that land use has long-term effects on soil microbiota structure and diversity, which are constantly altered by high levels of nutrient inputs related to human activities (Coller et al., 2019).

The land use change for agricultural or aquaculture purposes, occurs more frequently worldwide; between 2000 and 2016, anthropogenic impacts were responsible for 62% of the global loss of wetland and mangrove area, and the cultivation of shrimp, rice and palm oil were responsible for almost half of these global losses (De Lacerda et al., 2021).

Shrimp farming is one of the fastest developing financial exercises in the coastal areas of Asian and Pacific locations, which contribute more than 85% of the world's farmed shrimp. Due to the favorable climate and its accessibility, shrimp aquaculture has mostly grown in the tropical and subtropical coastal lowlands (Shahriar et al., 2019).

For the case of Ecuador, shrimp farming started in this country approximately 50 years ago in a casual manner. The first shrimp farms were established in the southern part of the country and, since then, almost 220 000 hectares of production ponds have been developed, which today are part of an industry that is the first source of non-oil foreign exchange earnings in the country (Piedrahita, 2018). In 2016, Ecuador led the shrimp production in Latin America with 57% of total production, followed by Mexico and Brazil with 17 and 8%, respectively (Souto et al., 2021).

Despite its enormous economic contribution, shrimp farming is associated with problems such as loss of coastal wetlands due to pond construction, as well as poor management of waste materials, since pond waters are routinely and frequently discharged into adjacent coastal ecosystems, affecting bacterial communities and carbon and nitrogen metabolisms in the soil (Chen et al., 2020).

In addition to routine effluents, at the end of each crop rotation, the pond sediments containing pathogenic bacteria and nutrients derived from feed additives are completely dredged and wastewater in the form of sludge (dredge wastewater) is discharged into adjacent ecosystems; thus, intensive nutrient discharge into coastal ecosystems consequently affects the carbon and nitrogen metabolism in soils (Shahriar et al., 2019; Chen et al., 2020).

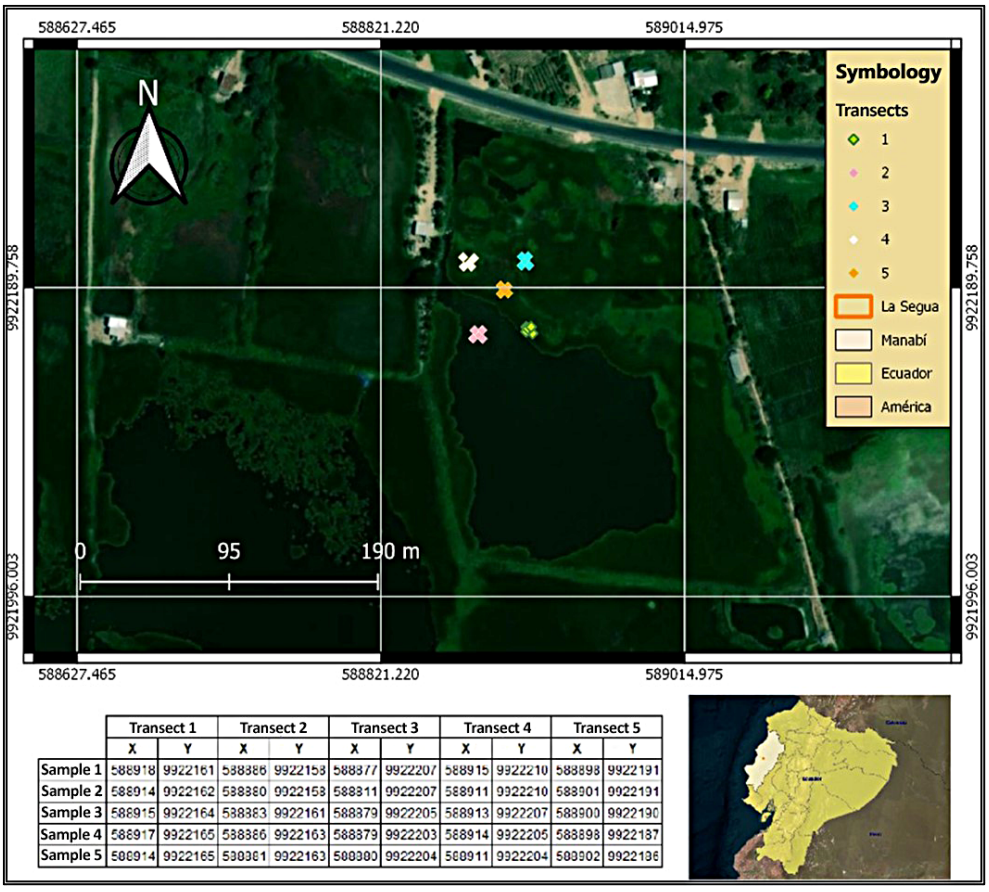
The growth rate of shrimp is directly proportional to feeding frequency; however, only a portion of the nutrients in the feed is consumed, assimilated and retained as shrimp biomass. Shrimp only incorporate 24 to 37% of nitrogen and 11 to 20% of phosphorus from the feed into their bodies. In addition, 15% of nitrogen losses occur during the first 2h of immersion of feed pellets in pond water; these unused nutrients will lead to a change in pH and dissolved oxygen (DO) in the water column and soil adjacent to the pond, causing eutrophication, bacterial and plankton blooms, and an increase in particulate organic matter (Alfiansah et al., 2018). Against this background, the objective of this research was to evaluate the influence of the bacterial microbiota in the shrimp pond bottom of the La Segua wetland on the organic matter content.

METHODOLOGY

This research is descriptive in nature and the field research method and documentary review were used for its development; moreover, interview and direct observation techniques were employed to collect relevant information on the state of the shrimp pond, the technologies applied and everything related to the production process as well as direct observation.

Study area

This research was carried out in the La Segua wetland, located between the limits of the towns of Chone and Tosagua (Manabí province, Ecuador). The wetland is highly seasonally dependent, since in the rainy season its extension reaches 1745 ha while in the dry season it is reduced to 525 ha; its average depth is 67 cm, its approximate



	Transect 1		Transect 2		Transect 3		Transect 4		Transect 5	
	X	Y	X	Y	X	Y	X	Y	X	Y
Sample 1	588918	9922161	588886	9922158	588877	9922207	588915	9922210	588898	9922191
Sample 2	588914	9922162	588880	9922158	588811	9922207	588911	9922210	588901	9922191
Sample 3	588915	9922164	588883	9922161	588879	9922205	588913	9922207	588900	9922190
Sample 4	588917	9922165	588886	9922163	588879	9922203	588914	9922205	588898	9922187
Sample 5	588914	9922165	588881	9922163	588880	9922204	588911	9922204	588902	9922186

Figure 1. Location of the study zone

altitude is 10–12 meters above sea level and the average temperature ranges between 26–27 °C (Rivera and Doumet, 2021). Figure 1 shows in detail the location of the transects, as well as the coordinates (UTM) of each sample obtained.

Description of the shrimp farm production process

In order to obtain the basic information on the production process developed in the shrimp farm, a semi-structured interview was conducted with the owner, identifying the associated activities, such as: larvae pre-selection, lagoon fertilization, filling, organic matter accumulation, feeding, larvae planting, lagoon management and harvesting. The information obtained was analyzed in detail to elaborate a general diagram of the described process.

Quantification of the bacterial microbiota existing in the soil of the shrimp farm

The sediment samples were obtained according to the English flag method, establishing 5 quadrants of 100 m^2, from each quadrant 5 subsamples were taken (1 in each corner and 1 in the center). These samples were collected randomly from an area of 10 m^2 adapting the methodology of Vivien et al. (2019), who recommend introducing a 10 ml syringe at a depth of 20 cm of the soil. The samples were stored at -20 °C in sterile airtight sealable plastic bags (Chen et al., 2019).

Bacteria were cultured on Sabouraud Dextrose and Triptic Soy Agar (TSA) medium. The stock solution for serial dilutions consisted of 1 g of the obtained soil (previously homogenized). This sample was diluted in 9 ml of distilled water, to obtain a better strain activation, 9 ml of sterile peptone water were also added.moreover, 9 ml of distilled water were added to this stock solution until serial dilutions of 10^{-1}, 10^{-2} and 10^{-3} were obtained.

The samples of 100 µl of 10^{-1} and 10^{-3} were grown on the medium employing a steel spreader; the plates were incubated at 30 °C for 24 h. Colony counting was performed by recording the existing types; subsequently, using the streaking method, each of the colony types obtained was purified by replating on a new culture medium until pure individual colonies were obtained.

For DNA extraction, the Powersoil® kit (Qiagen) was used according to factory specifications and adapting the criteria from Bravo (2018). Consequently, the samples were lyophilized and DNA concentration was measured employing a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Fisher); the extracted DNA, in its entirety, was stored at -20 °C (Chen et al., 2019). Applying the boiling method, bacterial genomic DNA was extracted by seeding pure colonies in culture broth for 18 h from the sediment obtained by centrifugation.

Bacterial strain identification was performed using the 16SrDNA gene, as well as the universal primers 16SrDNA27F and 16SrDNA1492R. In addition, bacterial DNA amplification was performed by PCR (with universal oligonucleotides) under the following conditions: 45 s for DNA denaturation at 94 °C, 30 s of coupling between primers and target DNA at 54 °C, and 90 s of extension at 72 °C; 30 cycles with 10-minute extension at 72 °C were performed using a Techne thermal cycler.

The sequences obtained were purified with Promega's Wizard PCR Clean Up System and their alignment was performed in the online software Blast (Basic Local Alignment Search Tool), and homology was determined by sequence comparison.

Determination of organic matter content in shrimp pond soils

The organic matter content was determined using the Walkley-Black titration method at the National Agricultural Research Institute of Ecuador (INIAP). The total organic matter content (expressed in %) was calculated by applying equation 1; the values obtained were categorized as detailed in Table 1.

Table 1. Concentrations of organic matter in the soil of aquaculture ponds (Torun et al., 2020)

(%) Organic matter	Interpretation
0–0.50	Very low, does not support good benthic growth
0.51–1.00	Low for fertilized ponds, but excellent for fed ponds
1.01–2.50	Optimal for fertilized ponds and acceptable for fed ponds
Más de 2.50	Excessive, prone to anaerobic bottom zones

$$\%MO = 100 - [(wf - wt/wts - wt) * 100] \quad (1)$$

where: $\%MO$ – organic matter concentration (%);
wt – crucible tare weight (g);
wf – final weight of the sample (g);
wts – crucible tare weight + (2 g) of the sample.

RESULTS AND DISCUSSION

Production process

According to its production and characteristics, the shrimp pond is semi-intensive (Salazar, 2019). On the other hand, its management is empirical, since those who manage the lagoon do not know about sustainable manufacturing techniques; in addition, the activities that are developed in the production process are detailed in Figure 2. It has already been pointed out that, globally, shrimp production technology is mostly extensive and semi-intensive, with much potential to improve efficiency through innovation and standardization of procedures, continuing the transition from artisanal to industrial scale (Jory, 2018).

The shrimp lagoon has a height of 1.5 m, an area of 4,965 m^2 and a volume of 7,447.6 m^3; filling is done with hydraulic pumps for 1 or 2 days, depending on the height of the water mirror. In contrast, this type of operation should follow a decanter-reservoir-lagoon flow (with an approximate duration of 4 to 5 days); in addition, the filling process should be slow and with strict supervision, since the filters should not be removed from the inlet and outlet structures during the first 30 days of cultivation and a filter management plan should be established to reduce the entry of undesirable organisms into the production system (Terkula & Kasana, 2021).

Quantification of bacterial microbiota

In the Sabouraud Dextrose medium, a total of 5.6×10^4 CFU/g background was quantified, corresponding to 5 colonies, the BRP of which presented 3.1×10^4 CFU/g background, this being the highest value; in contrast, BRG and CRM reached 1×10^3 CFU/g background. On the other hand, in the TSA medium, a value higher than 1.85×10^5 CFU/g background was obtained, the minute colonies of which were the highest with a value higher than 1 × 105 CFU/g background (Table 2).

All the colonies isolated in Sabouraud Dextrose belong to the Bacillus genus arranged in different strains and species; in contrast, in the TSA medium, bacteria belonging to the genera Bacillus, Exiguobacterium, Acinetobacter, Prolinoborus and Planococcus were identified, with a homology greater than 99% (Table 3).

The genus Bacillus has been associated with shrimp farming systems, as these probiotic bacteria have been found to help maintain good water quality through direct absorption or

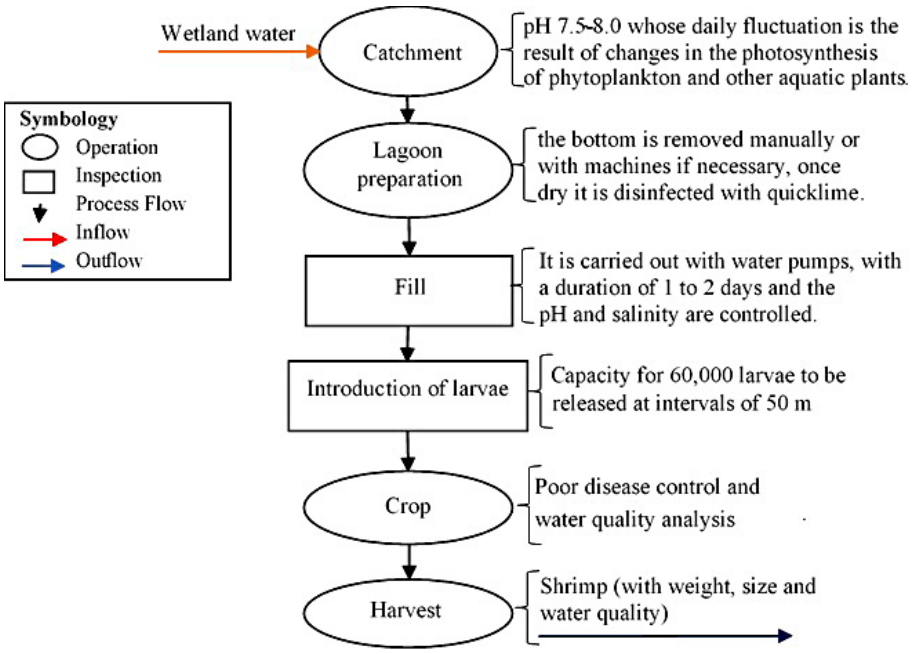


Figure 2. Diagram of the shrimp production process

Table 2. Colonies isolated in the culture media

Medium	Colonies	Count (UFC/g fondo)	Code
Sabouraud Dextrosa	BRG	$1\times1^{0}3$	1DX
	BRM	1.5×10^{3}	2DX
	CRM	1×10^{3}	3DX
	TRP	2.1×10^{4}	4DX
	BRP	3.1×10^{4}	5DX
	Total	5.6×10^{4}	
Triptic Soy Agar	CRM	3×10^{3}	6-1TS
	CRM		6-2TS
	BRM	1.2×10^{4}	7TS
	GrisRp c/pto	$7x10^{3}$	8TS
	BRP	2.46×10^{4}	9TS
	CIP	5×10^{3}	10TS
	BImP	7×10^{3}	11TS
	CRmP	2×10^{4}	12S
	GRisRp	6×10^{3}	13TS
	Tiny colonies	$>1\times10^{5}$	14TS 9-1 14TS 9-2
	Total	$>1.85\times10^{5}$	

Table 3. Isolated strains with their respective identification and percentage of sequence homology

Code	Molecular identification	Homología
1DX	*Bacillus aryabhattai strain HFBP06*	99.80%
	Bacillus megaterium strain FDU301	99.50%
2DX	*Bacillus aryabhattai strain HFBP06*	99.80%
	Bacillus megaterium strain FDU301	99.50%
3DX	*Bacillus sp. PN13* *Bacillus megaterium strain YM1C5*	99.93%
4DX	*Bacillus altitudinis strain NPB34b* *Bacillus altitudinis strain SCU11*	99.86%
5DX	*Bacillus altitudinis 41KF2b*	99.86%
6-1TS	*Bacillus marisflavi strain TF-11*	99.86%
6-2TS	*Bacillus marisflavi strain TF-11*	99.45%
7TS	*Bacillus aryabhattai B8W22* *Bacillus megaterium strain ATCC 14581*	99.79% 99.59%
8TS	*Bacillus altitudinis 41KF2b* *Bacillus stratosphericus strain 41KF2a*	99.93% 99.86%
9TS	*Bacillus altitudinis 41KF2b*	99.93%
10TS	*Bacillus altitudinis 41KF2b*	99.93%
11TS	*Exiguobacterium aquaticum strain IMTB-3094* *Exiguobacterium aurantiacum strain DSM 6208*	99.46% 99.26%
12S	*Acinetobacter lwoffii strain JCM 6840* *Acinetobacter lwoffii strain DSM 2403*	99.59% 99.31%
13TS	*Acinetobacter lwoffii strain DSM 2403 16S* *Prolinoborus fasciculus strain CIP 103579 (95% coverage)*	99.72% 99.79%
14TS 9-1	*Arthrobacter oryzae strain KV-651* *Arthrobacter humicola strain KV-653*	99.10% 98.61%
14TS 9-2	*Arthrobacter plakortidis strain AS/ASP6 (II)* *Planococcus maritimus strain TF-9*	99.17% 98.62%

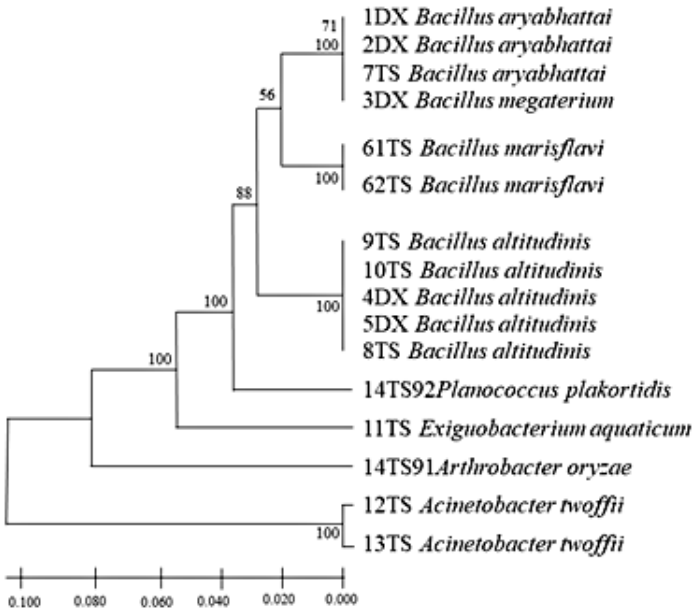


Figure 3. Phylogenetic tree of the identified strains

decomposition of organic matter (Zeng et al., 2020). Furthermore, in the shrimp farm soils located in Indonesia, Bacillus and Acinetobacter genera have dominated the bacterial communities (Alfiansah et al., 2018). Likewise, in wetland bottoms of Bogotá (Colombia), the bacteria of Bacillus genus have been found to be abundant (Castelblanco et al., 2020); agreeing with the findings of this research.

When analyzing the phylogenetic lineage of the species found, it is evident that the species *Bacillus aryabhattai* (1DX), *Bacillus aryabhattai* (2DX), *Bacillus aryabhattai* (7TS) and *Bacillus megaterium* (3DX) are in the same clade due to the high percentage of shared *homology; Similarly, Bacillus marisflavi* (61TS) *and Bacillus marisflavi* (62TS) have 100% homology similarity, thus sharing a clade (Figure 3).

Bacillus altitudinis (9TS), *Bacillus altitudinis* (10TS), *Bacillus altitudinis* (4DX), *Bacillus altitudinis* (5DX) and *Bacillus altitudinis* (8TS) have 100% similarity in the homology of their aligned sequences; Similarly, *Acinetobacter twoffii* (12TS) and *Acinetobacter twoffii* (13TS) belong to the same clade with 100% similarity in their homology; in turn, the other bacterial species sequenced did not show homology approximation with other species (Figure 3).

Determination of organic matter content

The organic matter content of the 5 samples analyzed is graphically summarized in Figure 4, showing that samples 1 and 5 have the highest percentage of organic matter with 2.20 and 2.00%, respectively, while the lowest value was obtained in sample 4 (1.00%). According to the criteria defined in Table 1, the highest values are within the optimum range for a fertilized lagoon, and the lowest level is excellent for a fed lagoon.

It should be noted that the decomposition of organic matter does not occur at the same rate, i.e., most of organic waste will decompose in a few weeks or months, but some of the material

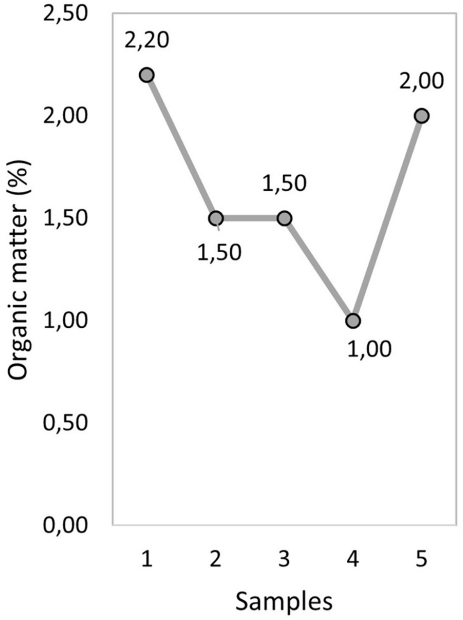


Figure 4. Distribution of organic matter content according to sampling zone

will persist for years; furthermore, decomposing microorganisms excrete organic compounds, and when they die they are converted into organic matter; thus, the excretions of microorganisms and resistant remains of decomposing organic matter form large complex molecules of humic substances. Hence, organic matter accumulates in the sediment of aquaculture ponds and, this material decomposes very slowly compared to the fresh organic matter that is deposited on the bottom during aquaculture cultures. (Boyd, 2016).

Given that a value of 2.20% (close to 2.50%) was obtained in the sample, this could be indicative of low dissolved oxygen levels due to inappropriate feeding practices in the lagoon, as food debris can become part of the sediment, creating the areas of low oxygen content. Another aspect to consider is the influence of microbial activity, which is closely related to organic matter content..

The microorganisms present in the aquaculture environment are indispensable for nutrient metabolism and energy cycling; specifically, Bacillus sp. and Pseudomonas sp. These are organic degraders, as they were isolated in Apostichopus japonicus nursery ponds and shown to degrade the chemical oxygen demand of the bottoms (Zhao et al., 2020). Similarly, the ability of Arthrobacter oryzae sp. (strain KV-651T) and Arthrobacter humicola sp. (strain KV-653T) to incorporate various carbon sources in their metabolic process and withstand up to 5% salinity has been demonstrated (Kageyama et al., 2008).

There is an axiomatic connection between bacterial communities and the ecological characteristics of the environment where aquaculture is developed, reaffirming the importance of studying these communities. It has been documented that certain bacteria can accelerate the decomposition of residual food and feces to purify water quality and reduce eutrophication levels; in addition, other microorganisms can convert toxic substances, such as ammonia, nitrite and hydrogen sulfide, into low-toxic or non-toxic formations to protect the survival of cultured species in aquaculture ponds (Zhao et al., 2020).

CONCLUSIONS

In the lagoon under study, the production process is managed in an empirical manner, lacking the techniques that contribute to the improvement of this process. It was determined that the Bacillus genus predominates in the shrimp farm soil, and there are also microorganisms of the genera Exiguobacterium, Acinetobacter, Prolinoborus, Arthrobacter, Planococcus, with 99% homology. The organic matter content determined in the shrimp pond soil is suitable for this activity, being dependent on the bacterial activity existing in this ecosystem.

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