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Assessment of fish coproducts *Sardina pilchardus* as the source of lactic acid bacteria

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ABSTRACT

Objective: To recover the waste (edges, heads and guts) of a species of pelagic fish *Sardina pilchardus* as a source of lactic bacteria.**Methods:** The microbiological control of the fish waste was carried out. Then, the fish waste was assessed as a source of bacteria of industrial interest among other lactic bacteria. The standard protocol for researches of these microorganisms was adopted which comprised enrichment, isolation, identification, purification and conservation.**Results:** The results of the microbiological control indicated the presence of some species as part of the normal flora of the fish. The physiological and biochemical characterization has presented 2 different groups of lactic bacteria: *Lactobacillus fermentum* and *Lactobacillus* spp.**Conclusions:** The assessment of fish waste can give us the opportunity to obtain different species of useful bacteria.

1. Introduction

Many developing countries have increasingly often used fish processing to meet demand for domestic or export requirements. Fish also plays an important role in the production of animal fodder and compounds for the pharmaceutical industry[1]. Fish processing is a crucial sector to meet the nutrient needs for humans. But it generates a large amount of waste estimated at 50% of the total volume. Meanwhile, it creates a problem of dumping of waste in different environments, which increases environmental pollution, in particular in the water environment. Fish coproducts are defined as: “unused parts and recoverable in the traditional production operations”[2]. During the processing of fish for human consumption, co-products including heads, viscera, falling trimming (thread), skin, scales, fins and tails are generated[1,3].

In many regions, most seafood processing waste from coastal factories are converted into fish meal or fertilizer, and all excess residues are discarded at sea, in coastal waters, directly applied on the ground or dumped in landfills[4]. These products are described as derivatives and not of finished products because they are generally marketed as ingredients, *i.e.* in the form of intermediate products for human nutrition, animal feed, die and cosmetic[3].

Our work therefore aims to recover the waste (edges, heads and

guts) of a species of pelagic fish, the common sardine *Sardina pilchardus* (*S. pilchardus*). We determine the possibility of using fish waste as a source of bacteria of industrial interest among other lactic bacteria.

2. Materials and methods

2.1. Biological material

The study included the waste of one widely consumed species of fish which was the common sardine (*S. pilchardus*). *S. pilchardus* belongs to the family *Clupeidae*. This is a small fish whose length does not exceed 20 cm and it has a fusiform body which slightly compressed on the flanks and covered with large scales. It also wears a short dorsal fin without spines.

2.2. Microbiological control of the waste

It is important to check if the waste of *S. pilchardus* complies with microbiological requirements specified in his monograph in the Pharmacopoeia[5].

A tube of sample (10 g fish waste) was introduced into and mixed with 9 mL of saline water or trypton salt broth medium thoroughly. Decimal dilutions were prepared from this solution by introducing 1 mL of stock solution in 9 mL saline water. The process was repeated until the desired dilution (the 6th dilution) was obtained.

Various microbiological analyses were applied; a count of the total aerobic mesophilic flora (TAMF), total yeasts and molds count (TYMC) and some pathogens such as *Staphylococcus aureus* (*S.*

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aureus), *Vibrio*, *Pseudomonas aeruginosa*, *Bacillus* were looking for. As for fecal contamination germ, a research for *Escherichia coli* (*E. coli*) is useful.

2.3. Fish waste as a source of lactic acid bacteria

To this point, the same standard protocol for the research of these microorganisms was adopted which comprised enrichment, isolation, identification, purification and conservation[6].

2.3.1. Enrichment and isolation

From a sample of fish waste, decimal dilutions were prepared. One milliliter of the dilution of 10^{-5} and 10^{-6} was placed in man rogosa sharpe (MRS) broth and incubated at 30 °C for 24–48 h. The positive result was indicated by the turbidity in the bouillon. MRS bouillon (0.1 mL) presented turbidity was seeded on MRS agar surface. The incubation was carried out under an aerobic conditions at 30 °C for 1–5 days.

2.3.2. Identification of lactic acid bacteria

Isolated bacterial strains were identified by physiological and biochemical characterizations according to recommended criteria[6-9]. First, the morphology of the isolated strains was studied. Then, the production of catalase, growth at different temperatures and their ability to produce gas from glucose were determined. Macroscopic observation was used to describe the appearance of colonies on agar medium obtained (size, pigmentation, contour, viscosity, etc.).

Microscopic examination was carried out by Gram stain on a young culture of 24 h. The characteristic shapes of microbial cells, their arrangement, the presence or absence of spore and Gram staining were determined.

2.3.3. Test of catalase production

A hydrogen peroxide drop was deposited on a colony for 48 h on agar medium. The result was immediate and characterized by a release of O_2 if the catalase was present[10].

2.3.4. Test of growth at different temperatures

The bacterial strains were seeded in their medium and their growth was tested at two temperatures (15 °C and 45 °C)[6]. The development of strains was assessed after one week of the incubation for cultures at 15 °C and after 24 and 48 h at 45 °C.

2.3.5. Test of CO_2 production

Isolated colonies were seeded on MRS broth modified without citrate and meat extract containing an inverted bell. Incubation was carried out at a temperature of 30 °C for mesophilic bacteria and at 45 °C for thermophilic bacteria for 24–48 h[11].

2.3.6. Purification and conservation of isolates

We conducted a series of seeding by streaks method to have pure cultures. The operation was repeated each time by randomly taking an isolated colony. This led to obtain a culture whose purity was estimated by microscopic observation after Gram staining. The short-term storage of purified isolates was achieved by plating on

inclined agar. After 24 h of incubation, the tubes were placed at 4 °C where they can be stored for several weeks[10].

3. Results

3.1. Microbiological control of the waste

According to the results, a sizeable presence of germs confirmed by macroscopic (colonies) and microscopic (Gram stain) observation was noticed (Table 1). Among these germs, *E. coli* species with a rate of 7.102 colony-forming unit (CFU)/mL, the sulfite-reducing clostridium and fecal contamination indicators were found. Their presences have resulted in a complete blackening of the medium. Then, *Staphylococci*, a Gram-positive bacteria, that can be arranged in a bunch of golden smooth colonies grew on Chapman agar. The enumeration TAMF and TYMC on preparations gave loads of 76.000 and 5.000 CFU/mL, respectively.

Table 1

Evaluation of the microbial load in the waste of *S. pilchardus*.

Germ	Amount (CFU/ mL)
Total aerobic mesophilic flora (TAMF)	76.000
<i>E. coli</i>	7.102
<i>Streptococcus</i>	104.000
Yeasts and molds (TYMC)	5.000

Vibrio was widespread in freshwater, marine and estuaries. Indeed, settlements in different sizes, beige and shiny have been identified on thiosulfate citrate bile salt sucrose agar turning from green to mid yellow. The results of Gram staining, oxydase test and fermentation of glucose confirmed the presence of *Vibrio* spp. The cetrimide agar has given rise to non-fluorescent white colonies under UV light, which did not correspond to the genus *Pseudomonas*. By contrary, Mossel agar presented colonies of different sizes, beige and other red shiny and smooth. The staining of the spore and the catalase test confirmed the presence of *Bacillus*.

3.2. Fish waste as a source of lactic acid bacteria

The seeding of 0.1 mL of the sample on the acidified MRS medium resulted in, after incubation at 30 °C for 24–48 h, a typical colonies of lactic acid bacteria. The identification results have presented 2 different groups of lactic bacteria (Figure 1).

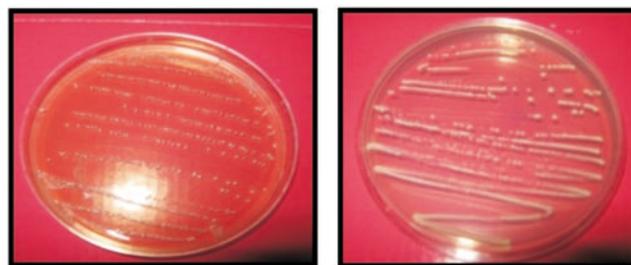


Figure 1. Macroscopic aspect of lactic acid bacteria isolated on MRS medium.

A group of bacteria (LR4), heterofermentatively grew at 15 °C and 45 °C, arginine dihydrolase positive, ferment lactose, raffinose, sucrose and ribose but mannitol, arabinose and xylose. This bacterium was pre- identified as *Lactobacillus fermentum* (*L. fermentum*).

A group (LR6) has atypical characters of *Betabacterium* group. They did not grow at 15 °C with hydrolyze esculin ferment mannitol, rhamnase, xylose and sorbitol. They were pre-identified to the genus *Lactobacillus* spp.

4. Discussion

In this study, it was useful to evaluate the microbial load in our sample to estimate the risk that may incur during a discharge of waste such as fishing waste and consequently explore the different recycling methods. Faecal contamination indicators were generally detected through the estimation of the presence and abundance in the waters of the microorganism (*E. coli*), which represented one of the most significant indicators of sewage contamination[12].

The samples with concentrations of *E. coli* ranging from 10⁶ to 10² CFU/mL were preliminary assayed to estimate the detection limit of the instrument as shown by Grossi *et al.*[13].

S. aureus was differentiated from most other species by the production of a coagulase[14]. In our study, the results of the Gram stain, catalase test and coagulase confirmed the actual presence of two types of species, *S. aureus* and *Staphylococcus saprophyticus*, which were coagulase-negative. These species were part of the normal flora of the fish.

The *Vibrio* was normal inhabitants of marine lives such as shellfish and fish[14]. It explained the presence of such seeds in our products. These results were consistent with the standards of the European Pharmacopoeia.

For the second part of our study, it was dedicated to confirm or deny the presence of lactic acid bacteria. So our second goal was to increase the focus on the operation of such waste as a source of bacteria of industrial interest or as a source of enzymes and antimicrobial substances.

The different steps of identification allowed us to determine two species: *L. fermentum* and *Lactobacillus* spp.

Recent studies reported that *L. fermentum* could present a positive effect in different sectors of agro-alimentary. The oral administration of *L. fermentum*, as a supplement or functional food, produced an increase in the intestinal activity[15].

Moreover, it has been reported that the administration of lactic acid bacteria can enhance metabolic activity markers in animal models of metabolic syndrome and diabetes[16].

In conclusion, the coproducts of *S. pilchardus* were found to have an interest as a source of lactic acid bacteria. The results of physiological and biochemical characterization has presented 2 different groups of lactic bacteria: *L. fermentum* and *Lactobacillus* spp. So, the assessment of fish waste can give us the opportunity to obtain different species of useful bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

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