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ORIGINAL ARTICLE

Valorization of the by-products of bluefin tuna *Thunnus thynnus* (Linnaeus, 1758) of the Algerian West Coast (Mostaganem): Use in Microbiology

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ABSTRACT
Among seafood by-products, tuna heads represent valuable biological resources able to generat various molecules of biological and nutritional interest. The aim of this work is to highlight these by-products (head, skin, viscera, tail, etc...) in the microbiology field. The obtained results, showed that the by-products of bluefin tuna Thunnus Thynnus sampled in the west coast of Algeria has a biological values with appreciable biochemical characteristics (protein: 17%; lipids: 14.5%; Ash: 07.14%). These by-products could replace the basic elements in the preparation of microbiology medium culture and therefore bacterial growth. Through this study we conclude that these by-products form a source of bacteria of industrial interest among them we have lactic bacteria of the genus Thermobacterium.
Key words: bluefin tuna; by-products, Microbiology, Lactic acid bacteria; West coast of Algeria.

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INTRODUCTION

The bluefin tuna *Thunnus thynnus* (Linnaeus, 1758) belongs to the family of Scombridae. Its maximum size could reach 4.5 m (and 680 Kg of weight). This big pelagic fish is frequent in Algerian fisheries and markets during the period from May until August (fishing period). The demand for bluefin tuna has exploded over the last thirty years, generating an important quantity of by-product. The by-products are estimated at 50% of the total weight of the fish [1]. These by-products or wastes are defined as unused parts, but can be salvageable [2]. Better management of these wastes during the threading, would allow a decrease in their impact on the environment. The valorization of these by-products is especially intended for the production of fishmeal, oil or mince for animal feed, nutraceuticals, pharmaceuticals, etc. [3].
Lactic acid bacteria is founded in the environment at the free form, they are present in different ecological niches such as milk, dairy products, plants, meat, fish, human and animal mucous membranes [4,5]. Among the lactic bacteria, *Lactobacillus* is the most widespread genus. This last comprises three subgenus: *Thermobacterium*, *Streptobacterium* and *Betabacterium* [6,7]. Lactic acid bacteria need an exogenous source of amino acids for growth because most of them cannot synthesize them from a simple nitrogen source. [8,9]. Several works have been undertaken on lactic bacteria, including the genus *Lactobacillus* as a bioprotector of food and marine products towards pathogenic germs. [10-12].

MATERIAL AND METHODS

Biological material

A sample of bluefin tuna by-products was obtained in fresh conditions from a fishery market located at Mostaganem City, Algeria in April 2017. After filleting one individual of 1.15 m in size almost 78 kg in weight, the rejected parts (head, ribs, tail) were collected. These by-products represent 42% of the total

weight of the fish (Fig 01). At the laboratory, the by-products were finely chopped and mixed with a laboratory blender (Waring Blendor, N ° 087137, USA) and kept at -20 °C until their examination.

Preparation of the protein isolate

The protein isolate refers to a product that is characterized by its protein richness with low ash quantities. In general, the chemical hydrolysis of the maceration is realized by NaOH (0.12 N, 70 °C, 120 min), to reach pH 12.5. Then, a solution of H_2O_2 (pH 3.5-4.5) is added to the mixture to reach a pH of 11.5 by lowering the temperature to 50 °C. The addition of HCl (concentrate) stabilizes the action of H_2O_2 and precipitates the proteins by reaching a pH of 4.5.After the first centrifugation, the obtained pellet is washed 2 to 3 times with an isopropanol solution (C_3H_8O) (at 60 °C, 15 min). A second centrifugation separates the pellet from the supernatant (oil and isopropanol). The resulting pellet is then washed 2 to 3 times until pH neutral, then dried in the universal oven at 50 °C and stored at -20 °C.

Biochemical characterization of the obtained products

The protein, lipid, ash, and moisture content of the tuna by-products and the protein isolate were determined using methods Nos. 984.13, 927.05, 920.39 B and 942.05, respectively [13] (AOAC, 2000). A factor of 6.25 was used to convert the value of nitrogen to protein. All Measurements were performed in triplicate and the results are the average of three values.

Preparation of culture media

The control culture media were prepared from Tryptone (TGEA: Tryptone Glucose Extract agar) and (MRS: Man agar, Rogosa, Sharpe). Other culture media were modified ($TGEA_M$ and MRS_M) and prepared with tuna by-products and protein isolate in place of Tryptone ($TGEA_{M1}$, MRS_{M1}; $TGEA_{M2}$, MRS_{M2}, respectively). For this, two bacteria were used [*Escherichia coli* (Gram negative) and a lactic bacterium *Lactobacillus bulgaricus* (Gram positive)].

Isolation of lactic acid bacteria from tuna by-products

A standard protocol for isolate and identify this type of bacteria was followed. A solution was prepared from 10 g of sample (by-product of tuna) in 90 mL of physiological water. From this stock solution, decimal dilutions 10^{-5} and 10^{-6} were incubated in MRS broth (at 30 °C for 24 to 48 h). A positive result is indicated by turbidity in the broth. From a positive tube, a sample of 0.1 mL was spread on the surface of MRS agar and incubated in anaerobic at 30 °C for 24 h to 5 days. The identification of lactic acid bacteria was realized by several tests: 1) A macroscopic observation which allows describing the appearance of the colonies, obtained on agar medium (size, pigmentation, contour, viscosity...); 2) Microscopic examination was released with a light microscope of a colonies after Gram stain. Determination of the characteristic forms of the microbial cells, their arrangement, the presence of spores and their Gram staining; 3) Catalytic activity consists on taking a colony from MRS agar and dissociated in a drop of oxygenated water (H_2O_2) at 10 volume. The result is immediate and is characterized by gaseous release (O_2) if catalase is present. [14] ; 4) A test of bacterial growth at different temperatures differentiates mesophilic from thermophilic lactic acid bacteria [15,16]. The developed strains were inoculated into MRS Broth at two different temperatures (at 15 °C for 7 days) and (at 45 °C for 24 to 48 h) and 5) The fermentative test was realized by seeding the strains into the MRS broth containing the Durham tubes and incubated (at 37 °C, for 24 to 48 h). The development of a heterofermentary bacteria is manifested by the appearance of gas in the Durham tubes, which is absent in homofermentary bacteria [17].

RESULT AND DISCUSSION

Biochemical analysis of tuna by-products and protein isolate

After the different steps of extraction of the protein isolate, the mass yield obtained has a low quantity of only 24%.

The chemical composition of the by-products and the protein isolate are presented in table 01. The results showed that the protein isolate is a potential source of protein (57.23%) and a low quantity of lipid and ash (8.32 and 7.21%, respectively), compared to the raw material (by-products).

The biochemical composition of proteins and lipids of the *T. Thynnus* by-products are a slight similar to those obtained by Nguyen et al [18] for the heads of *Thunnus albacares* (Bonnaterre, 1788) fishing in the Pacific Ocean (36.09% and 32.92% respectively) in relation to the dry matter. While the ash rate, obtained by the same authors was very high (28.78%) compared to our sample which was only 10.15% (Tab. 01).

Microbiological Analyses

Microbiological Study of modified culture media

MRS culture media prepared with bluefin tuna (MRS_{M1}) and protein isolate (MRS_{M2}) were of transparent color and a homogeneous structure than those of $MRS_{control}$. The colonies of the lactic bacteria were whitish, convex, smooth, on board regular and small in diameter of about 2 mm. In general, lactic bacteria

develop after only two days on MRS medium (Fig. 02) by degrading the essential components present in the medium such as proteins. In contrary, the appearance of these bacteria requires a slower incubation on both media MRS_{M1} and MRS_{M2} (3 and 4 days, respectively) compared to the $MRS_{control}$, which requires only 2 days for the appearance of the bacteria. The results obtained also showed that there is a difference in the number of bacterial colonies developed on the two culture media (MRS_{M1} and MRS_{M2}) compared to the $MRS_{control}$.

For the growth of *E. coli* (Fig. 03), the development of the bacteria on the three media ($TGEA_{control}$, $TGEA_{M1}$, and $TGEA_{M2}$) was important and similar compared to the number of colonies developed, but with a slight difference in the duration of bacterial appearance (6 to 8 h).

Table 01: Biochemical composition expressed in (%) of the by-products of *T. Thynnus* and of the protein isolate in relation to the dry matter.

Composition	Dry matter	Ash	Lipids	Protein
By-product of *T. thynnus*	70.3 ± 1.02	10.15 ± 0.98	31.29 ± 1.31	34.85 ± 0.88
Protein isolate	96.1 ± 0.45	07.21 ± 0.09	08.32 ± 0.71	57.23 ± 1.67

Values are mean ± SD (n = 3)

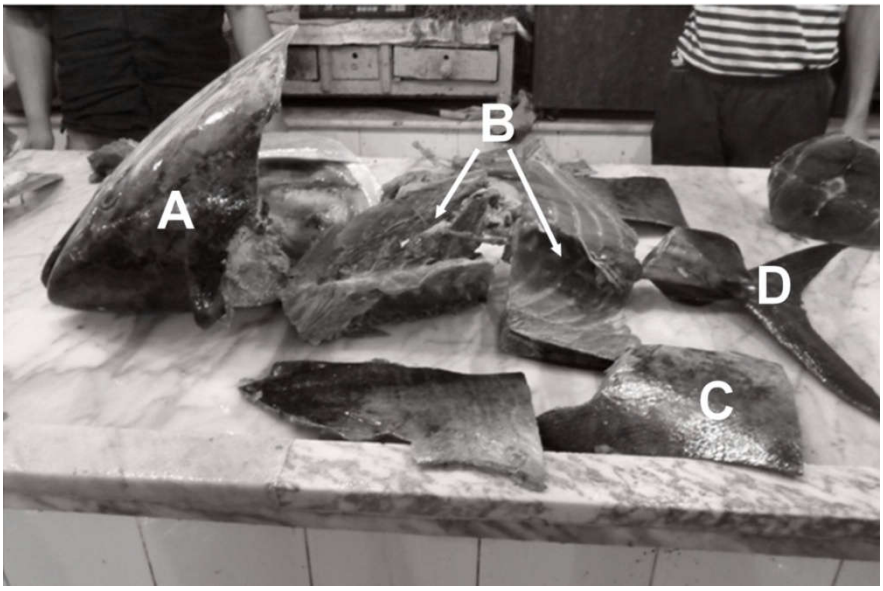

Figure 01: The By-products of bluefin tuna (*T. Thynnus*) recovered after filleting (the rejected part: (a) head, (B) skin, (C) ribs, and (D) tail).

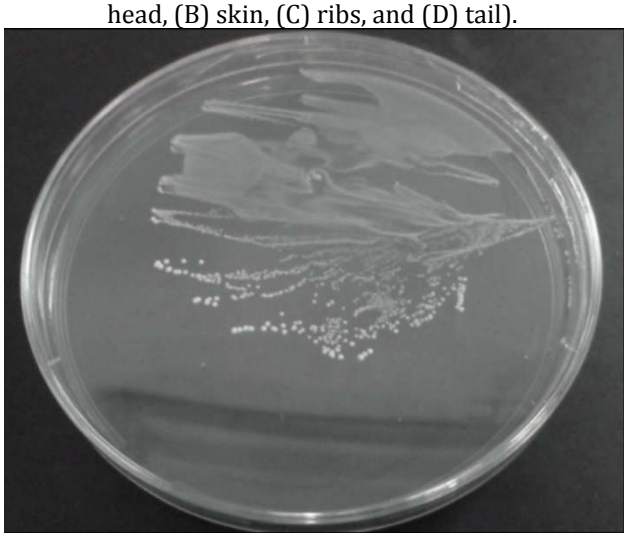

Figure 02: Macroscopic aspect of the development of the lactic bacterium *Lactobacillus bulgaricus* on MRS agar.

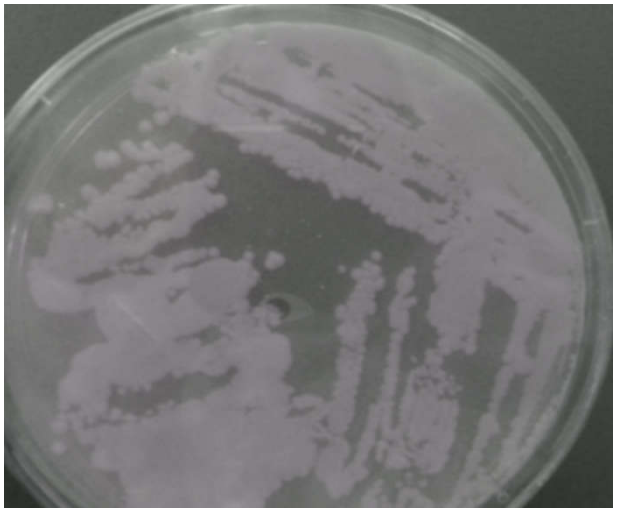

Figure 03: Macroscopic aspect of *E. coli* bacteria development on TGEA agar.

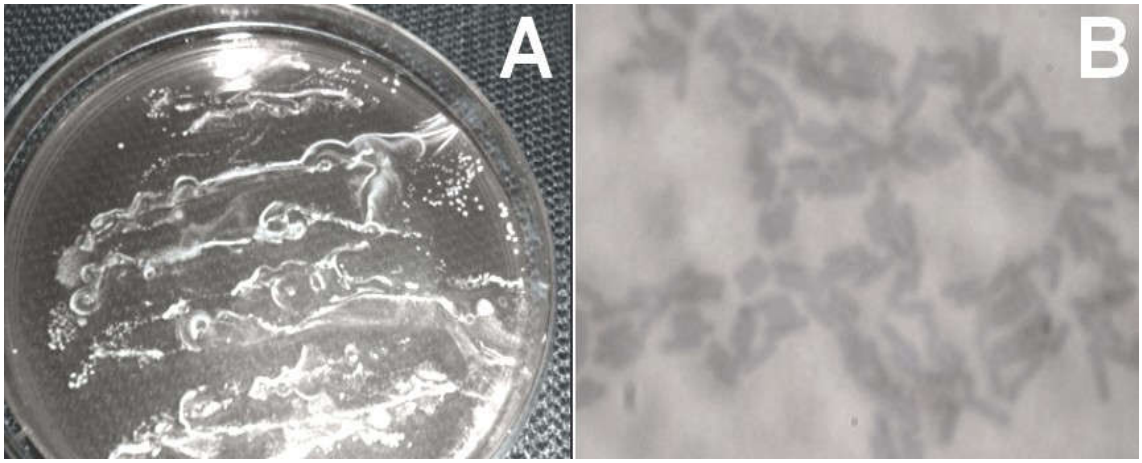

Figure 04: Macroscopic appearance of the lactic bacterium isolated in MRS (A); Microscopic appearance of the lactic bacterium isolated after gram staining (B).

Isolation of lactic acid bacteria from by-products

On both MRS broth (dilutions of 10^{-5} and 10^{-6} of solution of bluefin tuna by-products) and after incubation (at 30 °C for 24 to 48 h), we obtained positive results indicated by turbidity in MRS broth. This conducts us to deepen further the analysis: Isolation on MRS agar and Gram staining to confirm the presence of the lactic bacterium.

Inoculation on MRS acidified Agar gave rise, after incubation of 24 to 48 h, to colonies characteristic of a single lactic acid bacteria (Fig. 04 A). The bacteria was also identified by microscopic observations which gave a bacteria (Gram positive) of a rod shaped (bacilli) (Fig. 04 B). The developed bacteria, is called thermophilic because it develops at 45 °C and not at 15 °C. It is also characterized by a negative catalase. Thus, it is a lactic bacterium of the *Thermobacterium* group.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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