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Short Communication

Fishery by-product as a nutrient source for bacteria and archaea growth media

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Abstract

A highly soluble fish protein hydrolysates (FPH) with an 80% protein (peptide size between 1.5 and 20 kDa) and a low free amino acid content was obtained from hake (*Merluccius hubssi*) filleting waste [Lat. Am. Appl. Res. 30 (2000) 241]. Assays with *Halobacterium salinarum*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus epidermidis* were performed in order to test that FPH as nutrient source for archaea and eubacteria culture media. Cell growth was evaluated by plate count, and by monitoring turbidity and nucleic acids content in liquid cultures. Neither cell growth nor generation times resulting from control and FPH cultures exhibited statistically significant differences at α: 0.05 suggesting that FPH can be used as an alternative substrate for microorganism cultural purposes.
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Keywords: Fish protein hydrolysate; Fish waste; Culture media; Bacteria; Archaea

1. Introduction

Food industry wastes are an important environmental contamination source. Research has been carried out in order to develop methods to transform these wastes into useful products (Perea et al., 1993; Kristinsson and Rasco, 2000; Larsen et al., 2000; Guérard et al., 2001; Coello et al., 2002; Laufenberg et al., 2003). Probably more than 50% of the remaining material from the total fish capture is not used as food and involves near 32 million tons of waste (Kristinsson and Rasco, 2000). Because this secondary raw material includes head, viscera, skin, bone and some muscle tissue, it is an important protein source. However environmental regulations are becoming stricter, requiring new methods for discarding fish waste. An interesting alternative is hydrolyzing waste to obtain fish protein hydrolysates (FPH), containing proteins with desirable functional properties. Protein hydrolystes have been obtained by different methods such as utilization of acids (Espe et al., 1992), organic solvents (Durand, 1976), exogenous proteases (Ritchie and Mackie, 1992; Baca et al., 1991; Guérard et al., 2001) or enzymatic autolysis (Cassia et al., 2000; Kristinsson and Rasco, 2000). Although FPH seems to be a suitable and economical protein source for animal (Kristinsson and Rasco, 2000) and some bacteria (Clausen et al., 1985; Dufoseé et al., 2001) taste defects specifically bitterness may limit this application in human nutrition (Kristinsson and Rasco, 2000).

In our laboratory a FPH was obtained by autolysis of fishery wastes. It yields an amino acid composition similar to the FAO/WHO (Food and Agricultural Organization—World Health Organization, 1985) standard with a low content of hydrophobic amino acids and no microbial contamination (Cassia et al., 2000). This FPH may be a suitable nutrient source for rats, pets, aquaculture, bacteria and other commercially grown organisms. As nutrient sources represents the major cost in the production of microbial cell mass, the purpose of this work was to study the ability of the FPH prepared from hake (*Merluccius hubbsi*) waste to support bacterial growth.

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2. Methods

2.1. Preparation of fish protein hydrolysate

Fish protein hydrolysate (FPH) was obtained from hake (*M. hubbsi*) filleting waste, as described by Cassia et al. (2000). Briefly, fish wastes were minced, mixed with water and allowed to autolyse at 60 °C. After centrifugation the supernatant was freeze–dried to obtain solid FPH. The solubility in water of FPH was 100% at pH 6 or higher, and 80–90% at pH levels below 5. The FPH samples employed resulted from, at least, three batch preparations of 2–5 fish each.

2.2. Microorganisms

Three bacterial species and one archaea were employed: *Escherichia coli* XL 1 blue (Stratagene, La Jolla, CA, USA), *Bacillus subtilis*, *Staphylococcus epidermidis* and *Halobacterium salinarum* were strains isolated and identified by Servicio Nacional de Sanidad Animal (SENASA, Mar del Plata, Argentina).

2.3. Cultivation media and growth conditions

Luria–Bertani (LB) medium (Sambrook et al., 1989) was used as a reference for bacteria, and the halobacterial medium (DSM 97) (DasSarma et al., 1995) was used as the reference medium for archaea. The modified media contained FPH as the sole source of organic compounds and the same saline composition as the reference media. The pH of all the media were 7.2–7.5 before autoclaving at 121 °C for 20 min. The cultures were initially grown in the corresponding liquid reference medium in aerobic conditions with shaking (150 rpm, Lab-line ORBIT shaker no. 3591 Lab-line Instrument Inc., Melrose Park, IL, USA) until early exponential growth (A_{600} 0.3–0.4, spectrophotometer Beckman DU-530, Beckman Instruments Inc., Fullerton, CA, USA). These cultures were used as inoculum (1:500) for the following assays.

Plate counts were performed in reference or FPH media plus 1.5% agar. Pour plate technique was used for enumerating *E. coli* and *S. epidermidis*. The samples of *B. subtilis* and *H. salinarum*, were applied on the surface using a glass-spreading rod. The number of colonies was recorded after incubation (room at 37 °C) overnight for bacteria and 6 days for archaeas. These assays were performed independently at least four times using 2–8 plates each time.

Bacterial growth in liquid media was monitored by reading turbidity at 600 nm (A_{600}) and by extraction of nucleic acids and measuring absorbance at 260 nm (A_{260}) (Fleck and Munro, 1962). Briefly, to perform nucleic acid extraction cells pelleted from culture aliquots were washed with isotonic NaCl and centrifuged. Cells pelleted were treated with perchloric acid (0.2 N), and centrifuged. Pellets were subsequently resuspended in KOH (0.3 N) and incubated 40 min at 40 °C (water bath, Vicking Argentina). The reaction was stopped with cool perchloric acid (0.5 N) and samples centrifuged. Ribonucleic acid was measured in supernatant and pellet was resuspended in perchloric acid (0.5 N), incubated 1 h at 70 °C and centrifuged in order to measured DNA in supernatant. The experiments in liquid media were performed independently, at least three times. All the cultures were incubated at 37 °C (incubation room). The generation time (g) was calculated using A_{600} and A_{260} data from the lineal zone of the semilog propagation curves (Fig. 1).

2.4. Statistical analysis

Colony forming units (CFU) data were analyzed statistically as a randomized complete block design fitting a model: date + media + date × media (error) using analysis of variance: ANOVA (SAS, 1985). Analyzed data are average of 2–8 replicates performed in at least four independent experiments (date).

Generation time (g) data were analyzed statistically according to split-plot complete block randomized design, the model fitted was: date + media + date × media (main plot error) + measure + date × measure + media × measure (split-plot error) using generalized linear model: GLM (SAS, 1985). Analyzed data are replicates of g values (measure) obtained from at least three independent experiments (date). Means were compared using Duncan's multiple range test. The level of probability used to assess statistical significance was α: 0.05.

3. Results and discussion

3.1. Media composition

A highly soluble fish protein hydrolysates: FPH containing 80% protein (peptide size between 1.5 and 20 kDa) and a low free amino acids content was obtained in our laboratory from hake (*M. hubssi*) filleting waste (Cassia et al., 2000).The high quality of fish proteins has long been recognized, however, a great amount is used in poor quality products such as fish meal. Only about 50% of the fish processing waste with a high protein content (10–14% w/w) is typically recovered.

General purpose culture media contain digested proteins as the main source of organic carbon and nitrogen. They are usually supplied with meat or yeast extracts that also provide essential ions, minerals and vitamins (Merck, 1981). Taking into account the properties of the hake FPH prepared by us, we examined its potential as the sole source of organic carbon and nitrogen for bacteria and haloarchaea culture media. To

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