ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(4):910-913

ORIGINAL RESEARCH ARTICLE

Studies on Growth of Marine Bacteria Using Marine Fish Waste Medium

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Received 18 May 2012; Revised 10 Aug 2012; Accepted 20 Aug 2012

ABSTRACT

Peptones from fishes have been used only to a small extent and it is the most important ingredient of culture media. It would be advantageous if low-cost fish species and waste material could be converted into a byproduct of commercial value in such a way where peptones used as nitrogenous substances for the constituent in fermentation media. The present study made on marine peptone medium is possible to offer on impetus for further research in this field. This preliminary investigation provided baseline information on marine fish medium for further detailed research on using marine peptone as a suitable nutrient for large-scale industrial applications with environmental use in addition can be performed for its immense potential.

Key words: Peptones, Fish waste, Amino acids and Peptides.

1. INTRODUCTION

Nitrogen source is usually the most high-priced constituent of bacterial growth media. Organic nitrogen substrates such as peptones are widely used in many biological and biotechnological applications like microbial biomass production. Proteolytic micro-organisms can grow only in media containing peptones because of their rich source of amino acids and peptides, peptones have one of the most important ingredients of culture media [1]. Nowadays, the commercial peptones appear from the source of casein, soy, gelatin and meat. The peptone from fishes has been only used to small extent. It would be advantageous if low-cost fish species and waste material could be converted into a by-product of commercial value like peptones used as nitrogen substrates for fermentation media.

Seafood products are obtained from a wide variety of fishes. However, only a part of those fishes are usually used as food. Fish viscera, fins and heads represent an environmental problem to the fishing industry. Traditionally this material has been converted to powdered fish for animal feed [2]. The fish wastes contain high protein value. The composition of widespread profitable media for culturing marine bacteria is basically seawater with peptone and yeast extract, lacking the carbohydrates usually incorporated in "Terrestrial" media. Even though their essential simple preparation, these media are expensive. It would thus, be reasonable to assume that a similar medium prepared with seawater and peptones easily obtainable from fish residues and this significantly reduce culture costs. Particularly, fish protein hyrolysates have been reported as growth substrates for bacteria [3, 4].

One of the first reports on fish peptones for microbial media developed from red hake and from fishery by-product [5]. A new marine medium use of different fish peptones and comparative study of growth of selected species of marine bacteria [6], in this work they reported that fish peptones used for culturing marine bacteria are more appropriate than those from other commercial peptone for culturing marine bacteria. [7] reported that the *Sardinella aurita* heads and viscera flour enhanced protease production up to 100% more than commercial peptones when tested for protease production by *Bacillus subtilis.* Nereida Coello [8] reported that culture media were supplemented with fish silage at 40g/l the concentrations of biomass, served as a fermentation substrate for the extracellular production of L-lysine by *Corynebacterium glutamicum*. There are several reports pointed out about fish waste hydrolysis for lactic acid fermentation, recently reported by [9].

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At the present time, natural resources and the environment are under threat and are becoming increasingly protected by law. The catching and processing of fish generates a significant amount of waste. Traditionally, this substance has been transformed to powdered fish flour used for animal feed. Fish waste is also rich in potentially valuable oils, minerals, enzymes, pigments and flavors and so on, and these wastes may alternative uses in food, pharmaceutical, agricultural, aquacultural and industrial applications [10]. However, no study has been reported on the use of fish protein particularly from oil sardine (*Sardinella longiceps)* wastes used as growth substrates for bacteria subjected to any detailed study in the Indian context. Hence, the present study was undertaken to asses their suitability for preparing low price marine media using oil sardine (*Sardinella longiceps*) waste material and compare the results with obtained on standard growth substrate for culture of marine bacteria.

2. MATERIALS AND METHODS

2.1. Collection of samples:

Fish wastes (viscera, head, fins, bones) of oil sardine (*Sardinella longiceps*) were collected from Parangipettai landing centre and brought to the laboratory and stored at -20° C until used.

2.2. Preparation of fish peptone medium:

The stored fish wastes were minced by a grinder [11] and the homogenized samples were mixed with distilled water to make fish wastes (wet weight/water) in a ratio of 1:1. Half of the waste was defatted by using solvent mixture of Acetone and Methanol at the ratio 1: 2. Two types of fish waste slurry were taken. One is defatted fish waste slurry and another is slurry with lipid content (Sample – FS). Defatted fish waste slurry was taken and the initial pH of the slurry was set to 1 by the addition of (6Mol. HCl). The slurry was dried at 121°C for 20 min, and centrifuged at 4000rpm for 20 min. The supernatant was collected, that it is the defatted fish waste slurry (Sample - DFS). These FS and DFS samples were stored at -20° C until application and it was used as peptone source in culture media. Instead of using commercial peptone this marine peptone tested as a nutrient source (**Table 1**).

Table 1: Culture medium

Ingredients	CP	DFS	FS
Yeast extract	0.3g	0.3g	0.3g
Beef extract	0.3g	0.3g	0.3g
Agar	2g	2g	2g
Peptone	0.5g	1 ml	1 ml
pH	7±0.2	7±0.2	7±0.2
NaCl	0.5g	0.5	0.5g
Distilled water	100 ml	100 ml	100 ml

2.3. Microbiological tests

Sediment sample was collected from vellar estuary and it was serially diluted in sterilized distilled water upto 10^{-6}. Among those dilutions 10^{-3}, and 10^{-4} were taken and plated in three types of medium, (i) commercial peptone medium (CP), (ii) Defatted fish waste peptone medium (DFS), (iii) Fish waste peptone with fat content (FS) which are all prepared in distilled water. The inoculated plates were incubated at 27°C for 48 hours. After 48 hours development of colonies in three different medium were observed. Then the colonies were manually counted. The results were compared between three medium and expressed in colony forming units per ml (CFU ml^{-1}).

3. RESULTS AND DISCUSSION

The preliminary tests compared the variations in growth of bacterial colonies at 10^{-3} and 10^{-4} dilutions in three different medium like commercial peptone medium, defatted fish waste peptone medium and fatted fish waste peptone medium. The marine peptone source medium was tested with commercial peptone source. It is clear that, the effect of Defatted marine peptone medium at 10^{-3} dilution appeared more bacterial growth than fatted marine peptone and commercial peptone. Results of bacterial growth are shown in (**Table 2**) and Plates.

Table 2: Shows the growth of bacterial colonies in different culture

S. No	Dilution taken	Total number of colonies obtained in different culture medium (CFU ml^{-1})		
		XCP	DFS	FS
1	Control	-	-	-
2	10^{-3}	72	107	7
3	10^{-4}	5	26	3

XCP - Commercial peptone medium; DFS - defatted fish waste peptone sample; FS - Fish waste sample with fat content

In the present study, different peptone media was used for bacterial growth, it is clear that highest bacterial growth is seen in defatted fish waste peptone medium (DFS) at 10^{-3} dilution (Sediment sample). The nitrogen source is usually the most high-priced constituent of bacterial growth media. Proteolytic microorganisms can grow only in media containing peptones because of their rich source of amino acids and peptides. Peptones are one of the most important ingredients of culture media [12]. Nereida coello [13] reported that culture media supplemented with fish silage at 40g/l the concentrations of biomass, served as a fermentation substrate for the extracellular production of L-lysine by *Corynebacterium glutamicum*, showed fish silage promotes biomass and L-lysine production. Though there are several reports pointed about fish waste hydrolysis for lactic acid fermentation [14]. It was also examined

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that unhydrolyzed fish wastes were very poor in lactic acid production. When compared to commercial peptone the low cost nutrients of the fish waste hydrolysis had high performance in lactic acid production [15,16].

Subsequently fish peptones also used by new method based on Gompertz modeling of microbial growth, their performances appear comparable to the usual casein hydrolyzed at this point they tested nitrogen-limiting media, and evaluation of nitrogenous substrates (peptone from fish waste) for microbial growth. According to Ellouz *et al*. [3], the sardinelle waste peptone tested for protease production by *Bacillus subtilis* is useful more than commercial peptone.

Nowadays, natural resources and the environment are under threat and are becoming increasingly protected by law. The catching and processing of fish generates a significant amount of waste. Traditionally, this material has been converted to powdered fish flour used as animal feed. Fish waste is also rich in potentially valuable oils, minerals, enzymes, pigments and flavors etc., that have many alternative uses in food, pharmaceutical, agricultural, aquaculture and industrial applications.

In addition to fish meal and oil production there is potential in silage production, fertilizer, composing, fish protein hydrolysate and fish concentrate were used as nitrogen sources for the production of extracellular lipase. The marine media contain fish waste peptone were used in the place of using commercial peptone for microbiological analysis. It reduces culture costs. This advantage would be particularly pertinent when medium or large scale production is required. For example in the production of probiotics destined for aquaculture and it was also tested in biotransformation of fish waste by fermentation.

4. CONCLUSION

Organic nitrogen substrates such as peptones are widely used in many biological and biotechnological applications like microbial biomass production. Proteolytic micro-organisms can grow only in media containing peptones because of their rich source of amino acids and peptides. Peptones have one of the most important ingredients of culture media. Nowadays, the commercial peptones appear from the source of casein, soy, gelatin and meat. The peptones from fishes have been used only to a small extent. It would be advantageous if low-cost fish species and waste material could be converted into a by-product of commercial value like peptones used as nitrogen substrates for fermentation media.

Fish wastes of oil sardine (*Sardinella longiceps*) were collected from Parangipettai landing centre. Wastes were minced and one half of the waste slurry was defatted using solvents acetone and methanol. Further, the pH of the slurry was setted using 6 M HCl and then 10g of this sample was centrifuged to get purified protein and stored at -20°C until application in media.

The sediment sample collected from vellar estuary was serially diluted and plated using different peptone source and tested the growth of bacterial colonies. The results indicate that protein isolated from marine fish waste can be used as good peptone source for culturing marine microbes instead of other commercial peptone in culture medium. Thus, the present study made on marine peptone medium (oil sardine waste) is possible to offer an impetus for further research in this field. This preliminary investigation provided baseline information on marine fish waste medium, further detailed research on using marine peptone (fish waste) as suitable nutrients for large scale industrial applications with an environmental solution in addition can be done.

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