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SCIENCE DIRECT®
Bioresource Technology 97 (2006) 605–613
BIORESOURCE TECHNOLOGY

Preliminary tests on nisin and pediocin production using waste protein sources Factorial and kinetic studies

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Received 23 August 2004; received in revised form 10 January 2005; accepted 23 March 2005
Available online 23 May 2005

Abstract

Lactic acid bacteria, the object of current interest as bacteriocin producers, are microorganisms with complex requirements for peptidic sources, making them appropriate indicators for testing the suitability of formulations based on proteinaceous wastes for use as microbiological media. Different peptones obtained from visceral and fish muscle residues promoted growth of lactic acid bacteria when applied individually or in combination. Kinetic parameters and bacteriocin production were similar and, in some cases (pediocin), far superior (>500%) to those obtained with bactopeptones and commercial media specifically recommended for lactic acid bacteria growth. Visceral residues, especially when subjected to a brief process of autohydrolysis at 20 °C, were more efficient for bacterial growth than muscle, even when muscle was treated with pepsin.

Keywords: Nisin production; Pediocin production; Fish peptones; Waste medium

1. Introduction

Bacteriocins produced by lactic acid bacteria (LAB) are peptides degradable by intestinal proteases, with relatively specific antimicrobial activities and a number of applications in the field of food protection. In general, for LAB cultures semi-synthetic complex media are recommended, such as MRS, TGE, APT (e.g. Daba et al., 1993; Jensen and Hammer, 1993; De Vuyst, 1995). Although commercially available, these media are expensive, making the search for cheaper formulations for bacteriocin production attractive. Possible alternatives include residual media such as milk whey or mussel processing wastes (Goulhen et al., 1999; Guerra and Pastrana, 2002; Amiali et al., 1998; Vázquez et al., 2003).

The recommended media for LAB culture usually contain a surplus of proteins (tryptone, peptone, meat extract, yeast extract), a substantial proportion of which remain unconsumed, involving superfluous cost and hindering bacteriocin purification. If the role of the proteins is limited to the contribution of nitrogen, the problem could be resolved by using inorganic sources (Guerra and Pastrana, 2001), or by reducing the initial protein level to a value of the order of that consumed (Vázquez, 2001; Cabo, 1998). However, it has been suggested (e.g. Jensen and Hammer, 1993; De Vuyst, 1995) that the most important feature of these protein sources is their content of peptides that can act as inducers or precursors of bacteriocin biosynthesis, a hypothesis that explains the poor results of the aforementioned solutions.

The most common peptones—water-soluble, non-heat-coagulable protein hydrolysates, after Green et al. (1977)—in microbiological media are those derived from casein, soya, gelatine and meat. Peptones from fish are

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doi:10.1016/j.biortech.2005.03.020

uncommon, despite their good yields in areas such as the production of proteases by *Bacillus subtilis* (Ellouz et al., 2001), production of gastrine and epidermal growth factor (EGF) by mouse fibroblasts (Cancre et al., 1999), production of glycerol by *Saccharomyces cerevisiae* (Kurbanoglu and Kurbanoglu, 2003) and promotion of good microbial growth in other cases (De la Broise et al., 1998; Dufossé et al., 2001; Guo-Qiang and Page, 1994; Page, 1992; Green et al., 1977; Clausen et al., 1985; Gildberg et al., 1989; Vecht Lifshitz et al., 1990; Jassim et al., 1988; Dufossé et al., 1997).

The study described in this paper was an investigation of the use of protein hydrolysates obtained from residues of fishery products as peptide sources for the production of nisin and pediocin by *Lactococcus lactis* and *Pediococcus acidilactici*, respectively. The interchangeability of different products was studied with a factorial approximation, and yields studied by kinetic analysis. In both approaches, the comparison of the results with those obtained using a commercial bactopeptone, or with MRS medium, demonstrated the efficiency of the residues for the proposed objective.

2. Methods

2.1. Preparation of fish peptones

Raw materials used were viscera from rainbow trout (*Oncorhynchus mykiss*) and squid (*Loligo vulgaris*) and muscular wastes from swordfish (*Xiphias gladius*), sampled immediately after industrial processing and maintained at −20 °C until use. Storage did not exceed 15 days for the viscera. Each sample was triturated with 10% (v/w) distilled water, followed by determination of the proteolytic activity present in the homogenate. The trout homogenates at pH 6.5 were maintained for 6 h at 20 °C with orbital shaking at 100 rpm to promote hydrolysis with the enzymatic contents. Analogous treatment was applied to the squid homogenates, prolonging autohydrolysis until 24 h due to the low levels of proteolytic activity. Swordfish (muscle) homogenates, with trace proteolytic activity, were acidified to pH 2 and treated for 12 h with 0.1% (w/w) pepsin (Merck, 700 FIP-U/g) under identical conditions of temperature and shaking. The hydrolysates were sterilized by steam flow in an autoclave at (101 °C for 1 h), filtered with paper (Whatman No. 2), and the filtrates obtained (fish peptones, Table 1) stored at −20 °C until use in the cultures. For trout, a peptone was also prepared by omitting the autohydrolysis step.

2.2. Microbiological methods

The microorganisms used were *L. lactis* CECT 539 (abbreviated key Lc 1.04) from the Spanish Type Culture Collection, and *P. acidilactici* NRRL B-5627 (Pc 1.02), kindly provided by the Northern Regional Research Laboratory (Peoria, Illinois, USA). The methods for pediocin and nisin extraction and quantification have been described in detail previously (Cabo et al., 1999), using *Carnobacterium piscicola* CECT 4020 as an indicator. Stock cultures were stored at −50 °C in powdered skimmed milk suspension with 25% glycerol (Cabo et al., 2001a,b). Inocula (1% v/v) consisted of cellular suspensions from 12 (Lc 1.04) and 24 (Pc 1.02) hour-aged cultures on MRS medium, adjusted to an OD (700 nm) of 0.900. Incubations were carried out with orbital shaking at 200 rpm and 30 °C.

Table 1
Peptone extracts from fish wastes (see text)

	Protein (g/l)	Reducing sugars (g/l)
Th: Autohydrolyzed trout viscera	39.52	2.85
T: Trout viscera without autohydrolysis	39.89	2.55
Qh: Autohydrolyzed squid viscera	40.00	0.80
Sh: Sword fish muscle (pepsin hydrolysate)	40.60	–

The compositions of media used in the kinetic studies are shown in Table 2. For comparison, a medium (*D*) was used in which fish peptones were substituted for a commercial bactopeptone solution with an equivalent level of protein (Lowry), as well as a commercial MRS medium. The media used in the factorial experiments are described below in Section 2.4. In all cases, initial pH was adjusted to 7.0 and solutions sterilised at 121 °C for 15 min, with cultures carried out in duplicate. In the kinetic experiments, the microorganisms were grown in 300 ml Erlenmeyer flasks with 200 ml of medium. In the factorial approaches, 300 ml Erlenmeyer flasks with 72 ml medium were used.

2.3. Analytical methods

Proteolytic activity was estimated by the method of Barker and Worgan (1981). In microbiological assays,

Table 2
Compositions of media used in kinetic studies (g/l)

	FP media[a]	*D* medium	MRS medium
Glucose	20.00	20.00	20.00
Yeast extract	2.00	2.00	4.00
Sodium acetate	3.00	3.00	5.00
Ammonium citrate	2.00	2.00	2.00
K_2HPO_4	2.00	2.00	2.00
$MgSO_4$	0.20	0.20	0.20
$MnSO_4$	0.05	0.05	0.05
Tween 80	–	–	1.00
Meat extract	–	–	8.00
Bactopeptone	–	10.00	10.00
Fish peptone[a] protein (Lowry)	10.00	–	–

[a] The four types of fish peptones defined in Table 1.