International Journal of Food Microbiology 141 (2010) 28–31

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Some growth parameters and antimicrobial activity of a bacteriocin-producing strain *Pediococcus acidilactici* 13

Evrim Gunes Altuntas [a], Serap Cosansu [b], Kamuran Ayhan [a,*]

[a] Ankara University, Engineering Faculty, Food Engineering Department, Diskapi Campus, 06110, Diskapi/Ankara, Turkey
[b] Department of Food Engineering, Faculty of Engineering, Sakarya University, Sakarya 54187, Turkey

ARTICLE INFO

Article history:
Received 10 February 2010
Received in revised form 19 April 2010
Accepted 23 April 2010

Keywords:
Pediococci
Growth parameters
Bacteriocin

ABSTRACT

Some growth parameters and bacteriocin production by the strain *Pediococcus acidilactici* 13, were screened in this study. The inhibitor substance produced by this strain showed strong antimicrobial activity at 204,800 AU/mL (Activity Units/mL) against the gram positive food borne pathogen, *Listeria monocytogenes*, when the incubation temperature was 37 °C and the initial pH of the medium, TGE (Trypton Glucose Yeast Extract), was 6.0. The bacteriocin-producing strain had considerable resistance NaCl, since the strain maintained to growth up to 10% NaCl concentrations at TGE broth and was able to grow in a wide temperature range (25–50 °C). The strain was identified as *P. acidilactici* with API 50 CHL, as well as with 16S rRNA gene sequencing.

1. Introduction

The preservation of foods by natural and microbiological methods may be a satisfactory approach solving economic losses due to microbial spoilage of raw materials and food products, to reduce the incidence of food borne illnesses, and to meet the food requirements of the growing world population (Galvez et al., 2008). Since many people are concerned about the safety of chemical preservatives are questioned with regard to their safety, the potential applications of bacteriocins from lactic acid bacteria (LAB) in food and health care have received increasing attention in recent years (Papagianni and Anastasiadou, 2009). Bacteriocins have a wide antibacterial spectrum with potential applications in foods, such as meat and fish products, fruits and vegetables, cereals and beverages (Cleveland et al., 2001; Ivanova et al., 2000). Although many bacteria can produce bacteriocins, those produced by LAB are of particular interest to the food industry, since these bacteria have GRAS (generally regarded as safe) status (Anastasiadou et al., 2008; Barefoot and Nettles, 1993; Elegado et al., 1997). LAB have been used in food production as an effective method for extending shelf life of foodstuffs by simple fermentation (Galvez et al., 2008). *Lactococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Lactobacillus* and *Carnobacterium* are the genera most commonly used as starter cultures in the fermentation processes of milk, meat and vegetable products (Albano et al., 2007; Hastings and Stiles, 1991; Martinez-Cuesta et al., 2001). These bacteriocin-producing bacteria are probably among the most promising natural food biopreservatives (Atanassova et al., 2001; Leroy et al., 2003).

Use of bacteriocins, or the organisms which produce them, or both, could be attractive to the food industry because of addressing to the consumer demand for natural (green label) products (Montville and Winkowski, 1997). The past few years have seen the emergence of class IIa bacteriocins as one of the most interesting groups of antimicrobial peptides for use in food preservation and medicine, as antibiotic complements in treating infectious diseases or antiviral agents (Drider et al., 2006) or therapeutic agents (Jasniewski et al., 2008). The bacteriocins produced by *Pediococcus* spp. are classified as class IIa bacteriocins and have high antimicrobial activity, especially against *L. monocytogenes* (Cintas et al., 1998; Cosansu et al., 2007; Kim et al., 2000; Mattila et al., 2003; Rodriguez et al., 2005). Another important characteristic of these bacteriocins is their narrow inhibitory spectrum which prevents inhibition of starter cultures more than class I bacteriocins such as nisin (De Carvalho et al., 2006; Schneider et al., 2006).

Little information is available on growth and production physiology, metabolism, fermentation kinetics and bioprocessing strategies for improved yields of bacteriocin-producing *Pediococcus* species (Anastasiadou et al., 2008; Papagianni and Anastasiadou, 2009). The objective of this study was to investigate the growth parameters, physiological characteristics and bacteriocin production of a bacteriocin-producing strain *Pediococcus acidilactici* 13 isolated from Turkish style sucuk (traditional dry-fermented sausage produced by spontaneous fermentation) have been studied in continuation with our previous work (Cosansu et al., 2007).

* Corresponding author. Tel.: +90 312 596 14 17; fax: +90 312 317 87 11.
E-mail address: egunes@eng.ankara.edu.tr (K. Ayhan).

0168-1605/$ – see front matter.
doi:10.1016/j.ijfoodmicro.2010.04.024

2. Materials and methods

2.1. Media and culture conditions

Previously isolated (Cosansu et al., 2007) bacteriocin-producing strain *P. acidilactici* 13 was cultured in TGE (Tryptone Glucose Yeast Extract) broth at 37 °C for 24 h. Stock cultures were maintained at −20 °C in TGE broth containing 15% (v/v) glycerol. The TGE broth contained (grams per liter of deionized distilled water): tryptone 10.0; glucose 10.0; yeast extract 10.0; $MgSO_4$ trace amount; $MnSO_4$ trace amount; and Tween 80 1.0 mL. All of the ingredients used in the medium were obtained from Merck, Darmstadt, Germany. The pH was adjusted to 6.0 with 1 N HCl (Merck, Darmstadt, Germany). The medium was sterilized by autoclaving at 121 °C for 15 min.

L. monocytogenes strain ATCC 7644 was used as the indicator strain for antimicrobial activity tests. The strain was obtained from the Culture Collections of the Department of Food Engineering, University of Ankara and was grown in tryptic soy broth (TSB, MERCK, Darmstadt,Germany) at 37 °C for 24 h.

2.2. Bacteriocin activity assay

The bacteriocin was quantified by the critical dilution method, as described by Biswas et al. (1991). The cell-free supernatant of the *P. acidilactici* 13 strain grown in TGE broth at 37 °C for 24 h was serially diluted (1:2 to 1:1024) using sterile distilled water. A 5 µL portion, in duplicate, from each dilution, was spotted directly onto TGE agar plates which were then overlaid with soft TGE agar medium seeded with *L. monocytogenes* cells (10^8 cfu/mL approximately) at 45 °C. The plates were incubated at 37 °C for 24 h and examined for zones of inhibition. The highest dilution that produced a distinct zone of inhibition was multiplied by 200 (1 mL/5 µL) to obtain the arbitrary units per milliliter (AU/mL).

Proteinase K application was done to prove that the antimicrobial substance produced by the strain originated from bacteriocin (Macherey-Nagel, Düren, Germany). To perform this treatment, 3 µL of 10 mg/mL of proteinase K solution was dropped onto the TGE agar containing *L. monocytogenes* which was previously spotted with the culture supernatant. Lack of inhibition zones when sensitive bacteria were used as indicators indicated that the antimicrobial compound was proteinaceous in nature.

2.3. NaCl resistance of the strain P. acidilactici 13

To test NaCl resistance of the strain, TGE broths were prepared by adding NaCl (MERCK, Darmstadt, Germany) in various amounts (1, 5, 6, 7, 8, 9, 10, 15%). Ten (10) mL portions of TGE broths were inoculated in triplicate with 100 µL of the active culture (24-h old) of the strain *P. acidilactici* 13. After overnight incubation at 37 °C for the absorbances of the cultures were measured at 600 nm with UV/VIS spectrophotometer (Shimadzu UV-1208, Japan).

2.4. Optimum and maximum growth temperature of P. acidilactici 13

TGE broths, inoculated with 100 µL of the active culture of the strain *P. acidilactici* 13, were incubated at different temperatures; 10, 20, 25, 30, 37, 45, 50 and 60 °C for 24 h and the absorbances of the cultures were measured at 600 nm with UV/VIS spectrophotometer.

2.5. Optimum temperature for bacteriocin production

To find the optimum temperature for bacteriocin production of the strain *P. acidilactici* 13, the strain was incubated at different temperatures, i.e. 30, 35, 37 and 40 °C for 24 h. At the end of the incubation period the tubes were centrifuged (Hettich, Germany) at 2600×*g* for 10 min to separate the supernatants of the cultures from the cells. The supernatants were tested for bacteriocin activity as explained by Biswas et al. (1991).

2.6. Optimum pH for bacteriocin production

Samples of TGE broth were prepared by adjusting pH to 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0 with 0.1 N or 1 N HCl and 0.1 N or 1 N NaOH before sterilization. The tubes containing 10 mL of pH adjusted TGE broth were inoculated with 100 µL of active culture of the strain *P. acidilactici* 13 and incubated at 37 °C for 24 h. Bacteriocin activities of the supernatants of the cultures were calculated as described above.

2.7. The growth curve and bacteriocin production during growth

To evaluate the growth of the bacteria in TGE broth at 37 °C, a turbidimetric method was used (Ignatova et al., 2009). Bacterial growth was measured as the absorbance of cell suspensions at 600 nm using UV/VIS spectrophotometer (Shimadzu UV-1208) at 0, 2nd, 4th, 6th, 8th, 10th, 12th, and 24th hour.

For determining bacteriocin production in relation with incubation time, the strain was inoculated into TGE broth and incubated at 37 °C for 48 h. The samples were aseptically withdrawn, in duplicates, from the culture vessel at 2-h intervals throughout the incubation period. The bacteriocin activities of each sample were calculated and the results were compared with the growth curve (Van Reenen et al., 1998).

2.8. Identification of the strain P. acidilactici 13

The strain *P. acidilactici* 13 was identified with both API 50 CHL biochemical test kit (Biomerieux, France) and 16S rRNA gene sequencing method using the facilities at Middle East Technical University, Turkey. The bacterial DNA was purified with Qiagen DNeasy Blood&Tissue Kit before PCR amplification assay. The PCR mixture contained 0.25 mM dNTP (Fermantes), Taq buffer (0.2X, Applied Biosystems) 1.5 mM $MgCl_2$, AmpliTaq Polimeraz (0,03 U, Applied Biosystems), primer (0,4 pmol, the primer which duplicates whole 16S gene). After PCR amplification, Macherey-Nagel NucleoSpin Extract II was used in order to clean the PCR product. The DNA gene sequencing was performed with Bigdye Cycle Sequencing kit v.3.1 in ABI 3130XL Genetic Analyzer.

3. Results and discussion

3.1. Physiological characteristics

Absorbance values at 600 nm of the 24-h cultures grown at different temperatures suggested that 37 °C was the maximum growing temperature of the strain (Fig. 1). These results are consistent with the previous studies by other researchers; they also reported that 37 °C (Wood and Holzapfel, 1995) or 40 °C (Papagianni and Anastasiadou, 2009) is the optimal temperature for *Pediococci* and the maximum and optimum

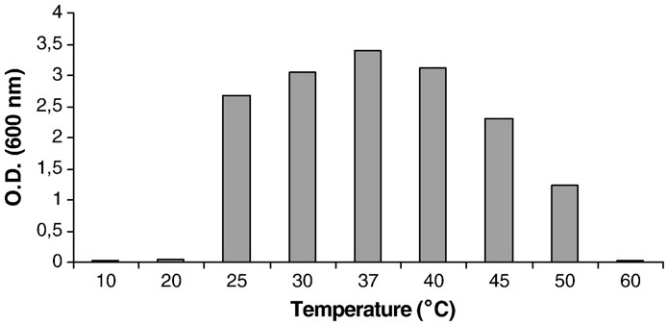

Fig. 1. O.D. values at 600 nm of the strain *Pediococcus acidilactici* 13 cultured at different temperatures for 24 h in TGE broth.