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Short Communication

Direct detection of an antimicrobial peptide of *Pediococcus acidilactici* in sodium dodecyl sulfate-polyacrylamide gel electrophoresis*

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SUMMARY

An SDS-PAGE technique is described that allows identification of the antimicrobial activity of a peptide secreted by a strain of *Pediococcus acidilactici*. This peptide has an antimicrobial property against several bacteria associated with food. This technique enables detection of the specific peptide (or protein) band(s) associated with the inhibitory effect which can then be eluted from the gel for further studies.

INTRODUCTION

Some food-grade lactic acid bacterial strains produce antimicrobial proteins that inhibit the growth of other bacteria, many of which are associated with food spoilage and health hazards of food origin [1,3,5,6,8]. Several unique properties, such as activity over a wide pH range and after high- or low-temperature treatment, make them suitable as biological preservatives to extend the shelf-life of refrigerated semipreserved foods and canned foods [1,8,9]. However, some basic information, such as amino acid composition, amino acid sequence, and mode of antimicrobial action, that would enable their effective use is not known. Available reports indicated that these compounds differ greatly in their molecular weights, ranging from 3500 [1] to 100 000 [2,5].

We obtained a strain of *Pediococcus acidilactici* by isolation from a fermented sausage and designated it as strain H. It was found to inhibit the growth of several food spoilage bacteria and food pathogens (Abstr. No. 143, Institute of Food Technologists Annual Meeting, 1987). Initial studies

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showed that the antimicrobial property of this strain is associated with a peptide of MW about 2700 that is secreted into the growth medium. We were interested in determining its mode of antimicrobial action, amino acid composition and possibly its amino acid sequence. In order to purify this peptide we used a procedure that included elution from SDS-PAGE. A direct assay technique was developed to identify the band associated with inhibitory activity directly on the gel. The method described in this short communication enabled us to identify and purify the active peptide from the gel for further studies.

MATERIALS AND METHODS

Culture and growth conditions

P. acidilactici strain H was grown at 30°C for 16 h to late exponential phase. Casein glucose broth (CG: casein, 1%; yeast extract, 0.5%; glucose, 1%; Tween 80, 0.1%; sodium acetate, 0.5%; magnesium sulphate, 0.01%; manganese sulphate, 0.005%; disodium phosphate, 0.2%) was made as a 10× concentrate and placed in a dialysis sac with a 14 000 molecular weight cut-off (MWCO) and dialyzed with deionized water (100 ml of concentrate in 900 ml water). The material outside the sac was adjusted to pH 6.8, autoclaved and used for the growth medium.

Preparation of antimicrobial agent

The culture was centrifuged and the supernatant medium was filtered through a 47-mm-diameter 0.45 μm pore membrane (GA-6, Gelman Sciences). An ammonium sulphate (70%) precipitation [6] was followed by dialysis of the precipitate in a dialysis membrane with a 1000 MWCO against deionized water. The dialysate (material inside the sac) was tested for its antimicrobial property against a sensitive indicator strain of *Lactobacillus plantarum* (strain WSO-39; obtained from Dr. M.A. Daeschell, Department of Food Science, North Carolina State University, Raleigh) by a disc assay method and for protein content by the Lowry method [7]. The disc assay method was done as follows: over a CG agar plate (CG broth plus 1.5% agar) a layer of 5 ml of CG soft agar, containing about^6 cells of the indicator organism, was poured. Then a sterile filter disc (6.25 mm) inoculated with 40 μl of the dialysate was placed on the soft agar. The plate was then incubated at 30°C for 18 h and examined for a zone of growth inhibition. The antimicrobial preparation was stored at 4°C until used in further studies.

Assay for antimicrobial activity in SDS-PAGE

The dialysate with antimicrobial activity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10–25% continuous gradient gel designed to purify low molecular weight proteins [4,11]. A 40 μl volume of the sample with antimicrobial activity, sample treated with trypsin, and molecular weight standards (MW-SDS-17, Sigma, St. Louis, MO) were applied in each of several wells on the slab gel. The trypsin treatment was done as follows: to 100 μl of dialysate were added 100 μl 8 mM phosphate buffer, pH 7.0, containing 20 μg of trypsin (T-8128, Sigma), and the mixture was incubated at 37°C for 1 h. All the samples were then mixed in a 1:1.5 ratio with sample buffer (4.6% SDS, 10% β-mercaptoethanol,

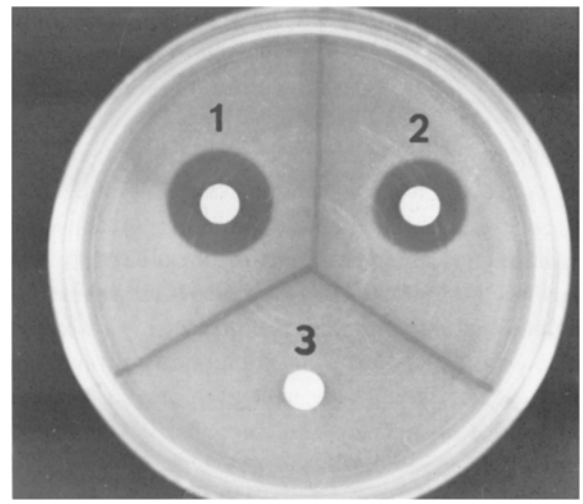

Fig. 1. Disc assay of antimicrobial activity of crude antimicrobial peptide from *P. acidilactici* H against *L. plantarum*: (1) untreated; (2) sample after heating at 121°C for 15 min; (3) sample after treatment with trypsin (200 μg/ml, 1 h at 37°C). Each disc contained 40 μl of dialysate of the $(NH_4)_2SO_4$ precipitate.

20% glycerol, 1.5% Tris, 1% bromophenol blue) and heated to 60°C for 15 min, cooled, and 40 μl aliquots were then examined by SDS-PAGE. After 4 h of electrophoresis at 40 mA, the gel was removed and cut into two vertical parts. One half containing the sample and molecular weight standard was stained with Coomassie Brilliant Blue R250. The other half of the gel containing the sample and the sample treated with trypsin was fixed immediately for 2 h in a solution of 20% isopropanol and 10% acetic acid and washed in deionized water for 6 h. This gel was placed into a sterile petri dish and overlaid with 10 ml of CG soft agar (CG broth plus 0.8% agar) containing about 10^6 cells of the indicator bacteria. The plate was incubated at 30°C for 18 h and examined for a zone of inhibition.

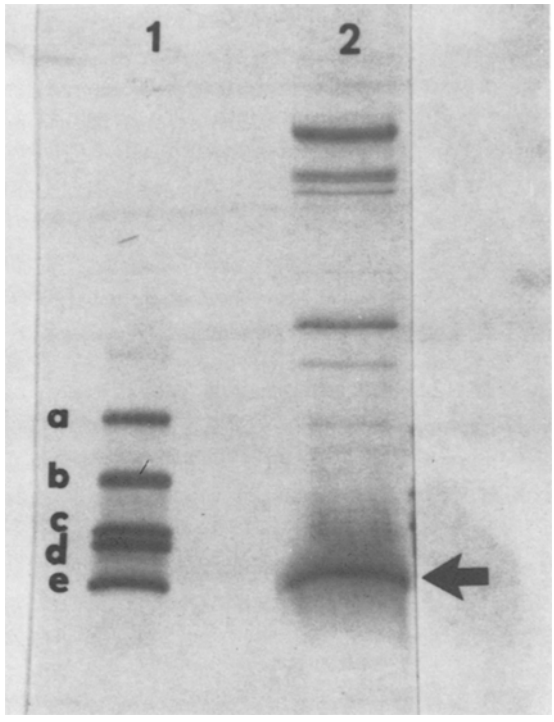

Fig. 2. The stained half of the SDS-PAGE gel showed bands formed by several proteins present in the $(NH_4)_2SO_4$ preparation (lane 2). The MW of the upper most band is about 50 000 and of the bottom one about 2700 (see arrow). Lane 1 contains the low-MW standards (MW-SDS-17, Sigma): a, 16 950; b, 14 400; c, 8160; d, 6210; e, 2510, which were used to calculate the MW of lower bands (16 000 and below). High-MW standards were used to calculate the MW of the upper bands (above 16 000, not presented).

RESULTS AND DISCUSSION

The inhibitory effect of the antimicrobial preparation from *P. acidilactici* strain H against *L. plantarum* is presented in Fig. 1. The peptide appeared to be active against this indicator strain. Heat treatment at 121°C for 15 min did not destroy its activity. However, prior treatment of the peptide with trypsin resulted in a loss of activity. In the gel stained with Coomassie blue, four major and several minor bands were observed with molecular weights ranging from approximately 50 000 to 2700 (see explanation in Fig. 2). In the gel that was used to determine antimicrobial property (Fig. 3) the zone of growth inhibition of *L. plantarum* corresponded with the lowermost band in the stained gel with a molecular weight of 2700. The antimicrobial activity was absent in the sample treated with trypsin. Protein in other bands did not have an antimicrobial property. This method enables us to identify the specific fraction of protein associated with antimicrobial activity.

Recently we have been able to purify the peptide from the gel section which corresponds to the zone

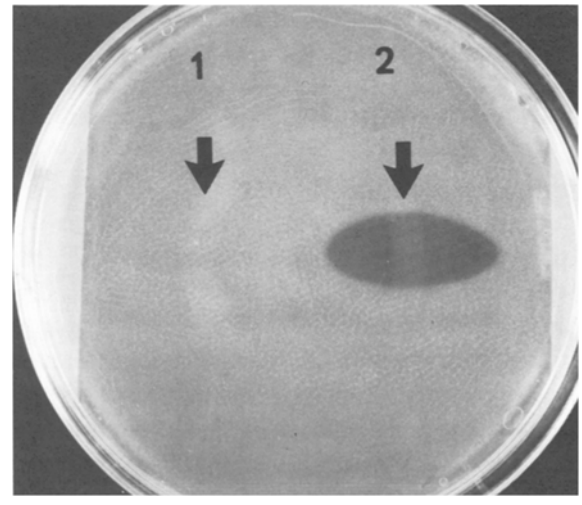

Fig. 3. The other half of the gel was overlaid with *L. plantarum* to determine which band(s) corresponds to the antimicrobial activity. Lane 2, showing the zone of growth inhibition (see arrow) corresponded with the lowermost band (MW 2700); other bands did not show antimicrobial activity. Lane 1 contained trypsin-treated sample and showed no growth inhibition.

of inhibition. This pure sample is now being used for the determination of amino acid composition and other studies.

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REFERENCES

1 Baily, F.J. and A. Hurst. 1971. Preparation of a highly active form of nisin from *Streptococcus lactis*. Can. J. Microbiol. 17:61–67.

2 Barefoot, S.F. and T.R. Klaenhammer. 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 45:1808–1815.

3 Daeschell, M.A. and T.R. Klaenhammer. 1985. Assocation of 13.6-megadalton plasmid in *Pediococcus pentosaceus* with bacteriocin activity. Appl. Environ. Microbiol. 50:1538–1541.

4 DeWald, D.B., L.D. Adams and J.D. Pearson. 1986. A non-urea electrophoretic gel system for resolution of polypeptides of Mr 2,000 to Mr 200,000. Anal. Biochem. 154:502–508.

5 Fleming, H.P., J.L. Etchells and R.N. Costilow. 1975. Microbial inhibition by an isolate of *Pediococcus* from cucumber brines. Appl. Microbiol. 30:1040–1042.

6 Graham, D.C. and L.L. McKay. 1985. Plasmid DNA in strains of *Pediococcus cerevisiae* and *Pediococcus pentosaceus*. Appl. Environ. Microbiol. 50:532–534.

7 Joerger, M.C. and T.R. Klaenhammer. 1986. Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by *Lactobacillus helveticus* 481. J. Bacteriol. 167:439–446.

8 Keene, J.H. 1966. Preparation and chemical properties of colicin 1. Can. J. Microbiol. 12:425–426.

9 Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265–275.

10 Tsai, H.-J. and W.E. Sandline. 1987. Conjugal transfer of nisin plasmid genes from *Streptococcus lactis* 7962 to *Leuconostoc dextranicum* 181. Appl. Environ. Microbiol. 53:352–357.

11 Walker, J.M. 1984. Gradient SDS polyacrylamine gel electrophoresis, Methods in Molecular Biology, Vol. 1, Proteins (Walker, J.M., ed.), pp. 57-61, The Humana Press Inc., Clifton, NJ.

12 White, R.J. and A. Hurst. 1968. The location of nisin in the producer organism, *Streptococcus lactis*. J. Gen. Microbiol. 53:171–179.