Chemistry of Natural Compounds, Vol. 37, No. 4, 2001

ISOLATION OF *L. casei* 925 a.k. CULTURE-MEDIUM COMPONENTS BACTERIOCINOGENIC FOR *Helicobacter pylori*

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A search was made for bacterial antagonists against the gastroduodenal pathogen H. pylori. *Protein fractions active against the pathogen were isolated from culture medium of lactobacterium.*

Key words: bactericidal, bacteriocin, bacteria.

Novel means are sought for the prophylaxis and treatment of gastroduodenal ailments (gastritis, stomach and duodenal ulcers) caused by the intestinal pathogen *Helicobacter pylori*. One of the directions involves antibiotics from microbes, in particular, bacteriocins from lactobacteria. The bacteriocins include low-molecular-weight proteins (MW 2.5-7 kDa) with bactericidal activity that can aggregate to form large associates (up to 180 kDa) [1, 2].

Our search for lactobacterial antagonists to clinical strains of *H. pylori* revealed that the most active strain was *Lactobacillus casei* 925 a.k., to which the pathogen was 100% sensitive. Therefore, we isolated and purified bactericidal components from the bacterium culture medium.

Protein was fractionated by ammonium sulfate in the first stage. This method is rather simple and accessible for concentrating protein in the initial isolation stage. Furthermore, it has a definite advantage because the high salt concentration protects the protein from proteolysis and effects from other bacteria. This is especially important for working with microorganisms.

Protein was precipitated by gradual salting out with 40, 50, 70, and 90% saturated ammonium sulfate. The *L. casei* culture medium was first purified of bacteria cells by centrifugation. The protein fractions obtained by salting out were lyophilized. The resulting fractions were tested for antagonistic activity against various clinical strains of *H. pylori*. The analytical results showed that fractions obtained using 40% (No. 1) and 90% (No. 4) saturated ammonium sulfate possessed antihelicobacter activity.

The active protein fractions (Nos. 1 and 4) were further analyzed by electrophoretic separation in 15% SDS-PAAG (Fig. 1). The electrophoregrams showed that fraction No. 4 gave one sharp band corresponding to 7000 Da. The absence of other bands in the track of this fraction and in that of fraction No. 1 could be due to the fact that small amounts of protein components are not colored by the less sensitive coumassie dye. Analytical separation by HPLC of fractions No. 1 and 4 confirmed this hypothesis.

The HPLC elution profile (Fig. 2) shows that hydrophobic components (No. 4) dominate the active protein fractions with more hydrophilic components (No. 1) also present.

Screening of the obtained chromatographic fractions for antibiotic activity will be described in subsequent articles.

Thus, the investigations discovered an active strain of lactobacteria that is an antagonist to *H. pylori*. Two active protein fractions with bactericidal activity are isolated from the culture medium of the bacterium by salting out. Electrophoresis in SDS-PAAG revealed the presence in active fraction No. 4 of a low-molecular-weight protein with MW 7 kDa. The HPLC elution profile of the active protein fractions indicates that the protein with antibiotic activity is not pure.

Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, fax (3712) 162 15 76. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 315-316, July-August, 2001. Original article submitted July 2, 2001.

0009-3130/01/3704-0371$25.00 ©2001 Plenum Publishing Corporation

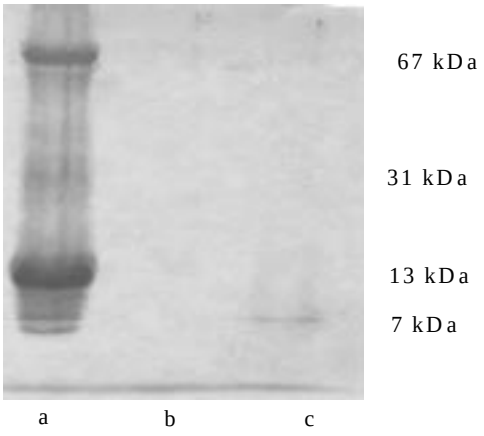

Fig. 1

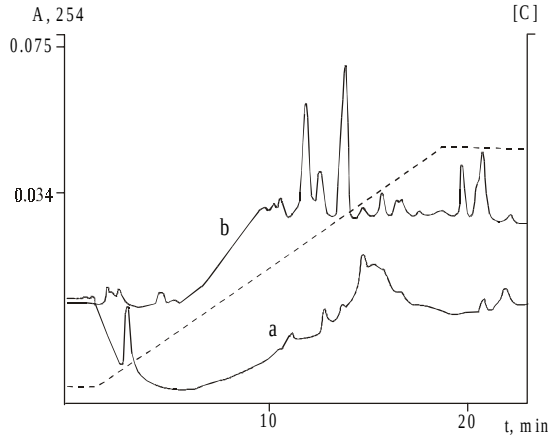

Fig. 2

Fig. 1. Electrophoregram for separation of protein fractions No. 1 and 4 in 15% SDS-PAAG. Markers (a), fraction No. 1 (b), fraction No. 4 (c).

Fig. 2. Chromatographic elution profile for separation of protein fractions No. 1 (a) and 4 (b) by HPLC with a linear gradient of isopropanol in trichloroacetic acid (14-77%) on a column of Bondapak C_{18}.

EXPERIMENTAL

Protein was salted out stepwise from the bacterium culture medium by 40, 50, 70, and 90% saturated ammonium sulfate with subsequent centrifugation (5000 rpm). Protein fractions were lyophilized and tested for antibiotic activity as described before [3].

Electrophoresis of active protein fractions was conducted by the Laemmli method in 15% PAAG containing SDS. Marker proteins were BSA (67 kDa), pancreatic DNA-ase (31 kDa), and pancreatic RNA-ase (13.7 kDa). The dye was coumassie G-250.

Active protein fractions were separated analytically by reverse-phase HPLC on a Beckman chromatograph (USA) using Gold 3.1 software and a μ-Bondapak C_{18} column (Millipore Corp.) with a linear concentration gradient of isopropanol (buffer A) in trichloroacetic acid (buffer B) (14-77%).

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