PROTICIN, A NEW PHOSPHORUS-CONTAINING ANTIBIOTIC. I

TAXONOMY, FERMENTATION, ISOLATION, AND BIOLOGICAL PROPERTIES

PAUL PRÄVE, DIETER SUKATSCH and LÁSZLÓ VÉRTESY

Farbwerke Hoechst AG, Frankfurt (Main), Germany

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Proticin is a phosphorus-containing, strongly unsaturated amorphous compound with broad activity spectrum, especially against Gram-negative pathogens. It is produced by fermentation of a strain which has been identified as a form of *Bacillus licheniformis*. The name proticin has been chosen to suggest a particular activity against *Proteus* bacteria.

Taxonomy

The strain *Bacillus* FH-G-439 was isolated from a soil sample collected on the bank of a Norwegian fjord. On the basis of its morphological and metabolic-physiological properties it was found to belong to the family Bacillaceae, morphological group I of the genus *Bacillus*, and was recognized to be a form of *B. licheniformis*[2] var. *mesentericus* nov. var. FH-G-439.

Taxonomically relevant were the differences from the reference strain ATCC (ATCC-No. 9259). The strain FH-G-439, which was found to stand between the forms mesentericus and *Bacillus licheniformis*, but to correspond more to the latter, agreed only to some extent, or not at all, with the ATCC reference strain in respect of the following parameters:

(a) colony form on nutrient agar (minute differences);
(b) mannitol fermentation with inorganic nitrogen (FH-G-439 +; licheniformis −);
(c) growth in 10 % NaCl (FH-G-439 −; licheniformis +);
(d) starch hydrolysis (with the reference strain only slight)

Table 1 shows utilization of carbon sources by FH-G-439.

Table 2 lists morphological, physiological, and other characteristics of FH-G-439.

On the basis of these taxonomical studies the strain FH-G-439 is to be designated as *Bacillus licheniformis* var. *mesentericus* nov. var. FH-G-439. It was deposited under ATCC No. 21 552.

Fermentation and Isolation

The antibiotic proticin is the product of *Bacillus* FH-G-439 in submerse culture (batch process).

Three-day old slants containing fully developed spores were rinsed with physiological saline solution and inoculated into 250 ml of nutrient solution in 1,000-ml Erlenmeyer flasks (5 tubes with a total of 50 ml of saline solution per flask). The

Table 1. Utilization of 1 % C sources by FH-G-439 (basic medium : 0.1 % beef peptone, 0.05 % NaCl, 0.0025 % phenol red)

	C source	Utilization		C source	Utilization
Organic nitrogen source	Fructose	+		Inulin	+
	Arabinose	+		Saccharose	+
	Mannose	±		Rhamnose	±
	Raffinose	+		Dulcitol	±
	Mannitol	+	Inorganic nitrogen source	Glucose	+
	Starch	+		Saccharose	+
	Glycerin	+		Glycerin	+
	Maltose	+		Arabinose	+
	Lactose	±		Xylose	+
	Xylose	±		Mannitol	+
	Glucose	+		Lactose	±
	Sorbitol	+		Rhamnose	+
	Galactose	±		Sorbitol	+
	Salicin	±			

Table 2. Morphological, physiological, and other characteristics of FH-G-439

Characteristics	FH-G-439	Characteristics	FH-G-439
Size of spores	0.5× 1.0 μ	Celulase	−
		NaCl 8 %	+
Size of rods	2.0× 6.0 μ	NaCl 9 %	−
Gram staining	+	Temperature +40°C	+
Motility	±	Temperature +50°C	−
Slime formation	−		
Gelatinase	+	pH of citrate	7.6
Protease	+	Indole	−
Amylase	+	VOGES-PROSKAUER test	+
Lipase	+		
Catalase	+	Methyl red test	−
Urease	−	H_2S formation	+
		NO_3 reduction	+
Cytochromoxydase	−	Citrate as C source	±
Phenylalaninedeaminase	−	Pigment	−
Lysinedecarboxylase	−	Anaerobic growth	±
Lecithinase	+	Vitamine requirement	−
Oxidase	−	7-Day growth in glucose	pH 4.9～7.2
Phophatase	±		

+ : yes, or positive, or present. − : no, or negative, or absent. ± : positive, or present, under specific conditions.

Table 3. Antibiotic spectrum of proticin

	MIC μg/ml
Proteus mirabilis	0.4
Escherichia coli	12.0
Salmonella typhimurium	3.0
Shigella flexneri	3.0
Streptococcus haemolyticus 380	0.4
Micrococcus pyogenes var. *aureus* FDA 209 P	50.0
Mycobacterium tuberculosis $H_{37}Rv$	5.0

flasks were agitated at 220 r.p.m. (amplitude 8 cm) for 2 days at 28°C. Two of the flasks were used as inoculum for a 30-liter fermentor tank filled with 10 liters of the following nutrient solution (at pH 7.2～7.4) : 4 % starch, 0.4 % corn steep liquor, 1 % glucose, 0.8 % $(NH_4)_2HPO_4$, 0.4 % soy bean meal, and 1 % casein peptone. This was allowed to ferment for 48 hours at 28°C under aeration of 500 liters air per hour, and the fermentor was harvested. For isolation 9 liters of the fermented solution were treated with 5 liters of butanol at pH 6.5 and subsequently stirred for 2 hours. The organic phase was then separated by centrifugation and concentrated carefully to 1/50 of the volume in vacuum. Dilution of the concentrate with petroleum ether (60～80°C) yielded a precipitate containing a major part of proticin. By the use of silica gel impregnated with sodium phosphate (pH 6.7) insured fractionation without losses. Chloroform with increasing additions of methanol acted as an eluant. Proticin was eluted with chloroform - methanol in the volume proportion 3 : 2. The crude antibiotic (600 mg) was further purified on Sephadex LH 20 (Pharmacia Fine Chemicals, Uppsala, Sweden) by gel chromatography[4]. The results on elementary analysis were 63.8 % C, 7.8 % H, 19.8 % O, 4.4 % P, and 3.2 % Na. Characteristic of proticin was the UV spectrum with the maxima at 284, 272.5, 264, and 235 nm ($E^{1\%}_{1cm}$ 415, 510, 395, and 1010). This absorption spectrum suggested a highly unsaturated compound with a conjugated triene.

Biological Properties

Proticin *in vitro* is especially active against a number of Gram-negative pathogens. Its activity was determined by measuring the minimum inhibitory concentration (MIC) in serial dilution test (Table 3).

The median lethal dose (LD_{50}) of proticin for mice was >150 mg/kg intravenously and 1,000 mg/kg subcutaneously.

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References

1) Farbwerke Hoechst AG : Ein Antibiotikum Proticin und Verfahren zu seiner Herstellung. Deutch. Patent 7,109,648
2) BERGEY's Manual for Determinative Bacteriology, 7 th edition. The Williams & Wilkins Co., 1957
3) BERGEY, M. E. & T. E. EBLE : The filipin complex. Biochemistry 7 : 653~659, 1968
4) VÉRTESY, L. : Proticin, a new phosphorus-containing antibiotic. II. Characterization and chemical studies. J. Antibiotics 25 : 4~10, 1972