Abalone Juice. Fractionation and Antibacterial Spectrum. (26155)

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The antibacterial activity of crude fresh abalone juice has been reported(1,2). The active substance (or substances) was thermostable, non-dialyzable and not easily diffusible, suggesting a macromolecule. In view of the widespread use of cellulose ion-exchange chromatography for separation and purification of proteins from animal or other materials(3,4), it seemed advantageous to use this procedure for isolation of the antimicrobial material present in abalone juice. This report describes the isolation of several fractions and results of tests on the *in vitro* antibacterial action of these against *Staphylococcus aureus* and other pathogenic bacteria.

Materials and methods have been previously described(1,2) except the following additions: *Test organisms.* Four additional strains of bacteria, *i.e., Salmonella paratyphi, A and B, Salmonella typhi* and *beta-hemolytic Streptococcus pyogenes*§ were used as test organisms. *Fractionation procedure.* One hundred ml of crude abalone juice were exhaustively dialyzed against distilled water at 4°C for 5 days. The small amount of insoluble material accumulating was removed by centrifugation and discarded. The dialyzed extract was adjusted to pH 7.8 and applied to a column packed with 50 g of the anion-exchanger diethylamino-ethylcellulose (Cellex-D)‖ equilibrated with 0.01 M tris (hydroxymethyl) aminomethane-phosphoric acid (tris-H_3PO_4) buffer at pH 7.6. The column was set up on an automatic fraction collector in the cold (4°C). Elution of material from the column was carried out with a series of Tris-H_3PO_4 buffers of varying ionic strength and pH (Table I). Fifteen ml samples of effluent were consecutively collected in each of 200 tubes at a rate of 3 ml per minute (Table I). The column was then washed with one liter of M NaCl to elute the residual highly bound protein. Several pools were made of various samples, dialyzed against distilled water for 48 hrs in the cold and lyophilized. The final lyophilized products were dissolved in pH 7.0 buffered saline, passed through a very fine glass fritted filter or heated in a water bath at 70°C for 30 minutes before assay.

Results. Activity of various fractions. The data in Table I demonstrate that several fractions isolated from abalone juice by ion-exchange chromatography contained marked antibacterial activity. The greatest activity was found in the first 4 pools of early eluates, corresponding to tubes 11-50. Eluate from tubes 1-10 (150 ml) after dialysis and lyophilization revealed a negligible amount of material not enough to test and it was discarded. The fifth pool showed some activity while all later eluates including the final M-NaCl wash were inactive. Two additional chromatographic experiments were performed with juice from 1-3 fresh frozen abalones. Similar results were obtained, although there was some variation in quantity of yield and intensity of activity of the product from different abalones. For example, from 100 ml of crude abalone juice used in the first chromatographic separation, 52 mg of active product was obtained from the first 4 pools, whereas, in the second experiment the yield was 200 mg of a product with less than ¼ the activity. Whether this was due to variation in technic or variation in the contents of the abalone juice awaits further study.

Relationship between concentration and antibacterial activity. A series of 10-fold dilutions in buffered saline was prepared from

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§ These cultures were kindly supplied by Dr. E. B. Seligmann, Division of Biologics Standards and Dr. P. Geisler, NIH Clinical Path. Lab., respectively.

‖ Purchased from California Biochemical Corp., Los Angeles.

TABLE I. Antibacterial Activity of Various Fractions from Abalone Juice.

Fraction No.	Pool from tubes	Eluent Tris-H_3PO_4 buffer Conc., molar	pH	Yield, mg*	Colonial growth of *Staphylococcus aureus* on plate† 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Control§
1	11- 19	.01	7.6	10	0	0	0	0	0	0	0
2	20- 30	.01	7.6	25	0	0	0	0	0	0	0
3	31- 40	.01	7.6	12	0	0	0	0	0	0	0
4	41- 50	.10	7.5	5	0	0	0	0	0	0	0
5	51- 89	.10	7.5	155	3	1	1	0	0	0	0
6	90-109	.25	3.7	115	3	3	2	0	0	0	0
7	110-149	.25	5.8	232	4	‖	3	0	0	0	0
8	150-179	.25	3.7	160	4	4	4	0	0	0	0
9	180-200	.25	3.7	125	4	4	2	0	0	0	0
10	M NaCl wash			4300	4	4	4	‖	4	0	0
	Control‡				4	4	4	4	0	0	0

1-4, etc. indicate degree of bacterial growth, 4 being heaviest growth. 0 = no bacterial growth.

* Lyophilized material.

† A series of 10-fold dilutions of *Staphylococcus aureus* culture (FDA 209 strain) was made and a loopful of each dilution was inoculated into a tube containing 1.8 ml of broth with 0.2 ml of abalone fraction. After 18 hr incubation a loopful from each tube was plated(2).

‡ Contained 0.2 ml of broth only.

§ Not inoculated.

‖ Tube broken.

a 100 mg % solution of fraction No. 2 (Table I), then tested *in vitro* against *Staphylococcus aureus* (Table II). It should be noted that concentration of the agent as low as 1 mg % showed activity. The same type of experiment was repeated with 2-fold dilutions of a 16 mg % solution of fraction No. 1 from the third chromatographic experiment with similar results.

Bactericidal activity of the product. *Staphylococcus aureus* inhibited for 48 hours in a broth culture by the presence of a fraction of abalone juice did not grow out when the culture was diluted with nutrient broth, 100 to 200 times and further incubated for a week. At this dilution, concentration of abalone fraction was far below that required for inhibition. It thus seems probable that the action of the agent under such *in vitro* conditions was bactericidal rather than bacteriostatic.

TABLE II. Antibacterial Activity of Pool 2 at Different Concentrations.

Final conc., mg %	Colonial growth of *Staphylococcus aureus* on plate* 10^{-0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	C†
100	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0
1	4	4	4	1	0	0	0	0
.1	4	4	4	4	4	4	0	0
.01	4	4	4	4	4	1	0	0
Control	4	4	4	4	4	1	0	0

1-4, etc. indicate degree of bacterial growth.
0 = no bacterial growth.

* See Table I. † Control—not inoculated.

Antibacterial spectrum. The data presented in Table III indicate that fraction 2 from abalone was not only active against a penicillin sensitive strain of *Staphylococcus aureus,* but also effective against other Gram-positive and Gram-negative pathogens including a penicillin-resistant strain of *Staphylococcus aureus.*

Discussion. The antimicrobial agent reported here differed from the usual antibiotics usually obtained from fluid cultures of molds or allied microorganisms. It was not dialyzable, yet thermostable as reported previously (2) and probably protein in nature as it gave a positive biuret test and was precipitated by trichloracetic acid and other protein precipitants. Possibly we are dealing with a new class of agent which might be widely distributed in nature, particularly in sea animals and can be isolated for therapeutic and prophylactic purposes.

With these initial studies as background, further work on purification and chemical

TABLE III. Antibacterial Spectrum of Pool 2 from Abalone Juice.

Test organism	Conc., mg %	Abalone juice pool 2	Colonial growth of test organisms on plate* 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	Control†
Staph. aureus, Pen. sensitive	5	+	0	0	0	0	0	0	0
		—	4	4	4	4	4	0	0
Staph. aureus, Pen. resistant	5	+	1	0	0	0	0	0	0
		—	4	4	4	4	4	0	0
Staph. aureus, Pen. resistant	10	+	0	0	0	0	0	0	
		—	4	4	4	4	4	0	0
Strep. pyogenes, beta hemolytic	5	+	2	0	0	0	0	0	0
		—	4	4	4	4	4	0	0
Salmonella paratyphi A	10	+	0	0	0	0	0	0	0
		—	4	4	4	4	4	4	0
Salmonella paratyphi B	10	+	1	0	0	0	0	0	0
		—	4	4	4	4	4	0	0
Salmonella typhi	10	+	1	1	0	0	0	0	0
		—	4	4	4	4	4	4	0

1-4, etc. indicate degree of bacterial growth. 0 = no bacterial growth.
* See Table I. † Not inoculated.

structure of the active agent and *in vivo* studies are in progress.

Summary. Ten fractions have been isolated from abalone juice by ion exchange chromatography (Cellex-D) and tested for *in vitro* antibacterial activity against *Staphylococcus aureus*. Four fractions of effluent showed high activity. One active fraction was tested and found effective against *Staphylococcus aureus*, (including a penicillin-resistant strain), *Streptococcus pyogenes*, beta-hemolytic strain, and *Salmonella typhi*, and *paratyphi A and B*. The action for *Staphylococcus aureus* seemingly was bactericidal.

The authors express thanks to Dr. C. W. Hiatt for valuable suggestions and to Dr. R. C. Woodworth, W. C. Jahnes, E. C. Martino and J. L. Rogers for technical assistance.

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Received August 31, 1960. P.S.E.B.M., 1960, v105.

Increased Nutritional Requirements of *Saccharomyces cerevisiae* as a Result of Incubation at 38°C.* (26156)

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During nutritional studies on yeasts, it was noted that certain strains grew poorly or not at all at 38°C in a chemically defined medium which supported excellent growth at 30°C. In contrast, some of the strains grew equally well at 30°C or 38°C when inoculated into a complex medium. In most instances where such a phenomenon had been demonstrated previously, an increase in temperature was accompanied by an increase in nutritional requirement(1-7). The present paper is concerned with identification of the substance(s) in the complex medium which

* This study was aided in part by a contract between the Office of Naval Research, Dept. of the Navy, and Univ. of Minnesota.