STUDIES OF TWO POLYPEPTIDE ANTIBIOTICS ELABORATED BY *Saccharomyces cerevisiae*[1]

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ABSTRACT

Two antibiotic substances (I_1 and I_2) isolated from yeast ferments have been shown to be polypeptides. I_1 contained glutamic acid, serine, glycine, alanine, valine, leucine, and tryptophan. I_2 contained the same compounds present in I_1 and in addition gamma-aminobutyric acid, aspartic acid, and phenylalanine. The infrared spectra of I_1 and I_2 were similar but not identical to that of gramicidin. The antibiotic substances were found to survive baking.

Motzel (3), Robinson *et al.* (6), and others (1,5) have reviewed the literature concerning the antibiotic substances elaborated by yeast.

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Parfentjev (5) isolated from alkaline water extracts of brewer's and baker's yeast a complex protein with anti-infectious properties. This yeast protein protected mice against infection by a number of microorganisms including such pathogens as *Proteus, Salmonella, Pseudomonas,* and *Brucella.* Robinson *et al.* (6) reported that the decrease in bacterial numbers in yeast ferments was due to the activity of antibiotic substances, two of which were isolated and designated I_1 and I_2. These antibiotics fluoresced blue and yellow and had R_f values of 0.24 and 0.68 in ethanol-concentrated ammonium hydroxide-water (80:5:15, v/v), respectively. They possessed antibiotic properties for *Staphylococcus aureus* L. 41, *Escherichia coli* L. 145, and mixed pre-ferment cultures.

The results presented in this paper deal with the purification and partial characterization of two antibiotic substances elaborated by *Saccharomyces cerevisiae* A.T.C.C. 9896 strain 139 during fermentation.

Materials and Methods

The pre-ferments were prepared as described by Robinson *et al.* (6) but from pure yeast culture and sterile materials. The procedure for isolation of the antibiotic substance was essentially that of Motzel (3,4).

The methods used for chromatographic separation of the antibiotics were described by Robinson *et al.* (6). One additional step in the present work, designed to ensure purity of the antibiotic, consisted of rechromatographing several times each separated component. The solvents used for purification consisted of ethanol-concentrated ammonium hydroxide-water (80:5:15, v/v) (Motzel, 3) and ethyl acetate-water-pyridine (40:40:18, v/v) (White and Secor, 7). The separated components were eluted with ethanol and concentrated in a rotary evaporator.

The infrared spectra of the purified antibiotic substances were established by use of the potassium bromide pellet technique (Gould, 2). Four milligrams of the antibiotic in 0.5 ml. of ethanol were combined with 500 mg. of potassium bromide to form a clear pellet. Known antibiotic substances were used for comparison.

Approximately 120 mg. of purified antibiotic were hydrolyzed in 50 ml. of 1.2*N* hydrochloric acid by autoclaving 19 hours at 15 lb. pressure. After being neutralized with sodium hydroxide, the hydrolysates were brought to dryness over a steam cone, extracted with 10 ml. of ethanol, and analyzed chromatographically. Each hydrolysate was placed on Whatman No. 4 paper and chromatographed first by

use of a descending technique and a solvent containing butanol-glacial acetic acid-water (4:1:5, v/v). The chromatograms were dried at room temperature for 3.5 hours, rotated 90°, and rechromatographed using a water-saturated phenol solvent. The atmosphere of the cabinet contained ammonia and hydrogen cyanide. The ammonia was evolved from 100 ml. of 0.2*N* ammonium hydroxide. The hydrogen cyanide was evolved by treating 100 mg. of potassium cyanide with dilute sulfuric acid. The chromatograms were air-dried, sprayed with 1% ethanolic ninhydrin, and heated to detect the amino acids.

Results and Discussion

The results of the ultraviolet spectra analyses of freshly prepared and purified antibiotics, I_1 and I_2, appear in Fig. 1. The ultraviolet spectrogram of I_2 which is similar to that of Y_1 obtained by Motzel (3) suggests a smaller amount of an aromatic amino acid than is present in I_1 because of lack of absorption at 275 mμ. The spectrogram of I_1 is similar to that of Y_2 obtained by Motzel (3).

No changes in ultraviolet spectra were noted when the antibiotics in alcoholic solution were stored for 1 year in the refrigerator after being finally purified in the ethanol-concentrated ammonium hydroxide-water solvent. When ethyl acetate-water-pyridine was used as the final solvent, the ultraviolet spectrum of I_1 changed with time. The change consisted of increasing absorption at 255 mμ. The altera-

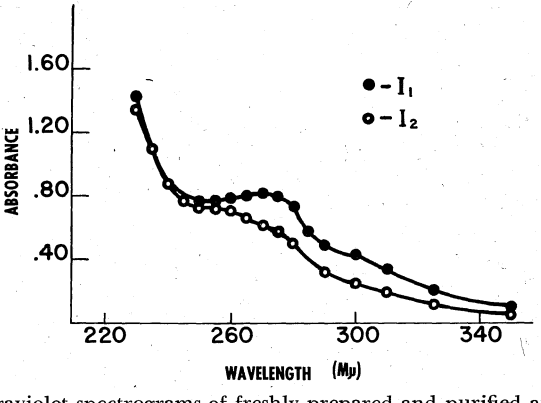
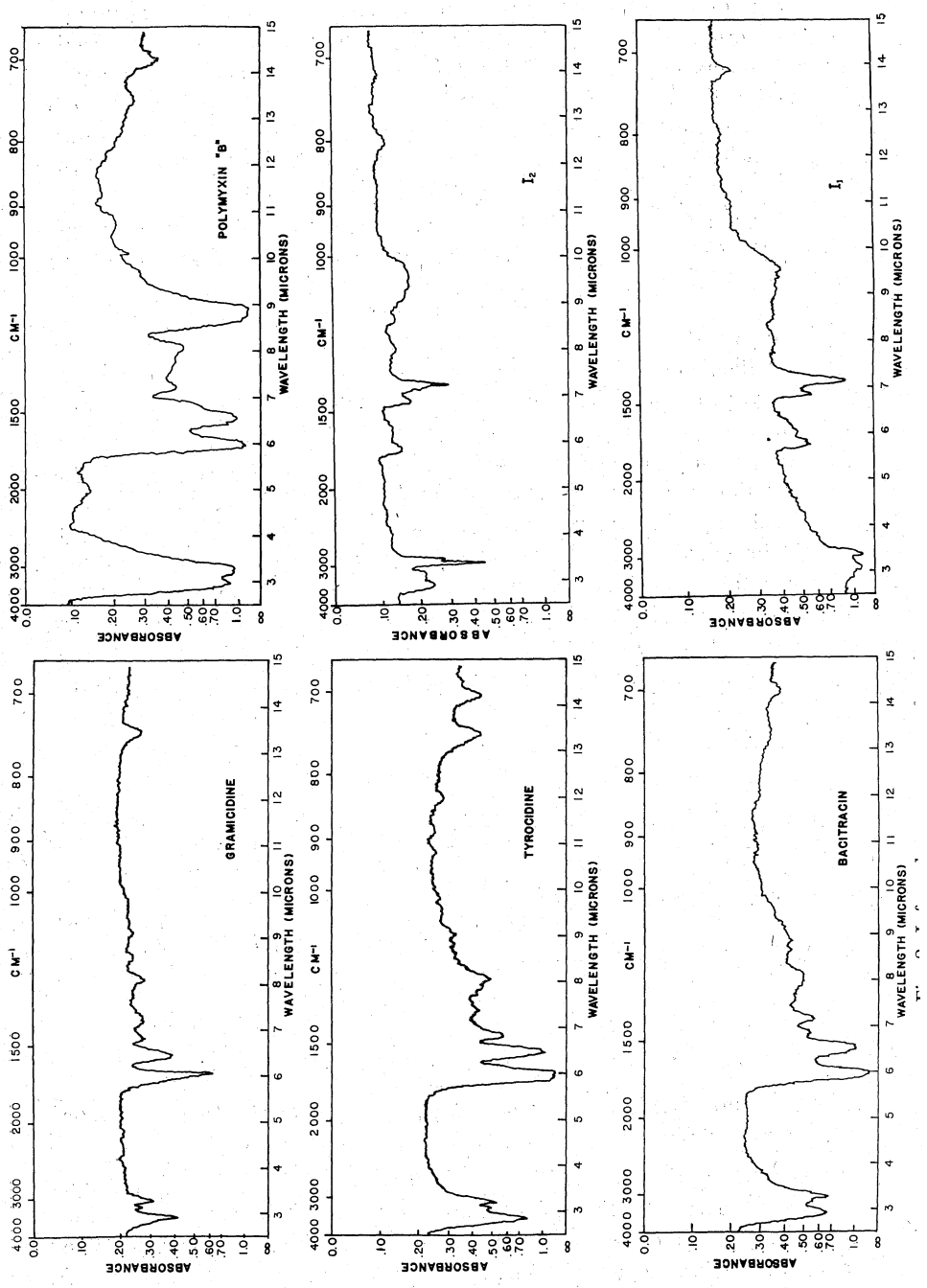


Fig. 1. Ultraviolet spectrograms of freshly prepared and purified antibiotics from *Saccharomyces cerevisiae*.

tion in the antibiotic with time was confirmed by infrared spectra.

A comparison of the infrared spectrograms of the two antibiotics, I_1 and I_2, with other common antibiotics is shown in Fig. 2. The curves for I_1 and I_2 are similar. A comparison of the infrared spectro-


POLYMYXIN "B"
I2
I1
GRAMICIDINE
TYROCIDINE
BACITRACIN
ABSORBANCE
WAVELENGTH (MICRONS)
CM-1

grams of I_1 and I_2 to that of gramicidin suggests that the uncharacterized antibiotics are peptides.

Chromatograms of hydrolyzed I_1 revealed the presence of glutamic acid, serine, glycine, alanine, valine, leucine, and tryptophan as well as some unidentified nitrogen-containing compounds. Chromatographic analyses of I_2 showed the presence of the same compounds present in I_1 and in addition phenylalanine, aspartic acid, and, gamma-aminobutyric acid. I_2 also contained at least four unidentified nitrogenous compounds. The antibiotic Y_1, isolated by Motzel (3), was shown to contain leucine, valine, alanine, glycine, and glutamic acid, while Y_2 contained the same amino acids as Y_1 plus gamma-aminobutyric acid.

The differences among the antibiotics obtained in the present work and those reported by Motzel (3,4) may be due to the different yeast sources used. Motzel (3) extracted the antibiotics from blocks of compressed yeast which were in the stationary phase. The present material was extracted from the fermenting liquor which was freed from yeast cells and residues.

To determine if the two antibiotic substances survived baking, loaves containing six and twelve times the quantity of antibiotic present in normal loaves were baked. These quantities of antibiotics were required in order to demonstrate adequately their presence in the bread, although these concentrations tended to reduce loaf volume and have an adverse effect on crumb odor and flavor. The same antibiotic substances survived the baking process as were in the ether

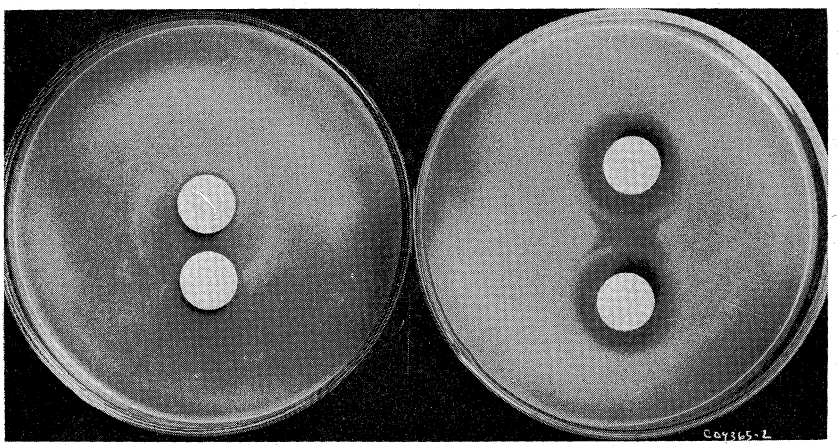


Fig. 3. Inhibition of *Staphylococcus aureus* L. 41 with ether extract of the crust. Left — crust of normal bread. Right — crust of bread to which extra antibiotics had been added.

extract of the pre-ferments. They had R_f values and amino acid compositions comparable to those of the antibiotic substances extracted from the pre-ferments. Likewise, the antibiotic substances extracted from the bread crust had the same inhibitory reaction, in 7-p.p.m. concentration (Fig. 3), to *Staphylococcus aureus* L. 41, as shown by Robinson *et al.* (6) for pre-ferments.

Quantities of I_1 and I_2 were obtained in chromatographically pure form and submitted to Alfred R. Stanley, National Institute of Health, for testing of their antitumor activity. The results showed no toxic or side effects on mice and were inactive against Sarcoma 180, Carcinoma 755, and leukemia 1210.

Acknowledgments

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