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## Effect of Rice Bran Extract upon Growth of Organisms from Several Genera.

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Salle and Dunn<sup>1</sup> have shown that 25% aqueous ethyl alcohol extracts of rice bran, from which the alcohol was subsequently removed by evaporation, contain a substance which stimulates the growth of *Escherichia coli*, but not *Alcaligenes fecalis*. This substance is related to the carbohydrates, though probably not a hexose, and is possibly identical with the "pantothenic acid" of Williams *et al.*<sup>2</sup>

The action of this extract on *E. coli* led us to suspect that other organisms would also be stimulated. Forty-one organisms, which could be readily cultured in liquid media, were selected. The preparation of the extract, inoculation, and measurement of growth were the same as in the previous experiments.<sup>1</sup> Experiments performed with those to be reported, but using extract prepared by the method of Williams, *i. e.*, extraction of the bran with 60% methyl alcohol, showed both extracts to have identical stimulating properties. The 25% ethyl alcohol extraction was adopted following a suggestion by Dr. S. Lepkovsky that the extraction of large amounts of rice bran might conveniently and fairly inexpensively be made by using the first steps of the method devised by Evans and himself<sup>3</sup> for the extraction of vitamin B.

In all the tables of results, the 3 columns under growth and under final pH show the average values obtained from duplicate determinations, and represent the results obtained with 0.0 cc., 0.1 cc., and 1.0 cc. of extract respectively. The amount of growth, expressed in cubic centimeters, was determined by centrifuging 10.0 cc. of the culture in Hopkins tubes. Original and final pH's were determined by means of the hydrogen electrode. The value for the original pH is the average of the pH of the 2 dilutions of the extract and the pH of the control. The variation of these 3 values was not large enough to warrant tabulation of each in the tables. Inoculation in each case was with 0.2 cc. of an 18-hour culture of

<sup>1</sup> Salle, A. J., and Dunn, R. W., *Proc. Soc. Exp. Biol. and Med.*, 1934, **32**, 168.

<sup>2</sup> Williams, R. J., Lyman, C. M., Goodyear, G. H., Truesdail, J. H., and Holaday, D., *J. Am. Chem. Soc.*, 1933, **55**, 2912.

<sup>3</sup> Evans, H. M., and Lepkovsky, S., *J. Nutrition*, 1931, **3**, 353.

the organisms. Aerobic growth determinations were incubated 8 hours, unless designated by an asterisk (\*), in which case the incubation time was 24 hours. Gas production during growth is indicated by a dagger (†). The incubation time of the anaerobes was 6 days.

TABLE I.

Organisms	Growth (cc.)			Original pH	Final pH		
	0.0 cc. extract	0.1 cc. extract	1.0 cc. extract		0.0 cc. extract	0.1 cc. extract	1.0 cc. extract
<i>Escherichia coli</i>	.009	.017	.058†	6.88	6.83	6.70	5.69
<i>Alcaligenes fecalis</i>	.003	.004	.004	7.17	7.32	7.35	7.35
<i>Aerobacter aerogenes</i>	.008	.017	.048†	6.76	6.70	6.55	6.13
<i>Eberthella typhi</i>	.002	.012	.030	6.76	6.78	6.59	5.65
<i>Salmonella paratyphi</i> (para A)	.003	.011	.028†	6.76	6.76	6.60	6.10
<i>Salmonella schottmuelleri</i> (para B)	.010	.018	.044†	6.76	6.79	6.65	5.60
<i>Salmonella enteritidis</i>	.004	.012	.032†	6.76	6.79	6.64	5.89
<i>Salmonella aertrycke</i>	.010	.019	.050†	6.76	6.78	6.62	5.82
<i>Salmonella suispestifer</i>	.005	.015	.030†	6.76	6.80	6.62	6.13
<i>Shigella dysenteriae</i> (Shiga)	.002	.003	.003	6.76	6.79	6.68	6.72
<i>Shigella paradysenteriae</i> (Flexner)	.002	.006	.008	6.76	6.80	6.63	6.65
<i>Shigella paradysenteriae</i> (Hiss)	.003	.009	.015	6.76	6.76	6.59	6.30
<i>Staphylococcus aureus*</i>	.001	.002	.003	6.85	6.87	6.80	6.80
<i>Staphylococcus albus*</i>	.003	.006	.029	6.85	6.83	6.71	6.22
<i>Sarcina lutea*</i>	.002	.002	.002	6.85	6.86	6.85	6.88
<i>Rhodococcus roseus*</i>	.001	.000	.000	6.85	6.85	—	—
<i>Gaffkya tetragena*</i>	.002	.006	.005	6.85	6.86	6.73	6.45
<i>Streptococcus fecalis*</i>	.002	.012	.040	6.97	6.99	6.82	5.60
<i>Streptococcus lactis*</i>	.001	.005	.060	6.97	6.97	6.85	5.74

Organisms reported in Table I were grown in a medium containing 6.0 cc. of buffer of pH 7.0, 5.0 cc. of infusion medium, extract, and 0.9% NaCl saline to make the total volume 12.0 cc. The aerobic organisms reported in Table II, Group I, and the anaerobic organisms reported in Table III, were grown in a medium containing 11.0 cc. of infusion medium, extract, and saline to make the total volume 12.0 cc. Anaerobic conditions were obtained by placing cultures in a desiccator over alkaline pyrogallol, evacuating, and replacing the evacuated air with hydrogen. A 1% glucose control was run with each of the anaerobic organisms. No observations were made to determine production of gas by the latter. Organisms included in Table II, Group 2 and Group 3, were grown in 5% glycerine-infusion and 5% blood-infusion media, respectively.

Of the 41 organisms reported, the following, according to Bergey, do not ferment carbohydrates: *Alcaligenes fecalis*, *Brucella melitensis*, *Brucella abortus*, *Sarcina lutea*, *Rhodococcus roseus*, *Neisseria*

TABLE II.

Organisms	Growth (cc.)			Original pH	Final pH		
	0.0 cc. extract	0.1 cc. extract	1.0 cc. extract		0.0 cc. extract	0.1 cc. extract	1.0 cc. extract
Group I							
<i>Klebsiella pneumoniae</i> (Friedlander)	.002	.020†	.040†	7.00	7.00	6.94	5.99
<i>Bacillus subtilis</i> *	.006	.010	.012†	7.00	7.05	6.98	6.83
<i>Proteus vulgaris</i>	.003	.010†	.040†	7.00	6.86	6.78	6.01
<i>Brucella melitensis</i> *	.002	.002	.002	7.00	7.00	6.99	7.03
<i>Brucella abortus</i> *	.002	.002	.002	7.00	6.99	7.00	7.05
<i>Lactobacillus acidophilus</i> *	.001	.003	.031	7.00	6.95	6.86	6.20
<i>Vibrio comma</i> (cholera)	.010	.017†	.032†	7.00	6.97	6.77	6.25
<i>Vibrio metchnikovii</i>	.009	.017†	.052†	7.00	7.11	6.86	6.47
Group II							
<i>Corynebacterium diphtheria</i>	.007	.009	.020	7.17	6.27	6.28	5.49
<i>Corynebacterium xerosis</i>	.006	.011	.013	7.17	6.72	6.35	6.18
<i>Corynebacterium hofmannii</i>	.001	.002	.005	7.17	5.95	5.64	5.62
Group III							
<i>Streptococcus</i> (type Alpha)	.010	.021	.051	7.38	6.39	5.82	4.41
<i>Streptococcus pyogenes</i>	.012	.018	.040	7.38	6.32	5.77	4.59
<i>Diplococcus pneumoniae</i>	.003	.004	.007	7.38	6.43	5.82	4.92
<i>Neisseria catarrhalis</i>	.023	.021	.023	7.38	7.38	7.64	7.41

*catarrhalis*, and *Clostridium tetani*. Of this group only *Clostridium tetani* (Table III) was stimulated by the extract. However, glucose also stimulated the growth of this organism, showing that the strain used possesses some carbohydrate-fermentative ability. Reddish and Rettger<sup>4</sup> have shown *Cl. tetani* to have weak saccharolytic properties. *Rhodococcus roseus* (Table I) not only was not stimulated, but failed to grow in the presence of the extract.

TABLE III.

Organisms	Growth (cc.)				Original pH	Final pH			
	0.0 cc. extract	0.1 cc. extract	1.0 cc. extract	1% glucose		0.0 cc. extract	0.1 cc. extract	1.0 cc. extract	1% glucose
<i>Clostridium welchii</i>	.001	.010	.020	.000	7.00	6.92	7.00	6.70	—
<i>Clostridium histolyticum</i>	.002	.005	.007	.000	7.00	6.96	7.05	7.01	—
<i>Clostridium oedematiens</i>	.004	.013	.025	.013	7.00	7.02	7.01	6.66	6.55
<i>Clostridium sporogenes</i>	.002	.010	.020	.010	7.00	6.88	6.90	6.39	6.37
<i>Clostridium botulinum</i> A	.006	.009	.021	.008	7.00	6.95	6.96	6.52	6.60
<i>Clostridium tetani</i>	.009	.009	.030	.023	7.40	7.39	7.27	6.84	5.57
<i>Vibron septique</i>	.001	.001	.060	.000	7.00	6.92	6.90	5.76	—

<sup>4</sup> Reddish, G. F., and Rettger, L. F., *J. Bact.*, 1924, 9, 13.

On the other hand, only one organism which ferments carbohydrates was not stimulated; namely, *Shigella dysenteriae* (Table I). This is probably explained by the fact that the organism is a weak carbohydrate-fermenter, producing acid from glucose only.

In each case, except that of *Clostridium histolyticum* (Table III), stimulation was accompanied by a decrease in final pH, while normal growth showed an increase in final pH.

*Clostridium histolyticum*, though fermenting several carbohydrates with the production of acid, but no gas, does not ferment glucose, and was therefore not stimulated by it. Since no gas is produced by it from carbohydrates, increased final pH was probably due to excess basic over acidic end-products of metabolism.

For the lack of growth of *Clostridium welchii*, *Clostridium histolyticum*, and *Vibrio septique* in 1% glucose-infusion medium no explanation is offered. Reinoculation failed to produce growth in any of the 3 cases. Further investigation was not undertaken.

Attempts to purify the extract, which is admittedly a complex material, have been unsuccessful. Due to lack of equipment, the electrolysis method of Williams was not employed.

*Conclusion.* 1. Since 34 out of 35 known carbohydrate-fermenting organisms were stimulated by rice bran extract, and all 5 of the non-carbohydrate-fermenters were not stimulated, a further confirmation of the conclusion of our first paper on the subject is shown; namely, that the stimulating agent, possibly pantothenic acid, is related to the carbohydrates. 2. A quantitative study of the growth obtained seems to justify the use of rice bran extracts in bacteriological laboratories and related commercial fields.