

solution of Na_2CO_3 was added with shaking followed by 1 c.c. of amyl alcohol and 1 c.c. of distilled water. The green colour separated in the amyl-alcohol layer and could be evaluated in a Lovibond Tintometer.

(ii) Procedure for water-soluble derivatives (*Synkamin* and *Synkanit*): 0.5 to 0.15 mgm. of the substance dissolved in 0.5 c.c. of water was treated with 0.1 c.c. of the reagent and heated in a water-bath at 70°C . for about 3 minutes. It was next cooled and 0.3 c.c. of 20 per cent. sodium carbonate solution was added followed by 1 c.c. of amyl alcohol when the green colour separated in the amyl alcohol layer.

This reaction could be used to detect even 0.005 mgm. of the quinone, but a good working range for quantitative work would be 0.05 to 0.15 mgm. of the quinone.

Further work is in progress and a detailed report will be published elsewhere.

Department of Physiology, D. V. S. REDDY.
Madura Medical College, V. SRINIVASAN.
December 3, 1947.

I. Reddy, D. V. S., *Proc. Indian Sci. Cong.*, 1946, 87. 2. —, *Medical Digest*, 1945, 13, 239. 3. *Nouvelle Sci.*, 1941, 93, 358.

INFLUENCE OF EXTRACTS OF GERMINATED INDIAN PULSES ON THE FORMATION OF AMYLASE BY *BACILLUS SUBTILIS*

RAGHAVENDRA RAO AND SREENIVASAYA¹ have shown the possibility of replacing the expensive asparagine, an ideal source of organic nitrogen for micro-organisms, by aqueous extracts of etiolated seedlings of certain Indian pulses that are rich in asparagine. The present work was carried out to determine the overall efficiency of these complex sources of nitrogen on the formations of amylase by *B. subtilis* (N.C.T.C.: 2027 N). The extracts were prepared at different periods of germination (4th, 6th and 8th day), and the results of their analysis were found to concur with those of previous workers.¹ These extracts have been tried,

TABLE I

Effect of germinated pulse extract on production of Amylase by *B. subtilis* with extract containing 0.5 mg. nitrogen per 10 ml.

Days after commencement of germination	Enzyme Units per 10 ml. of medium			
	Green gram	Black gram	Bengal gram	Horse gram
4	7.5	8.6	7.9	6.0
6	9.0	12.0	11.4	8.2
8	18.0	15.0	23.7	12.0
With extract containing 1.0 mg. nitrogen per 10 ml.				
4	9.2	10.5	10.0	9.5
6	12.8	18.0	19.8	14.8
8	20.0	21.6	30.0	22.2

by the method already described,² at two levels of nitrogen (0.5 and 1.0 mg. in 10 ml. of culture medium). The results are given in Table I.

The results show that, at both levels of nitrogen, the enzyme formed increases steadily with germination during the period of observation. The highest activity is obtained with the Bengal gram extract, both at 1.0 mg. and 0.5 mg. level of nitrogen.

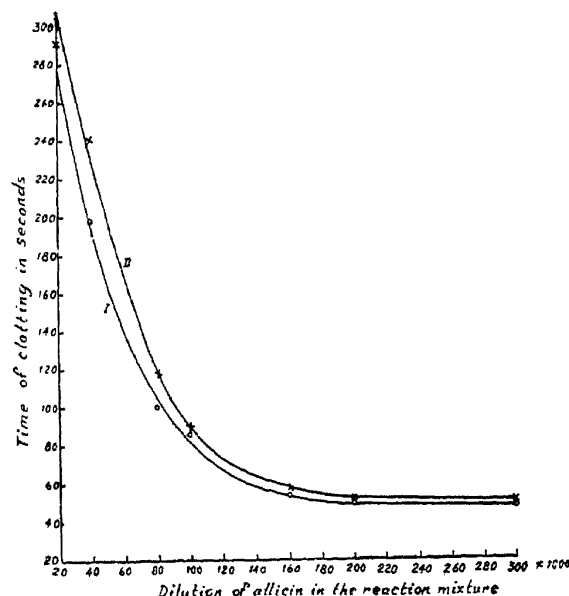
The author's thanks are due to Mr. M. Sreenivasaya and Prof. V. Subrahmanyam for their interest in this investigation.

Section of Fermentation Technology,
(later) Dept. of Biochemistry, B. S. LULLA.
Indian Institute of Science,
Bangalore,
December 12, 1947.

1. Raghavendra Rao and Sreenivasaya, *Curr. Sci.*, 1946, 15, 25. 2. Lulla, B. S. (reference of the above paper), *Ibid.*, 1948, 17, 2.

EFFECT OF ANTIBIOTICS ON THE MILK-CLOTTING ENZYMES OF *CARICA PAPAYA* AND *FICUS CARICA*

SEVERAL instances of sulphhydryl groups inactivating anti-microbial agents have been recorded in literature.¹⁻⁶ This has led to the view that the majority of the antibiotics act possibly by reacting with the -SH groupings of enzyme systems in the bacteria. Activation studies using glutathione, cysteine and other thiol compounds, and reversible inhibition by copper and maleic acid support the evidence in favour of the -SH nature of papain⁷ and the milk-clotting enzyme of *Ficus carica*.⁸ In view of this, it was of interest to study the effect of antibiotics on these enzyme systems.



Curve I after 5 minutes incubation
Curve II after 15 minutes incubation

Different dilutions each of penicillin and alliin were mixed with each enzyme (1:1 v/v),

incubated at 37° C. for different periods and the milk-clotting activities of the mixtures determined.⁹ Typical results obtained with penicillin-papain system are presented in Table I.

TABLE I
Inhibition of the milk-clotting activity of papain by Penicillin

Penicillin in O.U. per c.c. (cup plate method)*	Time in seconds for clotting after incubating the mixture for		
	15 minutes	30 minutes	60 minutes
Nil	31	31	31
500	70	111	140
250	54	83	125
100	40	45	50
50	39	41	45
20	32	35	37
10	32	33	34

* The clotting times with the mixtures before incubation were found to be 31 seconds for all the mixtures studied.

Similar results were obtained for the Ficus enzyme-penicillin systems also.

Complete and instantaneous inactivation of the enzymes resulted on incubation with 0.2 mg./c.c. of allicin. When the concentration of the antibiotic was brought gradually to about 3.5 µg, results similar to those for penicillin were obtained. In the accompanying figure, results with allicin and Ficus enzyme are presented.

The above observations confirm the -SH nature of the enzymes studied. Attention has already been drawn to the inhibiting activity of allicin¹⁰ on the starch-splitting activity of β-amylase, which has been shown to be another sulphhydryl enzyme.¹¹ These observations are being extended to other enzyme systems known to play an important role in cell-metabolism.

It is clear that the enzyme-inhibiting activities of antibiotics like allicin even in very high dilutions provides a quick and accurate method for their micro-assay.

Our thanks are due to Prof. V. Subrahmanyam for his active interest in the above work. We acknowledge with gratitude the generous support from the Council of Scientific and Industrial Research, New Delhi.

R. RAGHUNANDANA RAO.
C. R. KRISHNA MURTI.

Dept. of Biochemistry,
Indian Institute of Science,
Bangalore,
December 4, 1947.

1. Cavallito and Bailey, *Sci.*, 1944, **100**, 300.
2. Eagle, *J. Pharm.*, 1939, **66**, 436.
3. Fildes, *Brit. J. Expt. Path.*, 1940, **21**, 67.
4. Atkinson and Stanley, *Australian Journal Expt. Biol and Med. Sc.*, 1943, **21**, 255.
5. Geiger and Conn, *J. Amer. Chem. Soc.*, 1945, **67**, 112.
6. Cavallito *et al.*, *J. Bact.*, 1945, **50**, 61.
7. Ganapathi and Sastri, *Curr. Sci.*, 1940, **9**, 413.
8. Krishna Murti, C. R., *et al.*, *Sci. and Cult.* (under

9. Balls and Hoover, *J. Biol. Chem.*, 1937, **121**, 737.
10. Rao, R. R., *et al.*, *J. Sc. Ind. Res.*, 1946, **5B**, 31.
11. Weill, C. E., and Caldwell, M. L., *J. Amer. Chem. Soc.*, 1945, **67**, 214.

UTILIZATION OF DESIZING WASHINGS FOR THE CULTURE OF INDUSTRIALLY IMPORTANT MICRO-ORGANISMS

For desizing textiles, the common practice is to impregnate the sized cloth with an active amylase solution. After standing overnight the cloth is washed in running water. The washings contain about 1 per cent. sugar besides dextrans and other soluble matter. Our studies in the preparation of bacterial amylase show that the washings could be utilised as a source of carbon for the cultivation of the industrially important micro-organisms.

Bacteria (*B. subtilis*, N.C.T.C., 2027 N), Yeast (*Torula utilis*, N.C.T.C., 3050) and actinomyces (*Actinomyces griseus*, Waksman's strain), which had been previously standardized for amylase formation,¹ food yeast manufacture,² and streptomycin production,³ respectively were employed as test organisms for determining the efficiency of these waste liquids. The cultural conditions for the growth of these organisms were the same, except that the carbon supply in the culture medium was substituted by the equivalent amount of the desized washings on the basis of sugar present. The viscosimetric method* was followed for determining the amylase activity. Yeast growth was observed by Turbidity measurements. The cup assay method, using a susceptible strain of *B. subtilis*, was employed for the assay of streptomycin. The comparative data obtained with the experimental and control media are given in Table I.

TABLE I

<i>B. subtilis</i>		Yeast growth		<i>Actinomyces griseus</i>	
Amylase units per 10 ml.* of medium		Galvanometric deflection		Units γ/c.c. on 10th day	
<i>Exptl.</i>	<i>Contl.</i>	<i>Exptl.</i>	<i>Contl.</i>	<i>Exptl.</i>	<i>Contl.</i>
100.0	120.0	200.0	176.0	100 γ	80 γ

* One Amylase unit is that quantity of enzyme which, acting on a two per cent. starch solution at pH 7.0 and at 40° C., reduces the viscosity by 25 per cent. in 90 minutes.

The results show that the desized waste is a useful source of assimilable form of carbon for the micro-organisms. The difficulty of transporting large volumes of the waste could be considerably reduced either by slightly modifying the process of washing of the desized cloth or by using the washings on the spot.

My sincere thanks are due to Prof. V. Subrahmanyam for his kind encouragement, and guidance in these observations, and to the late Mr. Slicer, Chief Chemist to Bangalore Wool-