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BACTERIAL ORIGIN OF ALKALINE L-SERINE DEHYDRATASE IN FRENCH BEANS

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Abstract—Two L-serine dehydratases with pH optima at pH 5 5 and 9 0, respectively, have been identified in homogenates of French bean seedlings The L-serine dehydratase activity with optimum at pH 9 0 increases as the plant extract ages and this correlates with bacterial growth in the extract, appearance of the enzyme in plant homogenates was retarded by the antibacterial agent, chloromycetin. The main bacteria associated with the plant extracts were identified as *Erwinia herbicola* and *Pseudomonas fluorescens* Examination of cell free extracts of these bacteria show that the *Pseudomonas fluorescens* contains an active L-serine dehydratase. Whole cells of the bacterium show optimum activity towards L-serine at pH 9 0 It is concluded that in French bean homogenates this L-serine dehydratase may be of bacterial origin, in contrast, the enzyme with optimum at pH 5 5 appears to be a plant enzyme

INTRODUCTION

OUR INTEREST in the L-serine dehydratases of plant tissues originates from its possible involvement in a route postulated for acetyl-coenzyme A synthesis in chloroplasts from glycollate via glycine and serine [1,2] L-serine dehydratase (L-serine hydro-lyase (deaminating) (E C 4 2 1.13)) catalyses the reaction

$$\text{L-serine} + H_2O \leftrightarrow \text{pyruvate} + NH_3 + H_2O$$

and requires pyridoxal phosphate and a suitable divalent metal ion (Mg^{2+} or Mn^{2+}) as cofactors. The properties of this enzyme have been studied in various organisms[3–6] other than higher plants A closely related enzyme, L-threonine dehydratase (E C.4 2 1 16), catalyses the analogous deamination of L-threonine. L-Threonine dehydratase is the principal enzyme in the pathway for the biosynthesis of L-isoleucine in microorganisms and plants, and its properties have been studied in detail in various organisms[3,7–9,11] including higher plants [10,12–14] Current opinion seems to be that both L-serine and L-threonine are deaminated by the same enzyme [4,6,15]

In higher plants two types of L-threonine dehydratase have been reported to occur in

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[10] S R MODI and R MAZUMDER, *Indian J Biochem* **3**, 215 (1966)
[11] J P CHANGEUX, *Cold Spring Harp Symp Quant Biol* **28**, 497 (1963)
[12] R K SHARMA, S R MODI and R MAZUMDER, *Ind J Biochem* **4**, 61 (1967)
[13] V S TOMOVA, Z S KAGAN and W L KRETOVICH, *Biokhimiya* **33**, 244 (1968)
[14] Z S KAGAN, E M SINELNIKOVA and W L KRETOVICH, *Enzymologia* **36**, 335 (1970).
[15] J HOSHINO and H KROGER, *Hoppe-Seylers Z Physiol Chem* **350**, 595 (1969)

subcellular particles from pea seedlings [13] One enzyme, termed biodegradative L-threonine dehydratase, since it is activated by AMP and is not inhibited by L-isoleucine, has a pH optimum at 6 2 The other enzyme, biosynthetic L-threonine dehydratase, has an optimum at pH 9 0 and possesses allosteric properties of the type designated system K by Monod *et al* [8] In general both types of enzyme are found in chlorophyll containing organisms such as higher plants and the green parasitic plants like *Viscum album* L. (European mistletoe) and *Cuscuta europeae* L (Dodder) [14] In *Azotobacter vinelandii*[16] only the pH 9 0 enzyme is present while in *Arceuthobium oxycedri* (Dwarf mistletoe) only the pH 6 0 enzyme is present, in saprophytes devoid of chlorophyll such as *Monotropa hypopitys* L (Indian pipe) and obligatory parasites devoid of chlorophyll such as *Lathraea squamaria* L (Toothwort) threonine dehydratases appeared to be absent [14] Extracts of pea seedlings were inactive towards L-serine as substrate at both pH's

A threonine dehydratase with optimum pH at 9 0 identified in the soluble supernatant of spinach leaf extracts[10,12] showed some activity towards L-serine and showed other differences to the pea seedling enzyme in kinetic studies These differences may reflect existence of separate pH 9 0 isoenzymes of biosynthetic L-threonine dehydratase such as exist in *E coli* [4] No studies of the activity of spinach preparations below pH 7 0 were reported

In the present investigation extracts of *Phaseolus vulgaris* (French bean) seedlings have been examined for serine dehydratase activity over the range pH 2 0–10 0 It soon became evident that a L-serine dehydratase with optimum activity at pH 9 0 present in the homogenates might be a property of bacteria associated with the plants rather than of subcellular particles from the plant cells This aspect of the investigations is reported here

RESULTS AND DISCUSSION

pH Optima and Stability of the Enzyme

Preliminary investigations revealed that most of the L-serine dehydratase activity in French bean extracts sedimented on centrifugation with the crude chloroplast and mitochondrial fractions Serine dehydratase activity studied in relation to pH showed two peaks of activity at pH 5 5 and pH 9 0 respectively (Fig 1a) The activity evidenced at pH 9 0 increased as the extract aged at laboratory temperature but was stabilized by storage of extracts at −15°, while the increase was much reduced by storage at 0–2° (Table 1) The

TABLE 1 EFFECT OF STORAGE AT VARIOUS TEMPERATURES ON L-SERINE DEHYDRATASE ACTIVITY AT pH 9 0

Temp	Day 2	5	7
0–2°	0 8	1 3	1 2
20°	1 4	1 7	1 3
−15°	0 7	0 7	0 7

The 20,000 *g* pellet from plant extracts was resuspended in 0 01 M phosphate buffer, pH 7 0, and divided into three portions Activities are expressed as μmoles pyruvate/hr/5 mg protein The activity on day 0 was 0 7 μmoles/hr/5 mg protein

[16] W L KRETOVICH, Z S KAGAN, E M SINELNIKOVA, V S TOMOVA and L P LOSENA, *Febs Absts.* 1028 (1969)

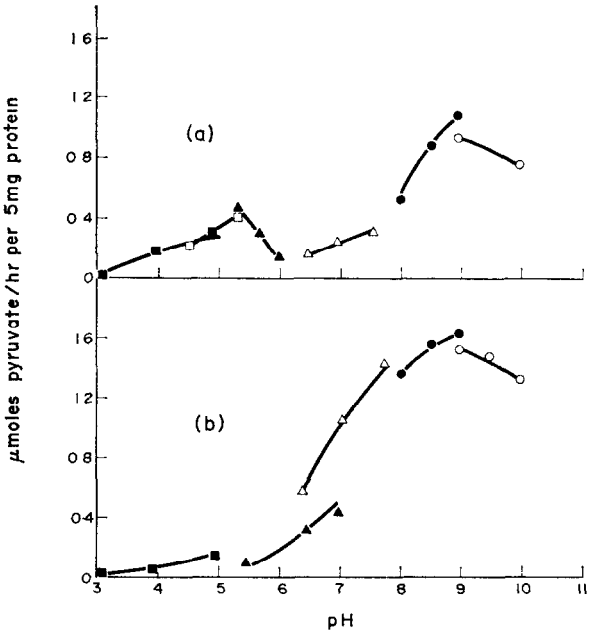

FIG 1

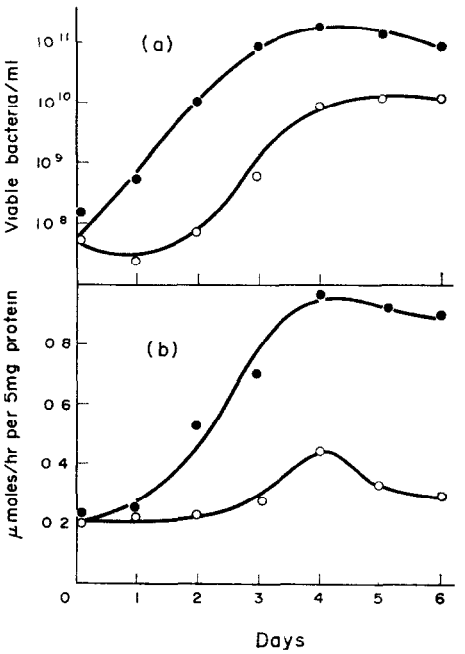

FIG 2

FIG 1 EFFECT OF AGE OF FRENCH BEAN HOMOGENATE ON pH OPTIMUM CURVE FOR L-SERINE DEHYDRATASE ACTIVITY

A plant extract (10 mg protein/ml) in 0 2 M phosphate buffer, pH 7 0, was divided into equal volumes One portion was assayed immediately (Fig 1a), the other was maintained at room temperature in the dark for five days before assay (Fig 1b) pH in individual assays was checked before and after incubation —○—○— carbonate, —●—●— tris, —△—△— phosphate, —▲—▲— maleate, —□—□— acetate, —■—■— citrate

FIG 2 RELATIONSHIPS OF BACTERIAL POPULATION AND L-SERINE DEHYDRATASE ACTIVITY AT pH 9 0 FOR FRENCH BEAN HOMOGENATES WITH AND WITHOUT ADDED CHLOROMYCETIN (2 mg/ml)

A French bean homogenate (10 mg protein/ml) was divided into two portions to one of which was added 2 mg/ml chloromycetin 1-ml samples of each incubation were assayed at 24 hr intervals for bacterial population (Fig 2a) and L-serine dehydratase activity (Fig 2b) —●—●— unsupplemented extract, —○—○— chloromycetin (2 mg/ml) present

level of enzyme fell between 5 and 7 days incubation at 20° possibly since enzyme synthesis (bacterial growth) has ceased or is now proceeding at a lower rate than loss of enzyme due to instability and other factors Similar data are given later in the studies summarised in Fig 2b. In aged extracts it is evident (Fig 3) that autolysis of bacteria would result in a shift in pH optimum of L-serine dehydratase towards lower pH's In contrast, activity at pH 5 5 steadily diminished as the extract aged and was entirely absent after a few days (Fig 1b) Storage at reduced temperatures did not stabilize the enzyme Occasionally in fresh extracts activity at pH 5 5 was greater than at pH 9 0 but typically, as in the case shown in the figure, the activity at pH 5 5 was only some half that observed at the higher pH Sonication of extracts for 2 × 30 sec slightly increased the observed activity at pH 9 0

Relationships of Bacterial Population and Enzyme Activity

Since activity at pH 9 0 increased on ageing we looked at the possibility that activity at this pH might be a reflection of bacterial contamination of the extracts The present paper

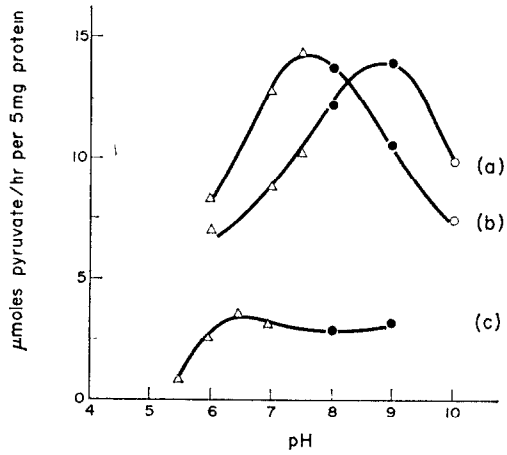

FIG 3 pH ACTIVITY CURVES FOR L-SERINE DEHYDRATASES OF *P fluorescens* AND *E herbicola* ISOLATED FROM FRENCH BEAN HOMOGENATES

For *P fluorescens* the L-serine dehydratase activity of both whole cells (a) and cell extracts (b) is shown, for *E herbicola* only the data for a cell free extract (c) is given For convenience only data for one *P fluorescens* is given since both types isolated from homogenates showed similar behaviour —○—○— carbonate (0 2 M), —●—●— tris (0 2 M), —△—△— phosphate (0 2 M)

reports this data, evidence that the pH 5 5 L-serine dehydratase is a plant enzyme and the significance of this finding will be discussed elsewhere

According to Wilson[17] chloromycetin is the only satisfactory inhibitor of bacterial growth in maize endosperm preparations, a number of other antibiotics proving less effective. We therefore supplemented freshly prepared plant extracts with 200 μg/ml or 2 mg/ml chloromycetin and examined the level of L-serine dehydratase activity at pH 9 0 over the succeeding 6 days In these experiments it was observed that in the presence of the higher level of chloromycetin the normally observed increase in L-serine dehydratase activity at pH 9 0 was delayed for some 2–3 days, the lower chloromycetin concentration had no appreciable effect

This observation suggested that bacterial contamination might be responsible for the observed increase in L-serine dehydratase activity To test this possibility we attempted to assess the typical bacterial populations of the plant extracts used in these investigations Plant extracts (10 mg protein/ml), with and without 2 mg/ml chloromycetin added, were maintained at room temperature in the dark for several days At 24 hr intervals samples of extract were examined for bacterial population and L-serine dehydratase activity This data, presented in Fig 2, showed that chloromycetin delayed both the increase in bacterial population and the appearance of L-serine dehydratase activity for some 48 hr In particular, growth of one of the bacteria, later shown to be *Erwinia herbicola*, was almost completely abolished After 48 hr the bacterial population increased but the final stationary population was significantly reduced compared to the control (chloromycetin absent) value, L-serine dehydratase levels were similarly reduced It was noted that activity of the enzyme increased

[17] C M WILSON, *Plant Physiol* **41**, 325 (1966)

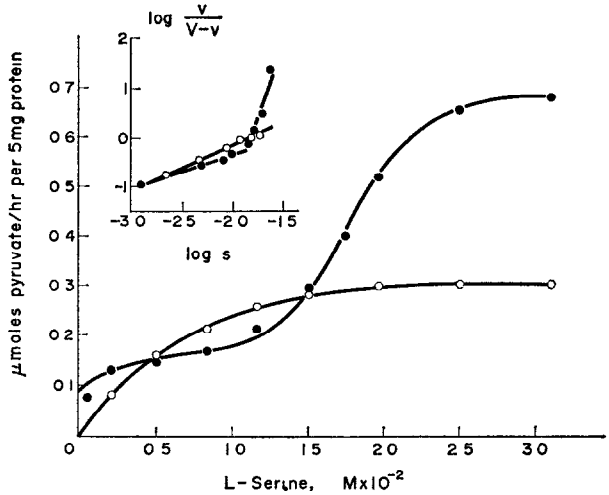

FIG 4 RELATIONSHIPS OF L-SERINE DEHYDRATASE ACTIVITY TO L-SERINE CONCENTRATION IN FRESH AND AGED FRENCH BEAN HOMOGENATES

A French bean homogenate was divided into two portions One portion was assayed immediately at pH 9 0 (—○—○—) while the second portion was assayed after ageing at room temp for 3 days at the same pH (—●—●—)

only some 5-fold over the course of the incubations whereas bacterial count increased 1000-fold. It may well be that L-serine dehydratase is present only at a particular phase of bacterial growth, or that enzymic activity is particularly subject to inhibition by cell metabolites, such as isoleucine, that accumulate Possibly, however, the *P. fluorescens* population is not homogeneous with regard to possession of L-serine dehydratase and that for some reason cells possessing the dehydratase grow less readily over the incubation period than accompanying populations If such cells are more susceptible to chloromycetin treatment this would explain the almost complete elimination of dehydratase activity following chloromycetin treatment while the effect on bacterial growth is largely a delay. We have noted that cultures of the major species of *P fluorescens* isolated from a plant homogenate can differ quite widely in activity towards L-serine Though these suggestions are speculative the data obtained does suggest that the increase in serine dehydratase activity at pH 9 0 was a property of bacteria associated with the original plant extract rather than attributable to activation of an enzyme present in plant tissues Accordingly the three types of bacteria shown to be present in the French bean extracts (*Erwinia herbicola* and two species of *Pseudomonas fluorescens* (see Methods) were separately cultured and whole cell and cell free extracts assayed for L-serine dehydratase activity. The results are summarised in Fig 3, the data showing that the *P fluorescens* possessed markedly higher levels of L-serine dehydratase activity than the *E. herbicola* Since on day 4 of the incubation one of the species of *P. fluorescens* comprised some three quarters of the contaminating bacteria the majority of the increased L-serine dehydratase activity of aged plant extracts can be assigned to this source. Whole cells of *P. fluorescens* showed a broad pH optimum at pH 9 0; however, in cell free extracts the pH optimum was approximately pH 7 5 This difference in pH optimum for

whole cells and cell free extracts presumably reflects the permeability kinetics of the substrate to whole cells In plant extracts prepared by the methods used in the present investigations, bacteria would not be disrupted, though this may have occurred to some extent in aged extracts through normal biological processes The bacterial population varied qualitatively as well as quantitatively during the course of incubations Initially *E herbicola* comprised some 50% of the colonies produced, but by day 4 of incubations the distribution was predominately (75%) the major species of *P fluorescens* with approximately equal quantities of *E herbicola* and the other species of *P fluorescens*

Kinetics of the enzyme A comparison of some kinetic parameters for the L-serine dehydratase of *P fluorescens* and the L-serine dehydratase present in freshly prepared plant extracts showed that the K_ms for serine were similar (K_ms 4–5 $\times$ 10^{-3} M) indicating that the enzyme originally present in the plant extract might have been due to contamination with *P fluorescens* Plots of reaction velocity versus L-serine concentration were hyperbolic in shape indicating a lack of homotropic cooperative interaction between L-serine molecules L-serine dehydratases have been reported in a wide variety of microorganisms and activity towards the substrate is evidenced by whole cells as well as by cell free extracts [5] The K_m for L-serine is usually within the range 3 0–5 0 $\times$ 10^{-3} M *P fluorescens* isolated from water has been shown to possess relatively high L-serine dehydratase activity [5] L-Serine dehydratases with activity below pH 6 0 have not been reported, the L-threonine dehydratase of pea seedlings showing optimal activity at pH 6 0 apparently possessing no activity towards L-serine as substrate [1,3]

Michaelis–Menten plots of kinetic data for two aged plant extracts showed anomalous behaviour (Fig 4) in that sigmoid plots, generally regarded as being typical of allosteric enzymes, were obtained These kinetic studies on aged plant extracts are given since it is interesting to note that sigmoid kinetics have been reported for biosynthetic L-threonine dehydratases of a number of plants [13,14] In the present studies on L-serine dehydratase the change from hyperbolic to sigmoid kinetics could be due to a number of causes Slaughter and Davies[18] have shown that inhibition of phosphoglycerate dehydrogenase by L-serine varied with both age of plant extract and assay pH, from hyperbolic kinetics with freshly prepared plant extracts to sigmoid kinetics with aged extracts The change was explained by stepwise degradation of the enzyme during extraction procedures This may be the case with L-serine dehydratase or alternatively the anomalous kinetic behaviour may be due to accumulation in aged extracts of metabolites such as L-isoleucine which would be expected to be allosteric effectors of the L-serine (L-threonine) dehydratases Though the data is not quoted in detail the activity of the French bean extracts used in the present work towards L-threonine as substrate (0 20 μmoles keto acid/hr/mg protein) was of the same order as that shown towards L-serine (0 25 μmoles keto acid/hr/mg protein) In *E coli* sigmoid kinetics for L-threonine dehydratase can be explained in terms of instability of the enzyme in the absence of threonine and in media of low ionic strength [19] In the same paper the kinetic data was similar to that obtained for aged plant extracts in that the plot of enzymic activity versus substrate concentration did not pass through the origin of the graph, indicating that endogenous substrate(s) in the extract had been converted to keto acid We did not obtain sigmoid kinetics with *P fluorescens* preparations or with freshly prepared plant extracts but since these incubations were well defined in terms of components these results were not unexpected and lend credence to the view that the sigmoid kinetics evidenced by aged plant

[18] J C SLAUGHTER and D D DAVIES, *Biochem J* **109**, 749 (1968)
[19] W M HARDING, *Arch Biochem Biophys* **129**, 57 (1969)

extracts and their contributing bacteria are due to presence of L-serine dehydratases from several sources and/or accumulation of allosteric modifiers of L-serine dehydratase as a result of normal metabolic processes within the associated plant and bacterial systems

The relationship of the L-threonine dehydratases reported in higher plants[10,12,13] to the L-serine dehydratase activity reported in this paper is difficult to ascertain The L-serine dehydratase present in French bean homogenates is active both towards L-serine and L-threonine as substrates, the higher affinity at pH 9 0 being shown towards L-serine The kinetics shown depend on the age of the extract (Fig 4) and the activity at pH 9 0 can largely, if not entirely, be attributed to bacterial contamination of the biological sample though the presence of a plant enzyme of similar properties cannot be entirely discounted. It is interesting to note that while the dehydratases from animals and microorganisms are usually more active towards L-threonine than L-serine a cell free extract of an unidentified *Pseudomonas* is reported[20] to show more activity towards L-serine In our view the possibility that bacteria have contributed, at least in part, to the L-threonine dehydratase activities reported for the pea and spinach homogenates cannot be ruled out The spinach L-threonine dehydratase does, however, show some properties which are not typical of the enzyme from commonly studied microorganisms, in particular, the enzyme loses activity rapidly at low temperatures unless stabilised by the presence of L-isoleucine [12]

Origin of Bacteria and Preventative Measures

Bacterial contamination of the final plant extract used in our investigations might arise from a number of sources, but the relatively high quantity of bacteria initially present suggests that they originate as part of the biological sample A check of the bacterial growth in buffers maintained under comparable conditions to those of the plant extracts for a considerable length of time (Table 2) showed that after ageing for several days only relatively low levels of bacteria were present. It is recognized that the centrifugation step in production of the 20,000 *g* crude mitochondrial fraction used in some assays would concentrate some ten-fold any bacteria present

Partial sterilization of the outer surface of the French bean leaves by wiping with cotton wool dampened with ethanol reduced bacterial populations in the 1000 *g* and 20,000 *g* fractions subsequently prepared by some 80% (Table 3) In agreement with other investigations[21,22] the figures show that the bacteria present in plant homogenates cling tenaciously

TABLE 2 BACTERIAL POPULATIONS OF SOLUTIONS STORED AT ROOM TEMPERATURE

Buffer	Start	Day 7	14	21
Phosphate pH 6	10^1	5×10^1	3×10^2	5×10^4
Phosphate pH 7	10^1	10^2	8×10^3	10^7
Carbonate pH 9	10^1	2×10^1	9×10^1	10^3
Phosphate pH 7 2 in 0 5 M Sucrose	10^2	10^3	10^6	—

All buffers were 0 2 M Figures indicate viable colony count per ml solution

[20] R. K. DART, *Biochem J* **107**, 29P (1968)
[21] C NING and H GEST, *Proc Natn Acad Sci U S A* **5**, 1823 (1966).
[22] A. A. APP and A T JAGENDORF, *Plant Physiol* **39**, 772 (1964)

TABLE 3 EFFECT OF ETHANOL STERILIZATION OF FRENCH BEAN LEAVES ON BACTERIAL CONTAMINATION OF SUBSEQUENTLY PREPARED EXTRACTS

	Bacterial population	
	Untreated leaves	Ethanol-treated leaves
Total homogenate	3×10^7	6×10^6
1000 *g* fraction	$1\,2 \times 10^7$	$2\,2 \times 10^6$
1000 *g* fraction (washed once)	$1\,1 \times 10^7$	—
15,000 *g* fraction	$1\,3 \times 10^7$	$2\,7 \times 10^6$

French bean leaves were homogenized in 0 01 M phosphate buffer, pH 7 0, and 1000 *g* and 15,000 *g* pellets prepared in sequence Bacterial population is expressed as number of bacteria per g wet weight French bean tissue

to subcellular particles and cannot be removed by washing or differential centrifugation Following homogenisation of plant tissues they appear to be distributed throughout the main membranous systems present Bacterial contamination of animal tissues is not so likely though contamination of mitochondria isolated from rat liver has been reported.[23,24]

Hallaway[25] has briefly reviewed reports in the literature on bacterial contamination of plant extracts. It is known that subcellular fractions from plant tissues frequently contain large numbers of bacteria; use of sterile buffers does not eliminate this contamination. The present work reinforces these observations, the main contaminating bacteria present in French bean homogenates being identified as *Erwinia herbicola*,[26] a common plant saprophyte found in soil, and two species of *Pseudomonas fluorescens* In other laboratory environments and in other plant species a different range of microorganisms might occur. Our observations suggest that in critical work it would be a sensible precaution to clean leaf surfaces, whenever possible, with some suitable sterilant, and to routinely use sterile buffers in preparative techniques involving centrifugation stages Supplementation of extracts with antibiotics to suppress bacterial growth in long term incubations is feasible though only chloromycetin is generally effective [27] In the present case *P fluorescens*, the main contaminating bacterium, was able to survive treatment with low (200 μg/ml) levels of chloromycetin

In studies of protein synthesis and related fields the problem of bacterial contamination has long been recognised since bacterial cells are generally some 20- to 100-fold more active than plant and animal cells in nucleic acid and protein synthesis [28] In studies of protein synthesis by plastid preparations it is reported that contributions of the order of 10% of observed incorporation of labelled amino acids into proteins can be attributed to bacteria [29] In a rat liver mitochondrial preparation containing 10^6 bacteria per ml some 90% of the amino acid incorporation in protein synthesis could be attributed to the bacteria present;

[23] L WHEELDON, *Biochem Biophys Res Commun* **24**, 407 (1966)
[24] D B RODYN, P J REIS and T S WORK, *Biochem J* **80**, 9 (1961)
[25] M HALLAWAY, in *Plant Cell Organelles* (edited by J B PRIDHAM), p 1, Academic Press, London (1968)
[26] D C GRAHAM and W HODGKISS, *J Appl Bact* **30**, 175 (1967)
[27] C J LEAVER and J EDELMAN, *Nature, Lond* **207**, 1000 (1965)
[28] D BOULTER, in *Biosynthetic Pathways in Higher Plants* (edited by J B PRIDHAM and T SWAIN), p 101 Academic Press, London (1965)
[29] M MARGULIES, *Plant Physiol* **43**, 504 (1968)

even when only 10^5 bacteria per ml were present these were responsible for 50% of the protein synthesising ability of the preparations [23]

However, other than in these fields of investigation, work on the enzymic complement of plant extracts or of subcellular components of plants has been less critical The present study shows that an enzymic activity which normally would be ascribed as being a complement of the plant cell can be largely, if not entirely, attributed to bacteria present on the surface of the plant which sediment with subcellular particles prepared by differential (and probably also gradient) centrifugation. The finding emphasizes the care that should be taken in studies on the enzymic complement of plant tissues especially in cases where the property being studied is not one uniquely associated with the photosynthetic process or when only small amounts of an activity known to be a property of microorganisms is demonstrated.

EXPERIMENTAL

Preparation of plant extracts Routinely some 40 g of leaves of 12-day-old French bean seedlings were washed in H_2O and cut into small pieces before being ground by pestle in 40 ml 0·5 M sucrose in 0 02 M phosphate buffer (pH 7 4) contained in a mortar chilled to 0–4° and maintained at this temperature. The resulting homogenate was squeezed through 4 layers of cheesecloth and the filtrate centrifuged at 300 *g* for 5 min The supernatant was decanted and centrifuged at 1000 *g* for 10 min to yield a crude chloroplast fraction and then at 20,000 *g* for 30 min to yield a crude mitochondrial fraction

Preparation of bacterial extracts Pure cultures of bacteria were prepared by isolation of colonies of each type of bacterium present from agar plates inoculated with plant extracts Samples of each bacterium were used to inoculate 250 ml Oxoid nutrient broth in 500 ml conical flasks, these then being incubated at 30° for 36–48 hr in a psychrotherm gyratory shaker (New Brunswick Scientific Co , New Jersey, U S A) operating at 275 rev/min A sample of bacteria was then removed from the liquid culture and grid dilution plated on sterile nutrient agar plates to check purity Bacteria were then harvested from liquid culture by batch centrifugation at 15,000 *g* for 15 min, and washed once in 0 01 M phosphate buffer, pH 7 1 Following centrifugation the bacterial pellet was resuspended in buffer and when cell free extracts were required bacteria were disrupted by sonic disintegration, the supernatant after centrifugation at 20,000 *g* for 15 min being termed the cell free bacterial extract Normal microbiological techniques were employed throughout

Protein estimation Protein concentrations of plant and bacterial extracts were determined by a modified biuret method[30] using crystalline bovine serum albumin as standard Prior to assay, samples were boiled with 1 0 ml 1 M NaOH and residual interference due to chlorophyll corrected for by inclusion of appropriate controls If cloudiness due to lipid was still present solutions were extracted with 2 0 ml Et_2O before spectrophotometric analysis

Assay of L-serine dehydratases Pyruvate, the product of the reaction, was assayed by a modification of the method described by Friedmann and Haugen [31] The incubation mixture, buffered to the pH at which activity was being measured, contained 100 μmoles L-serine, 80 nmoles pyridoxal phosphate and 0 5 ml plant or bacterial extract, in a total volume of 2 0 ml Incubations, usually of 60 min duration, were carried out at 35° under N_2. At the end of the incubation period 0 5 ml ice-cold 40% trichloroacetic acid was added and the mixture shaken before centrifugation at 1000 *g* for 10 min to remove precipitated protein 1 0 ml of supernatant was removed and added to 1 0 ml 10^{-3} M 2,4-dinitrophenyl hydrazine reagent After agitation the mixture was left at room temp for 20 min Finally, 1 0 ml EtOH and 5 0 ml 2 5 M NaOH were added with stirring and 5 min later the absorptivity of the solution at 515 nm was measured Activities were measured throughout by this procedure and are expressed as μmoles pyruvate produced/hr/5 mg protein When L-threonine dehydratase activity was measured L-threonine replaced L-serine in the assay medium, the keto acid produced in this case, α-oxobutyrate, was measured in the same way, correlation of absorptivity at 515 nm to keto acid present being made from the appropriate calibration curve.

Estimation of bacteria in plant extracts Estimation of bacteria in plant extracts was made by serial dilution. 1·0 ml of plant extract was transferred aseptically to 9 0 ml of sterile H_2O and any bacteria present thoroughly dispersed giving a 10^{-1} dilution Further 10-fold dilutions of this and resulting bacterial suspensions gave dilutions designated 10^{-2}, 10^{-3} etc From each dilution 0 1 ml bacterial suspension was removed and spread over the surface of an agar plate After incubation at 30° for 24 or 48 hr the number of colonies of bacteria present (in the range 30–300 colonies for a suitable dilution) was determined by visual inspection

Identification of bacteria Nearly all the bacteria identified following aerobic growth in Oxoid nutrient

[30] A. G Gornall, C. J. Bardawill and M M David, *J Biol Chem* **177**, 751 (1949).
[31] T E Friedmann and G E Haugen, *J Biol Chem* **147**, 415 (1943)

broth fell into one of three categories distinguished by the type of colony produced Present were 3–4 mm white faintly opaque colonies (15% total), 4–5 mm white opaque colonies (75% total), and 2–3 mm orange mucoid colonies (10% total) Application by Dr M E Rhodes (Department of Botany, University College of Wales, Aberystwyth) of standard microbiological tests identified these bacteria as two species of *Pseudomonas fluorescens* and *Erwinia herbicola* respectively A bacillus, probably *Bacillus stearothermophilus* was present as a minor contaminant in colonies of *E herbicola*, while another unidentified bacillus was present as a minor contaminant with the major *P fluorescens*

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Key Word Index—Phaseolus vulgaris, Leguminosae, bacterial contamination, *Pseudomonas fluorescens*, Serine (Threonine)dehydratase