Properties of an Auxin-Oxidising System from *Lactobacillus bulgaricus*

The enzymatic destruction of β-indolylacetic acid (IAA) in higher plants has been studied by several workers[1] but little is known about IAA degradation by bacterial cells[2]. Only 2 series of experiments need to be mentioned here: one on *Arthrobacter*[3] and the second on *Lactobacillus*[4,5]. The present study was undertaken in order to investigate some properties of an auxin-oxidasing system extracted from *Lactobacillus bulgaricus*.

Lactobacillus bulgaricus (strain 1416, Liebefeld Institute) was grown at 37 °C on *Lactobacillus* broth/0901 (Difco). For each series of growth measurements, tubes containing 10 ml of medium (with or without IAA) were inoculated with 1 ml of a young culture of *Lactobacillus* (standard: 0.416 mg of protein per inoculum) taken at the end of the lag phase of growth. The growth rate was expressed in terms of protein content variation. Protein was determined after precipitating the washed cells with trichloroacetic acid[5]. The preparation of IAA-oxidase and the measurement of induced IAA-oxidase activity in lyophilized *Lactobacillus* have already been described[5]. Bacterial suspension was centrifuged ($3.5 \times 10^3 g$; 15 min), prior to resuspension of the precipitate in buffer for a second centrifugation ($8 \times 10^3 g$; 10 min). The supernatant was made up to 10 ml with buffer. 2 ml of this active extract were mixed with 2 ml of H_2O and 4 ml of cold buffer. At zero time, 2 ml of IAA in H_2O (50 µg/ml) was added. The mixture was then incubated in a metabolic shaking incubator (28 °C) in complete darkness. Initial and residual IAA were determined by colorimetry[1]. The IAA-oxidase activity was expressed as µg of IAA destroyed per 0.1 mg of protein and per hour.

As can be seen in Table I, the growth of bacterial cells, expressed in terms of protein content, increased with time. Preincubation with IAA ($1 \times 10^{-5} M$) for 6 h caused a strong inhibition of growth. Preincubation for 12 h, however, resulted in a slight growth stimulation. The results suggest that exogenous IAA inhibits bacterial growth, that this inhibition is gradually decreased as the IAA is destroyed (at least partly by an exogenous oxidizing system), and that IAA destruction is followed by accelerated growth. These results are in agreement with other observations of the IAA's effect on bacterial[2,4,6] and root cells[7].

Results presented in Figure 1 show that the IAA-oxidase activity increases with the pre-incubation time. However, the exogenous IAA causes a stimulation of the IAA destruction which could be expressed in terms of an inductive reaction according to the previous observations on bacterial[3,5] and root cells[7,8].

The buffered solutions (Na_2HPO_4/KH_2PO_4) at different pH's were incubated at 28 °C. Phosphate buffers with pH below 5.0 were prepared by use of HCl N. The results (Figure 2A), for a pre-incubated culture of 8 h with $1 \times 10^{-5} M$ of IAA, clearly show that the optimum pH found was between 5.5 and 6.5. Consequently, a pH of 6.1 will be used. It may be noted that for IAA-oxidase from Lens[9], *Pisum*[10] and *Phaseolus*[11], the optimal pH's were between 6 and 7. For *Arthrobacter* preparations[3], the optimum IAA destruction was found at a pH of 6.5.

A culture of *Lactobacillus*, pre-incubated for 8 h with $1 \times 10^{-5} M$ of IAA and at pH 6.1, was used to study the

Table I. Growth (in terms of protein content) of *Lactobacillus* pre-incubated with (+) or without (—) IAA ($1 \times 10^{-5} M$)

Pre-incubation (h)	mg Protein/culture —IAA	+ IAA	Control (%)
0	0.54	0.54	0
6	2.91	1.84	—36.7
12	3.25	3.61	+11.0

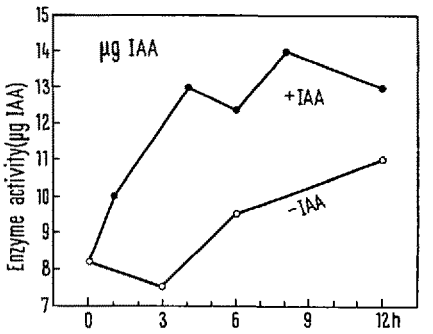

Fig. 1. Activity of an IAA-oxidasing system from *Lactobacillus* in relation to the duration (in h) of a pre-incubation with (+) or without (—) IAA ($1.10^{-5} M$). Enzyme activity expressed in µg of IAA destroyed per 60 min incubation (28 °C; pH = 6.1) and for 0.1 mg of protein.

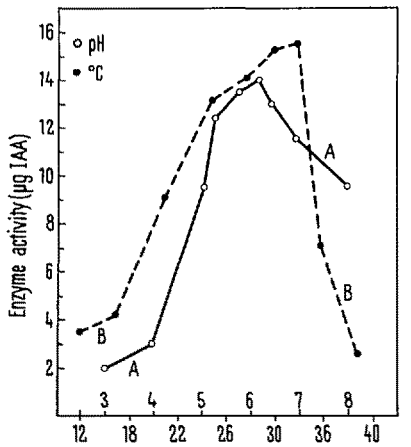

Fig. 2. Effects of pH (A) and temperature (B) on the activity of an IAA-oxidasing system from *Lactobacillus*. Enzyme activity expressed in µg of IAA destroyed per 60 min incubation and for 0.1 mg of protein. For pH variations (28 °C) and temperature variations (pH = 6.1).

[1] P. E. Pilet and T. Gaspar, *Le catabolisme auxinique* (Ed. Masson, Paris 1968).
[2] J. Rivière, Annls Inst. Pasteur, Paris, Suppl. *3*, 250 (1964).
[3] Y. Mino, Plant Cell Physiol. *9*, 169 (1968); *9*, 205 (1968); *11*, 129 (1970).
[4] B. Blanc and P. E. Pilet, C. r. Acad. Sci., Paris *261*, 2509 (1965).
[5] P. E. Pilet and B. Blanc, Nature, Lond. *217*, 285 (1968).
[6] N. Okabe and M. Goto, A. Rev. Phytopath. *1*, 397 (1963).
[7] P. E. Pilet, C. r. Acad. Sci., Paris *259*, 1183 (1964).
[8] A. W. Galston and L. Dalberg, Am. J. Bot. *41*, 373 (1954).
[9] P. E. Pilet, Physiologia Pl. *14*, 787 (1961).
[10] Y. W. Tang and J. Bonner, Archs Biochem. *13*, 11 (1947).
[11] A. C. Wagenknecht and R. H. Burris, Archs Biochem. *25*, 30 (1950).

effects of temperature. As shown in Figure 2B, temperature increases, when it rises from 16 to 32 °C; there is the activity of the IAA-destroying system. Further increase in temperature caused rapid inactivation of the system. Assays were carried out at 28 °C. Similar results were obtained for root cells[9].

To test the specificity of the auxin pretreatment, 3 IAA analogs (β-indolyl-carboxylic acid; β-indolylpropionic acid and DL-tryptophan) were used in a preincubation of 12 h, at the same concentration ($1 \times 10^{-5} M$) as IAA. For the study of the IAA destruction, a similar procedure was followed (10 ml of the active mixture, 2 ml of IAA – at 50 µg per ml – were added at zero time). The results summarized in Table II show that most IAA analogs inhibit the in vitro IAA destruction. Such observations are in agreement with those obtained with *Arthrobacter*[3].

Table II. Specificity of the auxin pre-treatment (12 h) on the IAA destruction (60 min; 28 °C; pH = 6.1) by *Lactobacillus*

Pre-treatment ($1 \times 10^{-5} M$)	µg IAA destroyed per 0.1 mg protein	Control (%)	IAA pre-treatment (%)
Control	11.2	0	—16.4
IAA	13.4	+10.7	0
β-indolylcarboxylic acid	9.5	—15.1	—29.1
β-indolylpropionic acid	8.5	—24.1	—36.1
DL-tryptophan	10.9	— 2.7	—18.6

The induction of a β-indolylacetic acid (IAA) inactivating enzyme in *Lactobacillus bulgaricus* was investigated. An IAA pretreatment of bacterial cells, which induces an IAA-oxidase, causes a strong inhibition of growth after 6 h, followed by a slight stimulation after 12 h. The optimal pH for the oxidizing system is 6.0 and the maximal IAA breakdown was observed at 32 °C. The ability of IAA to induce the oxidase was inhibited by some IAA analogues (β-indolyl carboxylic and β-indolyl propionic acids; DL-tryptophan). These results show that the induction system for IAA-oxidase has a high specificity for IAA. Properties of this enzyme-complex were found to be very similar to those of IAA-oxidase prepared from several plant tissues and explain the growth reactions of *Lactobacillus* incubated with IAA.

Résumé. Un traitement à l'acide β-indolylacétique (IAA) a pour effet d'induire – chez *Lactobacillus bulgaricus* – un système inactivant l'IAA. Le pH optimum de ce complexe enzymatique est de 6.0 et sa température optimale de réaction de 32 °C. Cette induction est inhibée par des analogues de l'IAA (acides β-indolyl carboxylique et β-indolyl propionique; DL-tryptophane). L'apparition de cette «IAA-oxydase» permet de rendre compte, dans une large mesure, des effets de l'IAA sur la croissance des microorganismes testés.

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Kinin-Releasing Activity of Pronase

REIS et al.[1] observed that after fractionation of pronase by paper electrophoresis at pH 8.6, a cathodic fraction was separated which when eluted and incubated with heat denatured plasma-preparation released bradykinin. Pronase, a commercial preparation from *Streptomyces griseus*, contains several peptidases (carboxy- and aminopeptidases) as well as proteinases active towards casein, of which one possesses in addition esterolytic activity towards N-benzoyl-L-arginine ethyl ester (BAEE)[2–4]. The specifity, inhibition and homology about the disulfide bridges of the latter enzyme suggest a close similarity with tryspin[5,6]. The experiments here reported were made in order to investigate which enzyme of pronase is responsible for the kinin-releasing activity.

Pronase P (Serva, Heidelberg, Germany) was separated into several fractions by column-chromatography on CM-Sephadex C-25 using a linear buffer gradient (Figure 1). 3 of the fractions obtained showed proteolytic activity

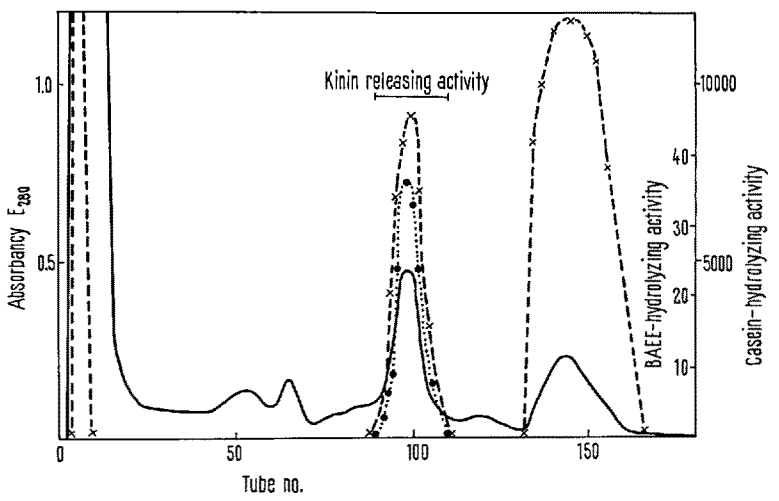

Fig. 1. Chromatography of pronase (300 mg) on CM-Sephadex C-25 (column 1.5 × 22 cm) equilibrated with 0.02 *M* ammonium acetate buffer pH 5.0. Elution was carried out using a linear buffer gradient (0.02–0.2 *M* ammonium acetate buffer pH 5.0) at a flow rate of 30 ml/h (5 ml per tube). BAEE hydrolyzing activity is expressed in µmoles BAEE hydrolyzed per min/ml, casein hydrolysis in units/ml.