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Effect of organic additives on activity of urease

C.S. FELICE[1]*, PURBAJ PANT[1], BHIM GURUNG[1], AKSHAYA NEUPANE[1] and BIJAY DHUNGEL[1]

[1]Department of Biotechnology, Koneru lakshmaiah College of Engineering Vaddeswaram- 522502, Guntur Dist, Andhra Pradesh (India).

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ABSTRACT

Urea has become the most used nitrogen fertilizer in the world, accounting for approximately 40% of the totally nitrogen supply. Its market share is increasing since it is the least expensive form of solid nitrogen fertilizer and its high nutrient content (46%N). Much of the nitrogen in fertilizer comes from urea, which bacteria degrade into ammonia and CO2 using urease.Its efficiency is however decreased by losses of nitrogen through ammonia volatilization by urease enzyme catalyzing it. In 1926 James Sumner showed that urease is a protein. Urease is found in bacteria, yeast and several higher plants. Urease is significant in the history of Enzymology as the first enzyme to be purified and crystallized. The present study was undertaken to study the isolation of the enzyme and the effect of various activators and inhibitors on the activity of the enzyme

Key words: Urea, urease, Estimation, Modulators, Inhibition, spectrophotometry.

INTRODUCTION

In 1926 James Sumner showed that urease is a protein. Urease is found in bacteria, yeast and several higher plants.urease is significant in the history of enzymology as the first enzyme to be purified and crystallized. James B. Sumner of Cornell University in (1946) received the Nobel Prize for his work with the enzyme urease, extracted from the jack bean. Urease is an enzyme that catalyzes the conversion of urea to ammonia and carbon dioxide. Certain bacteria that convert urea to ammonia as part of the nitrogen cycle contain this enzyme.Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB**)** has given nomenclature to the urease as E.C (3.5.1.5).Enzyme commission 3.5 of enzyme urease denotes its action that it is a hydrolase which breaks the bond by addition of water molecule(no:3) and specifically breaks carbon-nitrogen non peptide bond.(no:5). An unusual feature of urease is its dependence on nickel to grab onto and break up urea in the enzyme's active site. In 1982, Australian researchers Barry Marshall and Robin Warren discovered spiral-shaped bacteria in the stomach, later named Helicobacter pylori (H. pylori). After closely studying H. pylori's effect on the stomach, they proposed that the bacteria were the underlying cause of gastritis and peptic ulcers by using enzyme urease.

MATERIAL AND METHODS

Beans of *Dolichos lab lab,* Nesslers reagent,. Tris acetate buffer (10%TCA), 4. Sulphuric Acid (1N H2SO4), phosphate buffer (0.2M), ammonium sulphate solution, pure enzyme urease (0.1%)

EXPERIMENTAL

Urease enzyme was isolated from seeds of beans using mortar pestle and phosphate buffer and then stored at 4 C .Standardization of ammonium sulphate using Nessler's reagent will be done by drawing standard graph.Then determination of urease activity under various modulators with substrate (urea) by using the standard curve. Then

determination of pure urease activity under the same modulators. Then comparison of the results obtained.

Determination of urease activity

3 clean test tubes are taken and marked as control, test, blank. To the tubes control and test 2 ml of urea substrate were added for the blank 4.5 ml of phosphate buffer was added. The tubes were incubated for 10 min and add 0.5ml of enzyme to the test.. After 15 min 0.5 ml of enzyme was added to the tube control and immediately to all the tubes 0.5 ml of 10% trichloro acetic acid and 0.5 ml of 1N H2SO4 were added. All the tubes were centrifuged for 10 min at 2000rpm. After centrifugation 0.5 ml of supernatant was taken in a tube separately from all the test tubes. To the supernatant 0.5 ml of Nesslers reagent was added and the yellow colour developed was measured at 540 nm.

Table 1: Effect of Glycerol on activity of urease

Con. of glycerol	OD for 15	Conc. from graph
5%	0.20	0.146
10%	0.15	0.110
15%	0.13	0.095
20%	0.11	0.080

RESULTS AND DISCUSSION

After performing the said experiments, to the enzyme sample-varying conceentration of the inhibitor glycerol was taken and the activity of the enzyme was studied under specified and defined conditions. Enzyme activity is measured at various time intervals like 15min, 30min, 45min and maximum enzyme activity is shown at 30min, decrease for 45minutes. The results of analysis are presented in Table 1

CONCLUSION

Temperature fluctuations have significant effect on enzyme activity. Following conclusions are drawn for the enzyme activity and effect of inhibitors on enzyme activity for crude enzyme source and pure enzyme. Glycerol showed maximum inhibition at 20% concentration for crude enzyme source, and for pure enzyme maximum inhibition is shown at 20%.

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