JOURNAL OF PURE AND APPLIED MICROBIOLOGY, April 2009. Vol. 3(1), p. 379-381

Effect of Negative Modulators on Activity of Urease Isolated from Horse Gram

C.S. Felice, S. Vijaya Saradhi*, N.L.S.Bharani, V. Keerthana, P.S.H.R.K. Deepthi Sri and I. Jaya Chandra

Department of Biotechnology, Koneru Lakshmaiah College of Engineering, Vaddeswaram - 522 502, India.

(Received: 11 September 2008; accepted: 19 October 2008)

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 [1-9]. 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: Negative modulators, Urease, Nitrogen fertilizer, Horse Gram.

In 1926 James Sumner showed that urease is a protein. Urease [1-9] 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

* To whom all correspondence should be addressed.
E-mail: spsree@yahoo.com

enzyme's active site. In 1982, Australian researchers Barry Marshall and Robin Warren discovered spiral- shaped bacteria in the stomach, later named *Helicobacter 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

Macrotyloma uniflorum, 0.2M Phosphate buffer, Nessler's reagent, Ammonium Sulphate solution, Urea, Urease.

EXPERIMENTAL

Isolation of the enzyme

Urease enzyme was isolated from seeds of horse gram *(Macrotyloma uniflorum)* using mortar pestle and phosphate buffer and then stored at 4°C. Standardization of Ammonium Sulphate using Nessler's reagent is done by drawing standard graph. Then determination of urease activity under various modulators with substrate (urea) by using the standard curve. Then comparison of the urease activity under different modulators used.

Determination of urease activity

Six clean and dry volumetric flasks were taken along with control. To all these volumetric flask 10ml of Urea and 2.5ml of crude enzyme are added. Further 2.5ml of varying concentrations of NaCl solution (5%, 10%, 15% and 20 %) was added and then the volumetric flasks were incubated for 10 min. After incubation, the NH_3 evolved by the degradation of urea by urease, was estimated by adding 0.4ml of the Nessler's reagent and the yellow color chromogen developed was measured at 540 nm.

Similar tests were done taking 4 different concentrations of organic additives, 2.5 ml of Glycerol in 4 different concentrations (5%, 10%, 15% and 20 %) was added and crude enzyme activity was measured using Nessler's reagent.

Table 1. Effect of NaCl on activity of urease

Different Concentrations of NaCl solutions	O.D values after adding Nessler's reagent
Control (0%)	1.60
S1 (5%)	1.48
S2 (10%)	1.43
S3 (15%)	1.56
S4 (20%)	1.60

Table 2. Effect of Glycerol on activity of urease

Different Concentrations of NaCl solutions	O.D values after adding Nessler's reagent
Control (0%)	1.65
S1 (5%)	1.48
S2 (10%)	1.53
S3 (15%)	1.52
S4 (20%)	1.48

RESULTS AND DISCUSSION

Varying concentrations of the inhibitor were added to the enzyme solution and the activity in terms of absorbance was determined. The results of analysis for sodium chloride and Glycerol are represented in Table 1 and Table 2, respectively.

CONCLUSION

Temperature and pH have a predominant role to play in the activity of enzymes fluctuations have significant effect on enzyme activity. When the activity with the inhibitors was carefully studied it was found that glycerol showed maximum inhibition at 20% concentration for crude enzyme source, however for glycerol there was a steep and gradual decrease in activity while going from 5 to 20 %.

ACKNOWLEDGMENTS

The authors are grateful to Management of Koneru Lakshmaiah College of Engineering, Vaddeswaram, Guntur Dist, for their continuous support and encouragement and for providing the necessary facilities for carrying out the project.

REFERENCES

1. Burne, R.A. and Chen, Y.Y. Bacterial ureases in infectious diseases. *Microbes infect*. 2000; **2**: 533-542.
2. Mobley, H.L., Island, M.D. and Hausinger, R.p. Molecular biology of microbial, ureases. *Microbiol. Rev*. 1995; **59** 451-480.
3. Mobley, h.L., Island, M.D. And massad, g. Virulence determinants of uropathogenic, Escherichia coli and Proteus mirabilis. *Kidney Int. Suppl*. 1994; **47**: S129-136.
4. Mcgee, D.J. and Mobley, H.L. Mechanisms of *Helicobacter pylori* infection: bacterial, factors. *Curr. Top. Microbiol. Immunol*. 1999; **241**: 155-180.
5. Ha, N.C., OH, S.T., Sung, J.Y., Cha, K.A., Lee, M.H. and OH, B.H. Supramolecular assembly and acid resistance of *Helicobacter pylori* urease. *Nat. Struct. Biol*. 2001; **8**: 505-509.
6. P Bauerfeind, R Garner, B E Dunn, and H L Mobley, Synthesis and activity of *H. pylori* urease and catalase at low pH *Gut*. 1997; **40**(1): 25-30.
7. Susanne Klose, M.A. Tabatabai, *Soil Biology and Biochemistry*, 1999; **31**(2): 205-211.
8. M.A. Rivadeneyra, A. Gutierrez-Calderón, A.M. Rivadeneyra, A. Ramos-Cormenzana, A Study of Struvite Precipitation and Urease Activity in Bacteria Isolated from Patients with Urinary Infections and Their Possible Involvement in the Formation of Renal Calculi, *Urologia Internationalis*, 1999; **63**(3).
9. A. K. Sanyal, B. Banerjee, Urease activity in *Trichophyton Mentagrophytes*, *folia microbiologica*, 1983; **28**(2).