International Journal of Research in BioSciences
Vol. 3 Issue 2, pp. (53-57), April 2014
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Isolation, Partial Purification and Immobilization of Neuraminidase enzyme from *Vibrio cholera*

***Kumari C. K.[1], Diwedi K.[1], Singh S. K.[2], Shukla S.[2], Singh M.[2]**
[1]School of Life Science, ITM University, Gwalior, INDIA
[2]Division of Biotechnology, CytoGene Research & Development, Lucknow, INDIA

(Received April 10, 2014, Accepted April 24, 2014)

Abstract

Vibrio cholera was isolated from pond water on TCBS media. Enzyme (Neuraminidase) was isolated from Vibrio cholera and concentration was quantified using Lowry's method. The concentration is proportional to the absorbance. The difficult ranges of acetone were chosen for precipitation and enzyme quantification was performed for precipitated proteins obtained from their range. For acetone from 70% & 100% was chosen as from established sources it was found that 100% acetone give maximum concentration of enzyme. Enzyme sample obtained from precipitation with different acetone was then subjected to SDS-PAGE analysis. The band pattern of enzyme was observed on the basis of their molecular weight and purity after SDS-PAGE. The molecular weight of Neuraminidase was found to 50 kd. The purified enzyme was the subjected to immobilization with in sodium alginate beads.

Keywords: *Vibrio cholera,* Silica gel, Acetone, Neuraminidase, SDS-PAGE, Sodium Alginate.

Introduction

Vibriochloera is highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum. *Vibrio cholera*, the agent of cholera, is the most important. *Vibrio cholera* was first isolated in pure culture by Robert Koch in 1883, although it had been seen by other investigators, including Pacini, who is credited with describing it first in Florence, Italy, in 1854. Neuraminidase enzymes is produced by *Vibrio cholera,*are glycoside hydrolase enzymes (EC 3.2.1.18) that cleave the glycosidic linkages of neuraminic acids[1]. Neuraminidase enzymes are a large family, found in a range of organisms. The best-known neuraminidase is the viral neuraminidase, a drug target for the prevention of the spread of influenza infection[2]. The viral neuraminidases are frequently used as antigenic determinants found on the surface of the Influenza virus. Some variants of the influenza neuraminidase confer more virulence to the virus than others. Other homologs are found in mammalian cells, which have a range of functions. At least four Mammalian Sialidase homologs have been described in the human genome (see NEU1, NEU2, NEU3, NEU4)[3].Neuraminidases occurring on the surfaces of bacteria and other microorganisms are not as well characterized as those found on influenza viruses. However, these enzymes are known to contribute to the virulence of some bacteria. For example, the bacterium Pseudomonas aeruginosa produces a neuraminidase that appears to facilitate the formation of biofilms in the respiratory tracts of animals[4-6]**.** Biofilm production is believed to contribute to the pathogenicity of this organism.Drugs called neuraminidase inhibitors, which include oseltamivir (Tamiflu) and zanamivir (Relenza), inhibit the release of influenza A and B viruses from host cells. This inhibition stops the process of viral replication. Neuraminidase inhibitors are commonly used in both the prevention and the treatment of influenza[7].

Materials and Methods

Sample Collection

Vibrio species were isolated from pond water which was used for Neuraminidase enzyme production.

Fermentation Media for Neuraminidase Enzyme Production

Fermentation media was prepared for production of Neuraminidase form *Vibrio species.* (Alanine: 2gm, Glucose 1.5gm, Calcium Chloride: 1.25gm, Magnesium Chloride: 1.25gm, Sodium Phosphate Monobasic:1.69gm, Sodium Phosphate Dibasic:1.69gm, Sodium Chloride 1.25gm, Sodium Carbonate:1.25gm for 100 ml)

Purification of Neuraminidase

Acetone precipitation

The organism was inoculated in fermentation media for the production of Neuraminidase for 48 hours as described previously. The cells was separated by centrifugation (10000 rpm, 15 minutes), and the supernatant was fractionated by precipitation with Acetone at 70% and 100% of saturation. All subsequent steps will be carried out at 4°C.

Column chromatography

Column chromatography was prepared by washing it with distilled water and then with methanol, and then again with distilled water. Now Silica gel was poured in column and let it settle down after it get settled down cured extract was poured on it. After this variable buffer starting from 5ml was poured into the column and the variables was collected in the test tubes. Same step was done repeated for each buffer. These variables will be stored at 4^{o}C.

Quantification of Neuraminidase

Protease estimation by Lowry's method

0.2ml of sample was pipette into series of test tubes, leaving blank test tube. Then add distilled water with different concentration to make up the volume 2ml including blank. Add 5ml of alkaline copper sulphate solution in each test tube and allow standing for 15 min at room temperature. Then 0.5 ml of Folin - Ciocalteau reagent was added mixed well and keep in water bath at 100^{o}C for 15 min and cool at room temperature. Blue colour was developed. Now absorbance will be read at 620nm.

Molecular weight determination of Neuraminidase

Electrophoresis of enzyme

SDS PAGE electrophoresis was performed for different purification stage of enzyme in order to determine the molecular weight of Neuraminidase enzyme.

Immobilization of Neuraminidase

Immobilization of Neuraminidase enzyme was done by 4% Sodium Alginate in 1.4% of Calcium Chloride Solution. The enzyme was stored as beads for future use at 4^{o}C.

Result and Discussion

Sample Collection

The pond water sample was collected for isolation of *Vibrio Cholera* on TCBS Media.

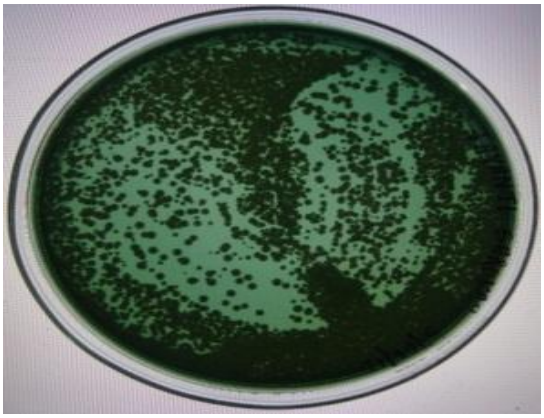


Figure 1: *Vibrio cholera*

Precipitation of Neuraminidase by Acetone

Precipitation of Enzyme extract was done at 70% and 100% concentration of Acetone. Best precipitation was obtained at 100% concentration of Acetone.

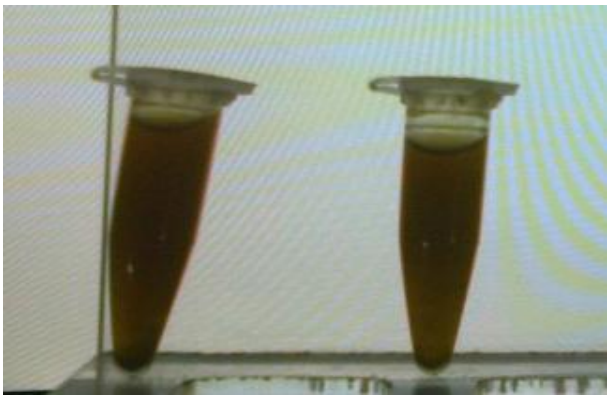


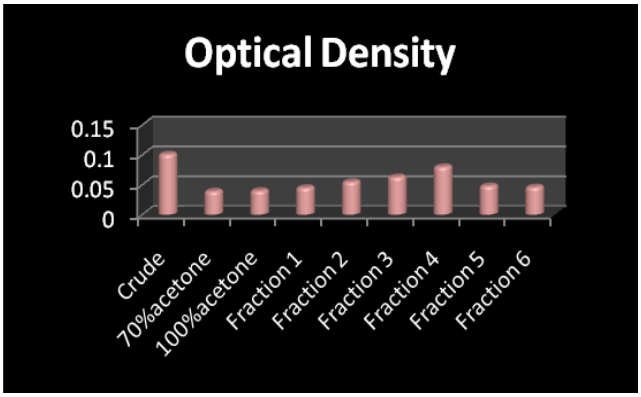
Figure 2: Precipitation by Acetone

Column Chromatography

Extract enzyme sample was partially purified by Column Chromatography using Silica Gel, different fraction were collected for estimation of enzyme.

Lowry Quantification of *Vibrio cholera* enzyme extract

All the samples of *Vibrio cholera* from crude to column chromatography fraction were quantified by Lowry's method at 600nm.



Graph 1: Lowry quantification of *Vibrio cholera*

Electrophoresis of enzyme

Molecular weight of different fraction of protein by SDS-PAGE electrophoresis was performed.

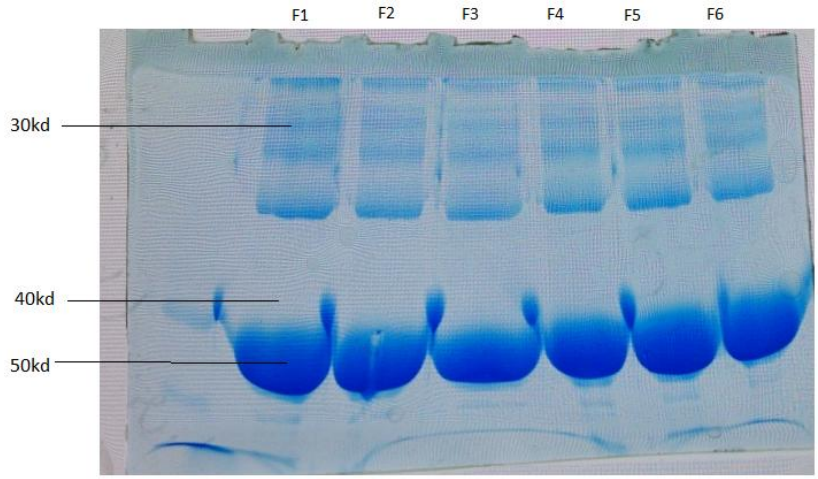
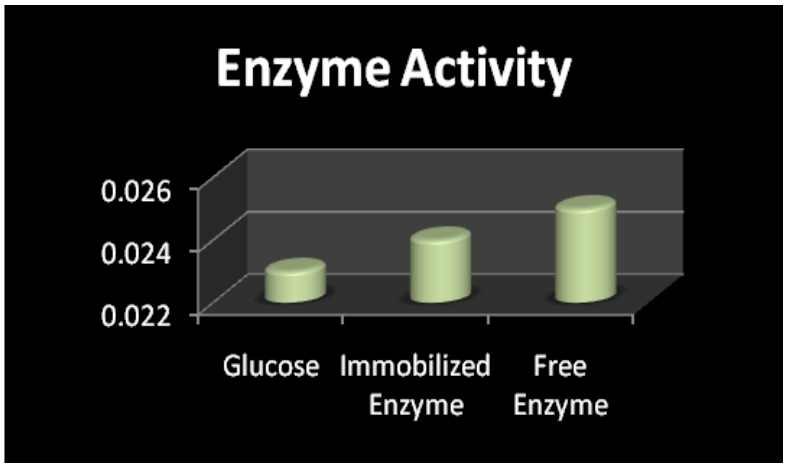


Figure 3: SDS-PAGE of *Vibrio cholera*

Immobilization of Enzyme

The purified Neuraminidase enzyme was immobilized in Sodium alginate Beads, so that it can be stored for further use at different range of temperature and pH. All the purified samples of different enzymes extracted were immobilized by sodium alginate and their activity were obtained against glucose.



Graph 2: Enzyme Activity of Immobilized Enzymes

Conclusion

Vibrio cholera was isolated from pond water on TCBS media. Enzyme (neuraminidase) concentration was quantified using Lowry's method. The concentration is proportional to the absorbance. The difficult ranges of acetone were chosen for precipitation and enzyme quantification was performed for precipitated proteins obtained from their range. For acetone from 70% & 100% was chosen as from established sources it was found that 100% acetone give maximum concentration of enzyme. Enzyme sample obtained from precipitation with different acetone was then subjected to SDS-PAGE analysis. The band pattern of enzyme was observed on the basis of their molecular weight and purity after SDS-PAGE. The molecular weight of Neuraminidase was found to 50 Kd. The purified enzyme was the subjected to immobilization with in sodium alginate beads.

Acknowledgement

I am thankful to CytoGene Research & Development, Lucknow for providing me with necessary facilities for the work, I am also thankful to the almighty without whose consent is not possible.

References

1. Morris J.G. Jr, Black R.E. Cholera and other vibrioses in the United States. N Engl J Med; 312:343 **(1985)**.

2. Hlady W.G., Klontz K.C., The epidemiology of Vibrio infections in Florida, 1981-1993. J Infect Dis; 173:1176**(1996)**.

3. Joseph S.W., Colwell R.R., Kaper J.B., Vibrio parahaemolyticus and related halophilic Vibrios. Crit Rev Microbiol; 10:77 **(1982)**.

4. Miwatani T., Takeda Y., Vibrio parahaemolyticus: A causative bacterium of food poisoning, Saikon Publishing Co, Tokyo **(1976)**.

5. Sakazaki R., Tamura K., Kato T., Studies on the enteropathogenic, facultative lyhalophilic bacterium, Vibrio parahaemolyticus. 3. Enteropathogenicity. Jpn J Med SciBiol; 21: 325 **(1968)**.

6. Miyamoto Y., Kato T., Obara Y., In vitro hemolytic characteristic of Vibrio parahaemolyticus: its close correlation with human pathogenicity. J. Bacteriol; 100:1147**(1969)**.

7. Sanyal S.C., Sen P.C., Human volunteer study on the pathogenicity of Vibrio parahaemolyticus. In: International Symposium on Vibrio parahaemolyticus, Saikon Publishing Company Ltd, Tokyo. 227, **(1973)**.