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Extraction and partial purification of urease enzyme from Jack fruit

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ABSTRACT

Jackfruit (*Artrocarpus Heterophyllus*) has a place with family Moraceae is a basic piece of normal Indian eating routine. Urease, one of the very productive known catalysts catalyzes the hydrolysis of urea into NH_3 and carbon dioxide. The present study aimed to extract urease enzyme from jackfruit. The protein extraction was done from jackfruit, after dialysis and precipitation, isolated urease was characterised by determining optimum pH and temperature and finally confirmed by the protein isolation technique, SDS-PAGE gel electrophoresis. The optimum temperature of urease activity was found to be 40 degree Celsius. The optimum pH of urease activity was found to be 6.9. The molecular weight of partially purified urease is 90 KDa. Thus, urease enzyme isolated from jackfruit source showed potential activity on physiological functions in human beings. Since plant urease enzyme is used as a vaccine. In future; the study can be extended in purifying the enzyme and it can be used for clinical aspects.

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INTRODUCTION

Ureases have a place in superfamily of amidohydrolases and phosphotriesterases. It's a protein catalyzes the hydrolysis of urea into carbon dioxide and smelling salts (Holm, Sander, 1997). The jackfruit tree is appropriate to tropical swamps, and its natural product is the biggest tree-borne organic product, coming to as much as 35 kg (80 lb) in weight, 90 cm (35 in) long, and 50 cm (20 in) in width (Pradeepkumar, 2008, Spada *et al.,* 2017). 100 to 200 organic products can be created by a developed jackfruit. The jackfruit is a various organic product, made out of hundreds to thousands of individual blossoms, and it is the meaty petals that are eaten (Krajewaska, Barbara, 2009). In the tropical locales of the world jackfruit tree is a generally developed and well-known sustenance thing (Martin, Hausinger, 1992). The mash of jackfruit is made out of 2% protein, 1% fat 74% water and 23% starches. In a 100-gram partition, crude jackfruit gives 400 kJ (95 kcal) and is a rich source (at least 20% of the Day by day Esteem, DV) of vitamin B6 (25% DV). It contains direct levels (10-19% DV) of vitamin C and potassium, with no different supplements in noteworthy substance (Dixon *et al.,* 1979).

Jackfruits have a particular fruity and sweet smell. In an investigation of flavor volatiles in five jackfruit cultivars, the primary unstable intensifies that were identified were ethyl isovalerate, propyl isovalerate, butyl isovalerate, isobutyl isovalerate, 3-methylbutyl acetic acid derivation, 1-butanol, and 2-methylbutan-1-ol. An unopened and completely riped jackfruit is known to "discharge a solid fragrance," within the organic product depicted as possessing an aroma similar to pineapple and banana. In the wake of simmering, the seeds might be utilized as a business other option to chocolate smell (Jabri *et al.,* 1995). Indistinguishable subunits (~90 kDa each) make up the contagious and plant ureases, most normally amassed as trimers and hexamers. For instance, jack-bean urease has two auxiliary and one synergist subunits exhibit in jack bean urease.

The α subunit contains the dynamic site, it is made out of 840 amino acids for each atom (90 cysteines), its sub-atomic mass without Ni (II) particles adding up to 90.77 kDa. The mass of the hexamer with the 12 nickel particles is 545.34 kDa. It is fundamentally identified with the (αβγ) 3 trimer of bacterial ureases. Different cases of homohexameric structures of plant ureases are those of soybean, pigeon pea and cotton seeds proteins (Zambell *et al.,* 2011). The (alpha) subunits are the dynamic site of all ureases. It is a bis-μ-hydroxo dimeric nickel focus, with an interatomic separation of ~3.5 å (Mobley and Hausinger, 1989) attractive vulnerability tests have shown that, in jack bean urease, high turn octahedrally planned Ni (II) particles are pitifully antiferromagnetically coupled. X-beam ingestion spectroscopy (XAS) investigations of *Canavalia ensiformis* (jack bean), *Klebsiella aerogenes* and *Sporosarcina pasteurii* (some time ago known as *Bacillus pasteurii* (Rosenstein, 1986) affirm 5– 6 organize nickel particles with solely O/N ligands (two imidazoles for every nickel) (Agrawal *et al.,* 2011).

The water particles are situated towards the opening of the dynamic site and frame a tetrahedral bunch that fills the cavity site through hydrogen bonds, and it's here where urea binds to the dynamic site for the response, uprooting the water atoms. The amino corrosive deposits take an interest in the substrate authoritative, basically through hydrogen holding, balance out the synergist change state and quicken the response. Furthermore, the amino corrosive build ups engaged with the design of the dynamic site make part out of the portable fold of the site, which is said to go about as a door for the substrate (Tang *et al.,* 2009). Cysteine deposits are regular in the fold district of the catalysts, which have been resolved not to be basic in catalysis, albeit associated with situating other key build ups in the dynamic site suitably. In the structure of *Sporosarcina pasteurii* urease the fold was found in the open adaptation, while its shut compliance is evidently required for the response (Caron, Tyler, 2015).

Whenever thought about, the α subunits of *Helicobacter pylori* urease and other bacterial ureases line up with the jack bean ureases, proposing that all ureases are developmental variations of one tribal protein (Glibert *et al.,* 2006). Note that the coordination of urea to the dynamic site of urease has never been seen in a resting condition of the compound (Daigh *et al.,* 2014). Present day natural chemistry has expanded its interest for urease. Jack bean supper (Kumari *et al.,* 2016), watermelon seeds, and pea seeds have all demonstrated helpful wellsprings of urease. The point of this investigation is to extract the enzyme urease from jackfruit.

MATERIALS AND METHODS

Protein extraction from Jack fruit

Ten grams of Jack fruit was weighed and macerated in a mortar and pestle and then suspended in 10 ml of distilled water. Occasional stirred for 3 h and filtered using double layer cheese cloth. After 15 minutes of centrifuging of the filtrate, the supernatant was isolated and used as "crude extract".

Assay of urease

The urease from jack fruit was extracted and isolate was evaluated by estimating the measure of the breakdown item alkali by the "Phenol-Hypochlorite" strategy. Response time took into account urease compound action was 2 min soluble hypochlorite, phenol and sodium nitroprusside were included. The ammonia delivered in respond with soluble hypochlorite and phenol in nearness of impetus sodium nitroprusside, to frame an indophenol, which is blue in shading. The absorbance of indophenols was read at 625 nm.

Estimation of protein

Proteins were estimated by Lowry et al. using BSA as standard. Distinctive convergences of BSA were readied extending from (0 to 25) μg/mL. The straight alignment bend was utilized to decide the convergence of protein in the measure and evaluated for the first example.

Enzyme characterisation

Determination of optimum pH and temperature

Optimum pH and temperature of compound was analysed by testing the enzyme activity at various pH and temperature.

Ammonium sulphate precipitation and dialysis

Ammonium sulphate precipitation is a standout amongst the most usually utilized strategies for vast and lab scale protein refinement and fractionation that can be utilized to isolate proteins by modifying their dissolvability within the sight of a high salt fixation.

SDS-PAGE

The dialysed proteins in test were fractionated utilizing SDS-PAGE electrophoresis (Laemmli 1970). 30 μg protein tests was run on 12% diminished SDS-PAGE. After the run, gel was settled in recoloring answer for 30 min at room temperature. Thus the gel was destained for 45 min and afterwards photographed. SDS-PAGE was keep running with protein marker.

RESULTS AND DISCUSSION

The amount of urease present in jack fruit was 62.5 mg of nitrogen. The optimum pH of urease activity was found to be 6.9 (Figure 1). The optimum temperature of urease activity was found to be 40 degree Celsius (Figure 2). On gel pictures (Figure 3), it was seen that the Molecular weight (Mr) of the urease compound is 90kDa.

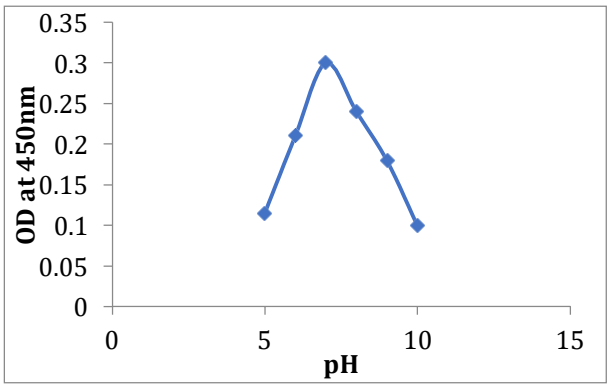

Figure 1: Impact of pH on Urease action

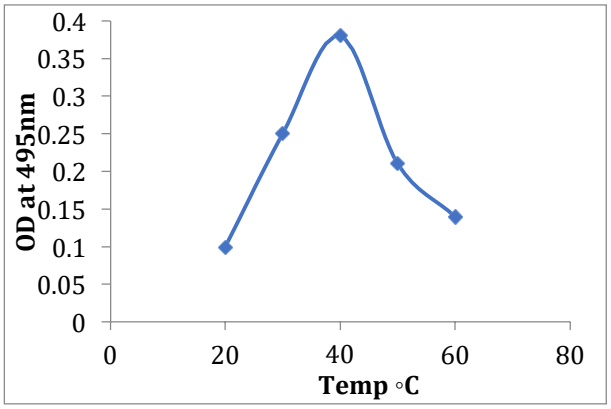

Figure 2: Impact of temperature on Urease action

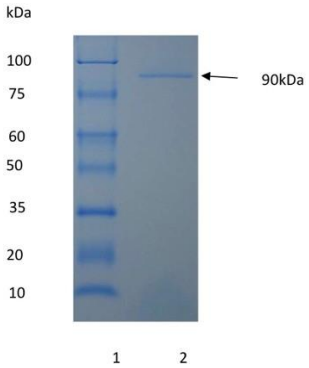

Figure 3: Enzyme isolation using SDS-PAGE

Bacterial ureases are the important risk factor for the pathogenesis of some medicinal conditions. They are related with hepatic encephalopathy, hepatic disease, stones, and peptic ulceration. Infection leads to urinary stones are a blend of struvite ($MgNH_4PO_4 \bullet 6H_2O$) and carbonate apatite [$Ca_2(PO_4)_6 \bullet CO_3$]. These polyvalent particles are solvent however end up insoluble when smelling salts is delivered from microbial urease amid urea hydrolysis, as this builds the encompassing conditions pH from roughly 6.5 to 9. The resultant alkalinization brings about stone crystallization. In people the microbial urease, *Proteus mirabilis*, is the most well-known in disease initiated urinary stones (Rosenstein, 1986).

Studies have demonstrated that *Helicobacter pylori* alongside cirrhosis of the liver reason hepatic encephalopathy and hepatic trance like state. As ureases they hydrolyze urea to create alkali and carbonic corrosive. As the microscopic organisms are limited to the stomach NH_3 delivered is promptly taken up by the circulatory framework from the gastric lumen. This outcomes in raised NH_3 levels in the blood and the clinical condition called as hyperammonemia. Hence, the annihilation of *Heliobacter pylori* indicate stamped diminishes in alkali levels (Agrawal *et al.,* 2011).

Urease protein fills in as a harmfulness factor and is in charge of pathogenesis in people. Urease movement of microbial sources has added to the improvement of numerous infection. Urease from plant source is utilized as immunization against microbial disease based on its inhibitory movement.

CONCLUSION

Thus, ureases from jackfruit source show potential activity upon gastrointestinal infections. In future, the study can be extended in purifying the enzyme and structurally it can be studied in detail. Plant urease thus can be applied for the treatment of many health disorders like gastric diseases and hypertension.

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