

# Production of urease enzyme from ureolytic yeast cell

Bharathi N\* and Meyyappan RM

Bio-Electro Chemical Laboratory,

Dept of Chemical Engineering, Annamalai University, Annamalai nagar-608002

Tamilnadu, India

\*[bharibt@gmail.com](mailto:bharibt@gmail.com)

**Abstract-** The objective of the present study is investigated the potentiality of isolated soil yeast *C. Tropicalis*, which produce urease enzyme and their conditions were carried. Parameters for growth conditions were studied. Urease producing *C.tropicalis* was identified by urease test and CHROMagar medium. Urease enzyme Crystals were confirmed by microscopic and macroscopic view.

## Introduction

Urease is a nickel dependent metalloenzyme which catalyzes the hydrolysis of urea to yield ammonia and carbamate, the latter compound spontaneously hydrolyzes to form carbonic acid and another molecule of ammonia. The best-studied urease is that from jack bean, which was identified as the first nickel metalloenzyme and urease from jack bean (*Canavalia ensiformis*) was the first enzyme to be crystallized. In 1926, James Sumner showed that urease is a protein. Urease is found in bacteria, yeast, and several higher plants. Urease is a cytosolic enzyme. Its major activity with some exceptions is associated with the soluble fractions of the cells. The best genetic data of plant ureases are available for soybean (*Glycine max*). Two urease iso-enzymes, a tissue-ubiquitous and embryo-specific encoded by two separate genes, as well as regulatory proteins encoded by unlinked genes were identified in soybean. The embryo-specific urease is an abundant seed protein in many plant species, including soybean, jack bean and *Arabidopsis*, while the other type of urease (called ubiquitous) is found in lower amounts in vegetative tissues of most plants. The enzyme urease occurs in a wide variety of tissues in humans, as well as in bacteria, yeasts, molds, plants and invertebrates. In 1926, Sumner was the first chemist who showed that urease was a protein of the globulin type with an iso-electric point of five.

**Microbial urease:** Microbial ureases hydrolyze urea to ammonia and carbon dioxide. There are many microbial sources for this enzyme including bacteria such as *Lactobacillus ruminis*, *Lactobacillus fermentum* and *Lactobacillus reuteri* and *Klebsiella aerogenes* and fungi such as *Rhizopus oryzae*. Filamentous fungi are the sources of about 40% of all available urease enzymes. Urease activity of an infectious microorganism can contribute to the development of urinary stones, pyelonephritis, gastric ulceration, and other diseases. In contrast to its pathogenic effects, microbial ureases are important enzymes in ruminant metabolism and in environmental transformations of certain nitrogenous compounds. Thus urease activities serve as an indicator of pathogenic potential and of drug resistance among some groups of bacteria (Banerjee and Aparna 2013).

## Materials and methods

### *Candida tropicalis*

From agricultural soil, sample was collected, using sterile water serially diluted and plated on

Sabouraud dextrose agar, *Candida tropicalis* colonies are cream colour with mycelial border. They are smooth, glabrous and yeast like in appearance.

Microscopic morphology shows spherical budding yeast like

No capsules present on Indian ink preparation.

Germ tube test shows negative, Hydrolysis of Urea shows positive, Growth on Cyclohexidine medium shows positive, Growth at 37<sup>0</sup> C shows positive

On CHROM agar medium shows bluish purple color (Ainscough and Kibbler, 1998).

#### **Urease test**

The media composition for urease test are; urea (20g/l), Na<sub>2</sub>HPO<sub>4</sub> (9.5g/l), KH<sub>2</sub>PO<sub>4</sub> (9.1g/l), SD broth (0.1g/l) and 0.01g phenol. pH was made to 7. This test detects the ability of organism to produce urease enzyme. This enzyme converts urea to ammonia and CO<sub>2</sub>, which convert the environment alkaline and turns pink colour referred as urease positive (Bharathi and Meyyappan, 2014).

#### **Incubation period**

Incubation was carried out for a time period ranging from 8 to 64 h with 8 h interval and the urease production was estimated

To determine the optimal pH for maximum urease, the production was tested ranging from 5 to 8 with the interval of pH 0.5. To know the optimal temperature for maximum urease, the production ranging between 20 to 40°C with the interval of 5°C. The effect of varying salt concentrations was checked between 5 to 50 ppt with the interval of 5 ppt since the strain is of marine origin.

**Inoculum size** Optimization of inoculum size with varying concentrations of addition 10 to 50 ml of  $6-8 \times 10^7$  cells/ml is one important factor for maximizing urease production and time conception by earlier production.

#### **Enzyme assay**

In the semi-quantitative screening method, the enzyme production was evaluated by visual inspection of the phenol red color change caused by ammonia liberation in the culture tubes.

The amount of color change intensity was correlated to the enzyme production. In the quantitative urease assay, the enzyme activity in media supernatants and homogenized mycelia was measured by Weatherburn method [21] with some modifications, i.e. Na<sub>2</sub> HPO<sub>4</sub> was used instead of NaOH in alkaline hypochlorite solution and the 20 minute time for color development was elongated to 30 minutes. The reactions were done in micro tubes containing 100 µl of sample, 500 µl of 50 mM urea, and 500 µl of 100 mM potassium phosphate buffer (pH 8.0) in a total volume of 1.1 ml.

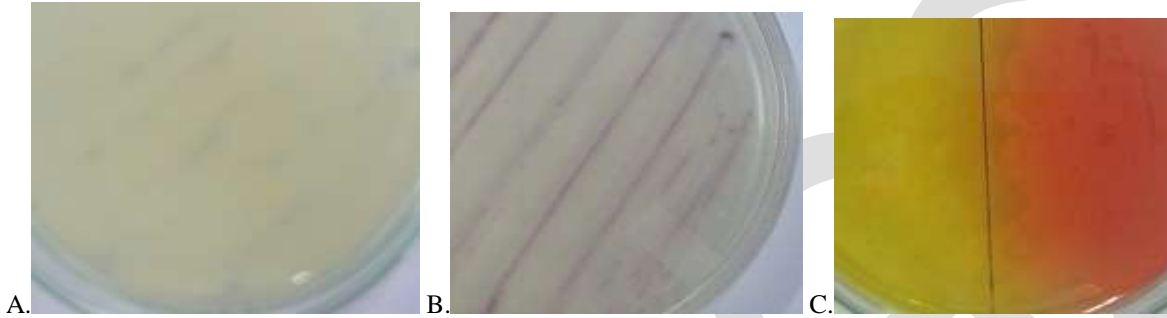
The reaction mixture was incubated in a shaking water bath at 37°C for 30 min. The reaction stopped by transferring 50 µl of reaction mixture to the tubes containing 500 µl of phenol-sodium nitroprusside solution (0.05 g sodium nitroprusside + 1 g phenol/100 ml distilled water). Five hundred micro liters of alkaline hypochlorite (3.56 g Na<sub>2</sub>HPO<sub>4</sub> + 1 ml sodium hypochlorite + 100 ml distilled water) was added to the tubes, and incubated at room temperature for 30 min. Finally, the optical density of the color complex was measured at 630 nm against the blank (500 µl phenol nitroprusside sodium + 500 µl sodium hypochlorite + 50 µl distilled water) with a spectronic 20D + spectrophotometer and compared to a standard curve prepared with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Controls used for the enzyme reactions were reaction mixture without substrate and reaction mixture without incubation. One unit of urease activity was defined as the amount of enzyme liberating 1 µmol NH<sub>3</sub> from urea per minute, under the above assay conditions (Natarajan, 1995).

## Results

The collected samples were serially diluted and plated on urea agar with pH 6.5 with phenol red pH indicator and incubated at 37°C for 48 h. After incubation, colonies showed pink colour are urease producers (that is) confirm hydrolysis of urea resulting ammonia, which forms an alkaline environment.

The isolated strains were identified as *Candida tropicalis*, strains were grown in urea broth and after incubation at 37°C for 48 h with pH 6.5, the broth was centrifuged and the cell free supernatant was used for phenol hypochlorite assay. Showed a maximum urease activity of 1.82U/ ml

### Isolation of *Candida tropicalis* from soil

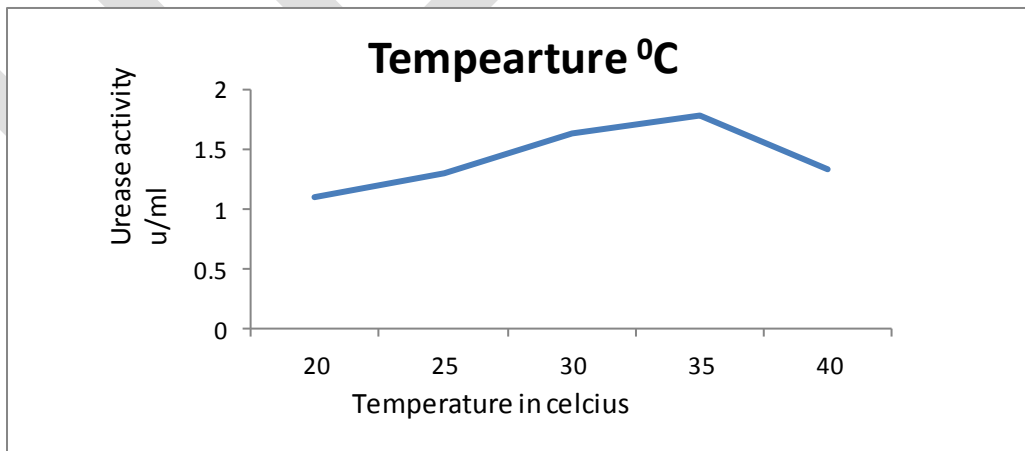
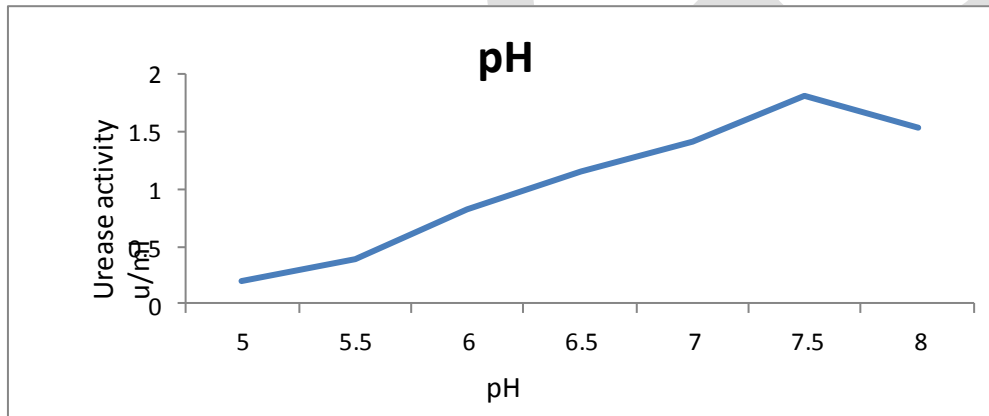
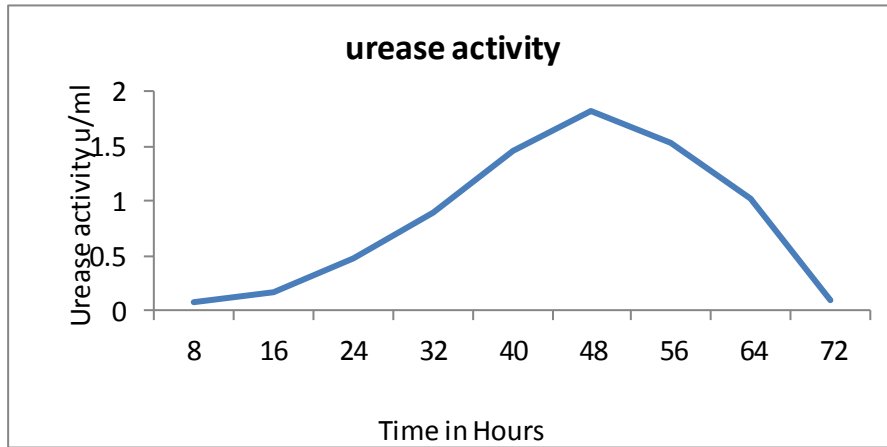


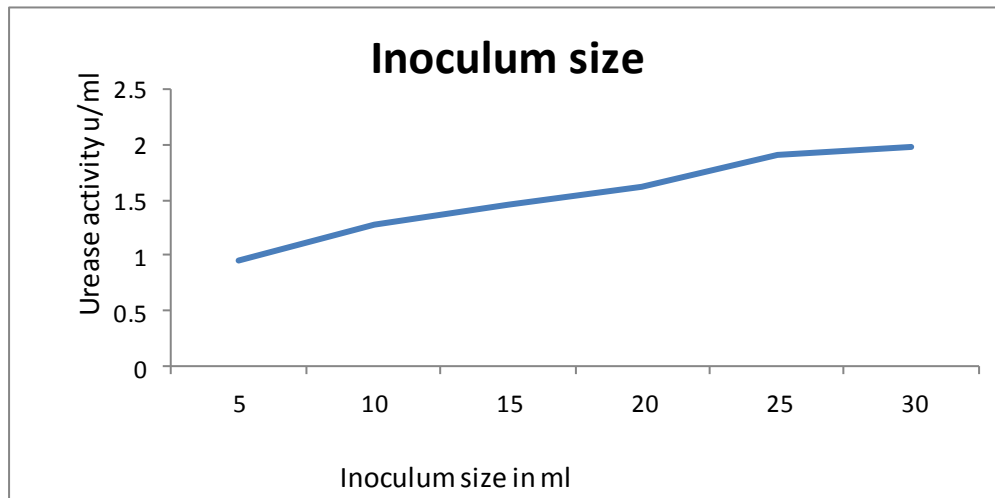
- A. Isolation of *C.tropicalis* from soil
- B. On CHROMagar medium shows bluish purple
- C. Urease positive

### Crystallization of urease enzyme



- C. Macroscopic view of urease crystal
- D. Microscopic view of urease crystal





The carried above conditions favours Urease enzyme production sufficiently.

### Conclusion

We conclude that the isolated soil yeast *C.tropicalis* can able to produce sufficient amount of urease enzyme for many applications. Under favourable conditions as period, pH, temperature for 48hrs, 7.5pH and 35<sup>0</sup>C respectively. Urease producing *Candida tropicalis* yeast cell was facultative sometimes. In agricultural field, they are capable for enzyme production and also favour soil texture and plant growth.

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