



Molecular Identification and Production of Urease Enzyme by *Proteus vulgaris* (ATCC 336)

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ABSTRACT: Urease is fit for urea hydrolysis. This compound is across the board. It is found in the regular habitat (water and soil) and in human body, where its event is associated with protein corruption. The samples were collected from an intense Mangrove grown location of Vellar estuary, Port Novo, India. Standard protocols were followed for Isolation Method, Screening of urease Production, Urease Plate assay, Quantitative Biochemical Assay, DNA Isolation Procedure, PCR Amplification, DNA sequencer and Phylogenetic tree reconstruction. The results were prominent in strains such as EMS 22 with 1.17U/ml, EMS 19 with 0.95U/ml, EMS 15 with 0.55U/ml, EMS 10 with .73U/ml, EMS 7 with 1.57U/ml, EMS5 with 1.07U/ml, EMS 2 with 1.37U/ml and EMS 17 with 2.98u/ml being the highest yielding strain.

Keywords: Urease, mangrove, DNA isolation, phylogenetic tree

I. INTRODUCTION

Enzymes are biochemicals which are used as a catalyst, they are produced by the human body. The use of enzyme has gained significant attenuation in the past recent years. The use of bio catalyst is changing the course of the entire industry which includes chemicals, textile and pharmaceutical. Enzymes are produced in almost all living cells. Every organism produces its own type of enzyme for its own purpose. Hence it is important to select a strain which yields maximum quantity of the protein. The production of enzymes from bacteria has also changed drastically in the past few years. *Proteus vulgaris* is a gram negative bacteria that is commonly found in soil and water. Urease is fit for urea hydrolysis. This compound is across the board: it is found in the regular habitat and in human body, where its event is associated with protein corruption. In people, urea is a factor of ordinary elements of kidneys [1, 2]. A sound grown-up discharges around 30 g of urea for every day [3]. Be that as it may, it is available in pee, as well as in blood serum, sweat and even in stomach [1, 3]. Hydrolysis of urea by urease is a perplexing procedure. In the initial step, one particle of smelling salts and one atom of carbamate show up. In water arrangement, carbamate immediately changes over into the second smelling salts atom and carbonic corrosive.

Urease production leads to precipitation of organic and inorganic compounds. Ureasases are nickel-containing, multisubunit compounds that catalyze the hydrolysis of urea to ammonia and carbon dioxide, with a net increment

in natural pH. They are profoundly monitored proteins found in various plants, microbes, parasites, and green growth [4]. In prokaryotes, urea hydrolysis can present assurance against executing in acidic situations [5] or can give smelling salts, which is a favored nitrogen hotspot for some microscopic organisms [6]. There is additionally confirm that a few living beings, for example, *Ureaplasma ureolyticum* [7] and some alkalophiles [8], may utilize ureolysis to produce a proton thought process constrain that can drive ATP combination. Since the natural substrate of this enzyme is urea, it was of interest to Molecular identification and Production of urease enzyme by *Proteus vulgaris* (ATCC 336).

II. MATERIALS AND METHODS

A. Sample Collection

The samples were collected from an intense Mangrove grown location of Vellar estuary (Latitude 11 29' N; Longitude 79 46'E), Port Novo, India. Surface sediment sample was collected using a sterile spatula and surface water sample was collected in pre-sterilised bottles allowing enough air space inside, so as to facilitate thorough mixing. The collected samples were transferred to pre-sterilized bottle containers and necessary precautionary measures were taken to minimize the contamination while handling the samples. The collected samples were kept in an ice box maintained at 4°C and samples were transferred and processed immediately in the lab.

B. Isolation Method

50% aged sea water prepared nutrient agar medium was prepared and poured on to sterile petri plates, after the agar solidifies, 2ml of serial diluted sample was spread on the agar plates using spread plate technique. Petri plates were incubated at room temperature.

C. Screening of urease Production

All the axenic bacterial strains were individually cultured in urea agar slants containing Phenol red indicator with pH 6.5 and the slants were incubated at 37°C for 48 hours [9]. After incubation, positive strains show pink colored colonies on yellow agar medium this is because of urea hydrolysis to ammonia form alkaline environment and yellow color of slants indicates negative results for urease production.

D. Urease Plate assay

Pink color forming bacteria from the slant cultures were individually tested for their urease producing potential in Well diffusion assay under the same urea agar medium conditions. Axenic cultures were broth cultured on urea broth (without added agar on urea agar medium) and incubated for 48 hrs. Cultured broth was centrifuged for 3000 rpm for 15 min. and 50µl of supernatant were used in well for zone formation. Based on maximum zone formation, strains were chosen for further studies.

E. Quantitative Biochemical Assay

Urease activity was measured by phenol-hypochlorite assay [10]. 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) giving a total volume of 1.1 ml. The reaction mixture was incubated at 37°C for 30 min in a shaking water bath. The reaction was stopped by transferring 50µl 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). 500 µl of alkaline hypochlorite (3.56 g Na₂HPO₄ + 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 colour complex was measured at 630 nm against the blank (500 ml phenol nitroprusside sodium + 500 ml sodium hypochlorite + 50 ml distilled water) in a spectrophotometer and compared to a standard curve prepared with (NH₄)₂SO₄. 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 mg NH₃ from urea per minute, under the above assay conditions [11]. The bacterium showing maximum enzyme activity on urease plate assay and quantitative biochemical assay was identified using molecular sequence method.

F. DNA Isolation Procedure

DNA extraction was performed from 2-mL bacterial cultures collected at the mid-exponential growth phase using the Roche Kit (Germany) according to the manufacturer's instructions.

Primers. Universal set of the primers

27F - 5'- AGAG TTTG ATCM TGGC TCAG -3'

1492R - 5'- GGTT ACCT TGTT ACGA CTT -3'

PCR Amplification. The PCR was done on a thermal cycler (Eppendorf) with 50µl reaction mix. The reaction mix contained 10× amplification buffer (5µl), 1.5mM MgCl₂ (5µl), 1µl of each forward and reverse primer, 1µl dNTP and 0.25µl Taq polymerase. After an initial denaturation at 95°C for 1min, amplification was carried out with 35 cycles of 35 s at 94°C, 40 s at 55°C, 2min at 72°C followed by a final extension for 8min at 72°C. The PCR products were analyzed by electrophoresis using 1.2% agarose gel (Genei).

DNA Sequencer. The PCR product was purified using the Qiagen PCR purification kit and then sequenced on an ABI Prism 377 automatic sequencer (Applied Biosystems, CA, USA).

Phylogenetic tree reconstruction: The evolutionary distances were computed using the maximum-composite-likelihood method (Tamura *et al.*, 2004) and the evolutionary analyses was conducted using MEGA7 software (Kumar *et al.*, 2016). The tree topologies were evaluated by bootstrap analyses based on 1,000 replicates and phylogenetic trees were inferred using the neighbour joining method.

III. RESULTS AND DISCUSSIONS

A. Microscopic observations

The microscopic images were observed under a phase contrast microscope. gram staining procedure was carried out for the bacterial cells (Fig. 1). The produced images of the bacterium to be rod shaped and red in colour. This proves that the bacteria is a gram negative bacteria. One of the few exceptional qualities of gram-negative microscopic organisms is the structure of the bacterial external film. The external layer of this layer involves a complex lipopolysaccharide (LPS) whose lipid partition goes about as an endotoxin. On the off chance that gram-negative microscopic organisms enter the circulatory framework, the liposaccharide can cause a poisonous response. This outcomes in fever, an expanded respiratory rate, and low pulse. This may prompt dangerous septic shock [12]. The external layer shields the microorganisms from a few anti-toxins, colors, and cleansers that would ordinarily harm either the inward film or the cell divider (made of peptidoglycan). The external layer gives these microbes protection from lysozyme and penicillin. The

periplasmic (space between the two cell films) likewise contains catalysts which separate or adjust anti-infection agents.

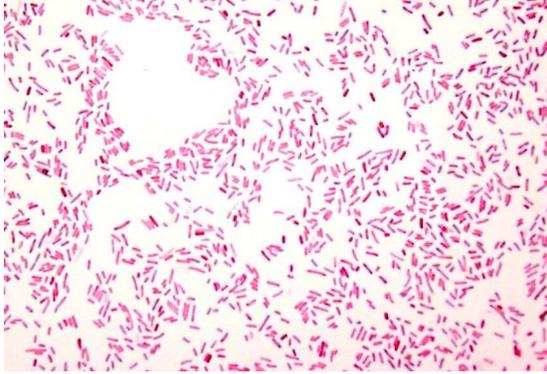


Fig. 1. Microscopic Observation of *Proteus vulgaris*.

Medications normally used to treat gram negative diseases incorporate amino, carboxy and ureido penicillins (ampicillin, amoxicillin, piperacillin, ticarcillin) these medications might be joined with beta-lactamase inhibitors to battle the nearness of proteins that can process these medications (known as beta-lactamases) in the peri-plasmic space. Different classes of medications that have gram negative range incorporate cephalosporins, monobactams (aztreonam), aminoglycosides, quinolones, macrolides, chloramphenicol, folate adversaries, and carbapenems [13]. The pathogenic ability of gram-negative microscopic organisms is frequently connected with specific segments of their layer, specifically, the LPS [14]. In people, the nearness of LPS triggers an intrinsic resistant reaction, enacting the invulnerable framework and creating cytokines (hormonal controllers).

B. Protease Production in Urea Agar

The selected organism (*Proteus vulgaris*) was cultured in urea agar and the colour change in the slants determine the presence of urease production. Red colour indicates positive urease production and yellow collar indicates negative urease production (Fig. 2).

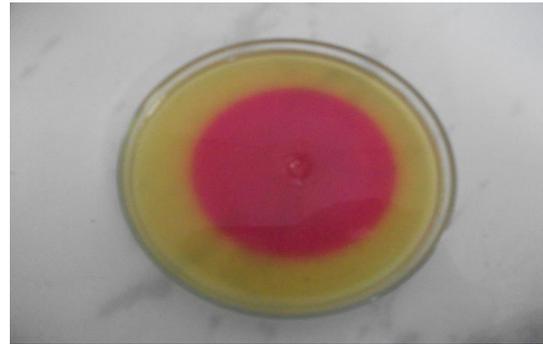


Fig. 2. Zone formed by *Proteus vulgaris* EMS 17.

C. Quantitative Biochemical Assay

The urease activity was measured by phenol-hypochlorite assay (Weatherburn 1967). The results were prominent in strains such as EMS 22 with 1.17U/ml, EMS 19 with 0.95U/ml, EMS 15 with 0.55U/ml, EMS 10 with .73U/ml, EMS 7 with 1.57U/ml, EMS5 with 1.07U/ml, EMS 2 with 1.37U/ml and EMS 17 with 2.98u/ml being the highest yielding strain.

D. Neighbour Joining Phylogenetic Tree

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 78.31352283 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [15]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method [16] and are in the units of the number of base substitutions per site. The analysis involved 12 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 1454 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [17].

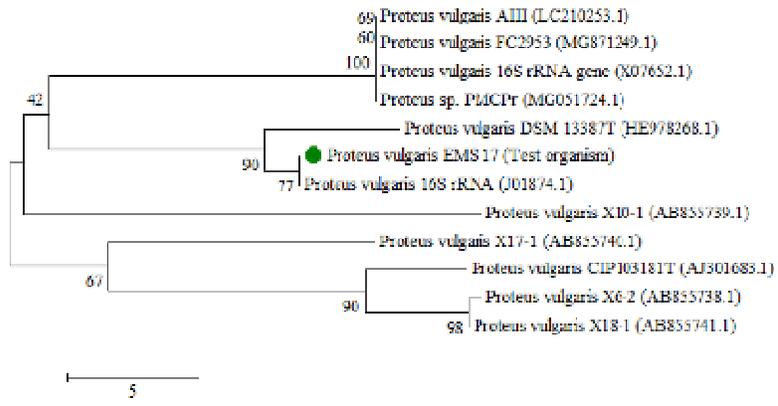


Fig. 3. Evolutionary Relationship of *Proteus vulgaris*.

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Fig. 4. Pure Cultured Nutrient agar plate of *Proteus vulgaris*.



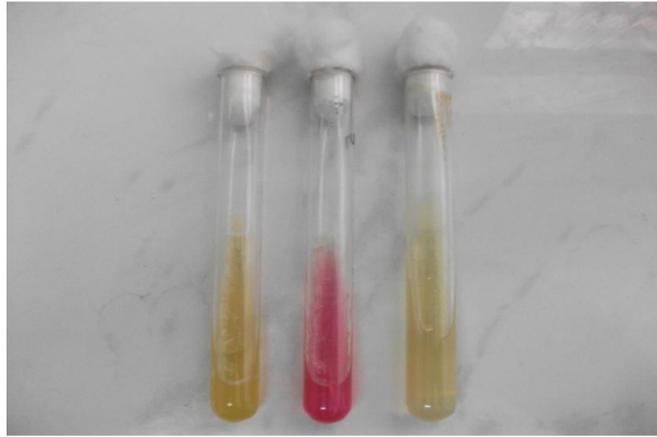


Fig. 5. Preliminary Screening of urease production on urea agar slants, recolour tubes indicates positive and yellow colour tubes indicates negative urease production.

Assembled data for NCBI submission

> *Proteus vulgaris* EMS 17,16S rRNA partial sequence of estuarine bacteria

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GTTTGATCATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAACAGGAG
AAAGCTTGCTTTCTTGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCCGTAGAGGGGG
ATAACTACTGGAAACGGTGGCTAATACCCGCATGACGTCTACGGACCAAAGCAGGGGTCTTCGGACCTT
GCGCTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGAGGTAATGGTCACCTAGGCGACGATCTCT
AGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCTACGGGAGGCAGC
AGTGGGCAATATTGCACAATGGGCGCAAGCCTGATGCAGCATGCCGCGTGTATGAAGAAGGCCTTAGG
GTTGTAAGTACTTTCAGCGGGGAGGAAGGTGATAAAGTTAATACCTTTGTCAATTGACGTTACCCGCA
GAAGAAGCACC GGCTAACTCCGTGCCAGCGCCGCGCTAATACGGAGGGTGCAAGCGTTAATCGGAATT
ACTGGGCGTAAAGCGCACGCAGGCGGCAATTAAGTCAGATGTGAAAGCCCCGAGCTCAACTTGGGAAC
TGCATCTGAAACTGGCTGGCAGAGTCTTGTAGAGGGGGGTAGAATTCACGTGTAGCGGTGAAATGCG
TAGAGATGTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAA
AGCGTGGGGACCAACAGGATTAGATACCTGGTAGTCCACGCTGTAAACGATGTGCGATTTAGAGGTTGT
GGTCTTGAACGTGGCTTCTGCAGCTAACCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGTTA
AACTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAA
GAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAGAGATAGAGGAGTGCCTTCGGGACGCTGAGAC
AGGTGCTGCATGGCTGTCGTCAGTCTGTTGTTGTGAAATGTTGGGTTAAGTCCC GCAACGAGCGCAACC
CTTATCCTTTGTTGCCAGCGGTAATGGCGGGA ACTCAAAGGAGACTGCGGTGATAAACCGGAGGAAG
GTGGGGATGACGTTAAGTCGTATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCAGAT
ACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAACTCATAAGTCTGTGCTAGTCCGGATTGGAGTCTG
CAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTTCCCG
GGCCTTGACACACCGCCCGTCACACCATGGAGTGGCTTGCAAAGAAGTAGGTAGCTTAACCTTCGGG
AGGGCGCTTACCA
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IV. CONCLUSION

Urease is a compound concentrated for quite a while. Its structure, combination and biochemical movement are known. There are likewise numerous investigations concerning urease poisonous impact on human tissues. In any case, its part in dependable immune system sicknesses is as yet questionable. In any case, the nearness of atomic mimicry between bacterial ureases and human proteins has been recommended. Proteins containing intentions, like irresistible operators, may work as autoantigens. In depicted autoantigenes, a few likenesses to ureases might be discovered. It was demonstrated that this compound fortifies antibodies

blend, however assurance of epitopes in urease protein might be troublesome and non-definitive. Subsequently examinations applying manufactured peptides could be extremely useful in mapping epitopes both in irresistible operators proteins and in deciding amino acids situated in epitopes which are fundamental for human humoral reaction. Urease, in spite of the fact that examined for quite a while, still is by all accounts an unexplored chemical. EMS 22 with 1.17U/ml, EMS 19 with 0.95U/ml, EMS 15 with 0.55U/ml, EMS 10 with .73U/ml, EMS 7 with 1.57U/ml, EMS5 with 1.07U/ml, EMS 2 with 1.37U/ml and EMS 17 with 2.98u/ml showed highest yield from the samples collected from

Mangrove grown location of Vellar estuary (Latitude 11 29' N; Longitude 79 46'E).

REFERENCES

- [1]. Newsholme E, Leech A. (2011). *Functional Biochemistry in Health and Disease*. 2nd ed. Chichester: John Wiley & Sons; 2011.
- [2]. Sirko A, Brodzik R. (2000). Plant ureases: Roles and regulation. *Acta Biochim. Pol.*, **47**(4): 1189–1195.
- [3]. Mobley H.L.T., Island M.D., Hausinger R.P. (1995). Molecular biology of microbial ureases. *Microbiol. Rev.*, **59**(3): 451–480.
- [4]. Mobley H. L.T., Hausinger R.P. (1989) Microbiol ureases: significance, regulation, and molecular characterization. *Microbiol. Rev.*, **53**: 85–108.
- [5]. Sissons C. H., Hancock E. M. (1993). Urease activity in *Streptococcus salivarius* at low pH. *Arch. Oral Biol.*, **38**: 507–516.
- [6]. Cruz-Ramos H., Glaser P., Wray J. L. V., Fisher S. H. (1997). The *Bacillus subtilis* ure ABC operon. *J. Bacteriol.* **179**: 3371–3373.
- [7]. Smith D. G., Russel W.C., Ingledew W. J., Thirkell D. (1993). Hydrolysis of urea by *Ureaplasma urealyticum* generates a transmembrane potential with resultant ATP synthesis. *J. Bacteriol.* **175**: 3253–3258.
- [8]. Jahns T. (1996). Ammonium/urea-dependent generation of a proton electrochemical potential and synthesis of ATP in *Bacillus pasteurii*. *J. Bacteriol.* **178**: 403–409.
- [9]. Balan, S. S., Fathima, F. and Jayalakshmi, S., (2012). Characterization of urease enzyme from marine bacterium *Klebsiella* species. *African J. Microbiol. Res.*, **6**(30) 5914-5923.
- [10]. Weatherburn, M.W., (1967). Phenol-hypochlorite reaction for determination of ammonia. *Anal Chem.*, **39**: 971–4.
- [11]. Smith, P.T., A. K. J. Douglas, and N. Goodman (1993). Isolation and characterization of urease from *Aspergillus niger*. *J. Gen. Microbiol.* **139**: 957-962
- [12]. Pellitier L.L. Jr, (2017). "Microbiology of the Circulatory System" "NCBI Bookshelf", April 18, 2017.
- [13]. <https://www.jwatch.org/id200312120000007/2003/12/12/gram-negative-bacteria-and-broad-spectrum>
- [14]. Baron S., Salton M.R., Kim K.S. (1996). "Structure". In Baron S, et al. *Baron's Medical Microbiology* (4th ed.). University of Texas Medical Branch.
- [15]. Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**: 783-791.
- [16]. Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA)* **101**: 11030-11035.
- [17]. Kumar S., Stecher G., and Tamura K., (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**: 1870-1874.

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