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Isolation and Characterisation on *Proteus vulgaris* from Mangrove Sediment of Vellar Estuary for Urease Production

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ABSTRACT: Urease is a virulence factor found in various pathogenic bacteria. It is essential in colonization of a host organism and in maintenance of bacterial cells in tissues. Due to its enzymatic activity, urease has a toxic effect on human cells. The present study focused on the production of urease by a *Proteus vulgaris* isolated from a sediment sample of Vellar estuary, Porto Novo, Tamil Nadu, India. Secondary screening for quantitative urease estimation was done by phenol-hypochlorite assay. Based on the maximum enzyme activity on urease slant and plate assay as well as quantitative biochemical enzyme assay, most potential strains were chosen for further studies. An excellent correlation was found between test and standard urease which evidenced the possibilities of its industrial applications as urea diagnosis and reduction. *Proteus vulgaris* is an ideal product for the industrial production and various applications like determining and reducing urea contaminants.

Keywords: Production; characterization; urease enzyme and *Proteus vulgaris*.

INTRODUCTION: Urease is capable of urea hydrolysis. This compound is widespread: it is found in the natural environment (water and soil) and in human body, where its occurrence is connected with protein degradation. In humans, urea is a factor of normal functions of kidneys. A healthy adult excretes about 30 g of urea per day. However, it is present not only in urine, but also in blood serum, sweat and even in stomach. Hydrolysis of urea by urease is a complex process. In the first step, one molecule of ammonia and one molecule of carbamate appear. In water solution, carbamate spontaneously converts into the second ammonia molecule and carbonic acid. Next ammonia is protonated. This process results in pH increase. Urease (urea amidohydrolase, EC 3.5.1.5) catalyzes the hydrolysis of urea into ammonia and carbamate and they play an important role in nitrogen metabolism (Karplus et al., 1997). Urease is a nickel-containing metalloenzyme first isolated from seeds of the jack bean plant in 1926. Urease is widely distributed in nature and is detected in microorganisms, plants and animals. There are many applications for the enzyme urease, it can be used as a diagnostic tool to detect the blood urea, to remove urea from alcoholic beverages and in urease conductometric biosensors for detection of heavy metal ions, biocalcification (Sarda et al., 2009), etc.

More number of microbial source for this enzyme including bacteria such as *Lactobacillus ruminis, corynebacterium lillium, Lactobacillus fermentum, Lactobacillus reuteri* (Kakimoto and Suzuki, 1992) *Bacillus pasteurii*, *Enterobacter* sp, *Klebseilla* spp (Rao et al., 1993) and fungi such as *Aspergillus niger, Aspergillus nidulans, Rhizipus oryzae* (Farley and Santosa, 2002) have been well characterized. When microorganisms utilize urea in urea medium, ammonia is formed during the incubation which makes these media alkaline. Consequently urease production can be detected using pH indicators. Although urea is the major substrate of urease, the enzyme is capable of hydrolyzing other substrates such as acetamide, formamide, N-methylurea and semicarbazide (Dixon et al., 1980). Urea hydrolysis has been widely used for the classification and identification of microorganisms, especially members of the family Enterobacteriaceae, *Pseudomonads*, *Haemoplhiltis* spp and other Gram-negative bacteria (Qadri et al., 1984).

Reports of urease produced by many different bacteria, fungi, plants and even some invertebrates. Microorganisms with ureolytic properties were found in soil and water as well as in human and animal bodies. Ureolytic bacteria may belong to symbiotic natural microflora or to pathogens. In facultative anaerobes

from intestinal microflora the level of this activity is diverse and species characteristic (Suzuki *et al.*, 1979; Zonia *et al.*, 1997). Molecular Identification and Production of Urease Enzyme by *Proteus vulgaris* (ATCC 336). (Rebekkah Shanthakumari and Boominathan (2018).

The present study focused on the production of urease by a *Proteus vulgaris* isolated from a sediment sample of Vellar estuary, Porto Novo, Tamil Nadu, India. Further, the objects of this study to screen, produce, purify and characterize the urease enzyme produced by this potential estuarine bacterium, *P. vulgaris*. The main objective of the present study is to study the urease producing potentials of the isolated bacterial strains from estuary mangrove water and sediment of Porto Novo.

MATERIAL AND METHOD: The present study was designed to isolate and characterize a potential urease producing estuarine bacterium from mangrove water and sediment samples of Vellar estuary, Porto Novo, Tamil Nadu. To carry out a thorough and detailed investigation, the present study adopted these standard methods to achieve the objective. The samples were collected from the above mentioned locations, serially diluted and spread plated on 50 % aged sea water prepared Nutrient agar plates. After 48hrs incubation at 37ºC, morphologically distinct strains were isolated, pure cultured and studied for urease production using urea agar slants containing Phenol red indicator at pH 6.5 (Balan et al., 2012). 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.

Secondary screening for quantitative urease estimation was done by phenol-hypochlorite assay (Weatherburn, 1967). Based on the maximum enzyme activity on urease slant and plate assay as well as quantitative biochemical enzyme assay, most potential strains were chosen for further studies. The most potential bacterium was molecular identified using 16S rRNA partial sequence method with the help of universal set of the primers, 27F - 5′- AGAG TTTG ATCM TGGC TCAG -3′ and 1492R - 5′- GGTT ACCT TGTT ACGA CTT -3′.

RESULTS AND DISCUSSION: Mangrove biological community is one of the world's most profitable environment that yields business woods items, improves waterfront waters, bolster seaside fisheries and ensure coastlines. In any case, mangroves get by under outrageous tides, state of high saltiness, high temperature, solid breezes, and sloppy and anaerobic soils. No other gathering of plants has been accounted for with such profoundly developed natural, morphological, physiological and organic adjustments to outrageous conditions.

The ecosystem is inhabited by wide range of organism and mostly by microorganisms that can be exploited for different purposes. However, the isolation of bacterial strains showing urease enzymes is not tapped with full exploitation. The function of urease in bacterial metabolism is not known with certainty. The enzyme is regarded as a detoxifying agent for the cell and as a catalyst whereby the bacterium derives nitrogen in the form of ammonia for the synthesis of amino acids. The present study has isolated different bacterial strains from estuary mangrove water and sediment of Port Novo (Table 1 & Table 2).

Table 1: Showing the urease producing potentials of the isolated bacterial strains from estuary mangrove water of Porto Novo.

S. No	Bacterial Strain ID	Results		
		Urea agar slants	Well diffusion assay	Biochemical enzyme assay
1	EMW 1	Negative	-	-
2	EMW 2	Negative	-	-
3	EMW 3	Positive	12mm	0.79U/ml
4	EMW 4	Negative	-	-
5	EMW 5	Negative	-	-
6	EMW 6	Negative	-	-
7	EMW 7	Positive	17mm	1.03U/ml
8	EMW 8	Negative	-	-
9	EMW 9	Positive	21mm	1.21U/ml
10	EMW 10	Negative	-	-
11	EMW 11	Positive	9mm	0.55U/ml

Table 2: Showing the urease producing potentials of the isolated bacterial strains from estuary mangrove sediment of Porto Novo.

S. No	Bacterial Strain ID	Results		
		Urea agar slants	Well diffusion assay	Biochemical enzyme assay
1	EMS 1	Negative	-	-
2	EMS 2	Positive	22mm	1.37U/ml
3	EMS 3	Negative	-	-
4	EMS 4	Negative	-	-
5	EMS 5	Positive	17mm	1.07U/ml
6	EMS 6	Negative	-	-
7	EMS 7	Positive	31mm	1.57U/ml
8	EMS 8	Negative	-	-
9	EMS 9	Negative	-	-
10	EMS 10	Positive	11mm	0.73U/ml
11	EMS 11	Negative	-	-
12	EMS 12	Negative	-	-
13	EMS 13	Negative	-	-
14	EMS 14	Negative	-	-
15	EMS 15	Positive	9mm	0.55U/ml
16	EMS 16	Negative	-	-
17	EMS 17	Positive	56mm	2.98U/ml
18	EMS 18	Negative	-	-
19	EMS 19	Positive	15mm	0.95U/ml
20	EMS 20	Negative	-	-
21	EMS 21	Negative	-	-
22	EMS 22	Positive	19mm	1.17U/ml
23	EMS 23	Negative	-	-

The mangrove water and sediment samples of Vellar estuary showed total heterotrophic bacterial count of 2.78×10^5 CFU/g and 2.3×10^7 CFU/g and there were 11 and 23 morphological distinct bacteria were isolated from mangrove water and sediment samples which were abbreviated as EMW 1 to EMW 11 (Estuarine Mangrove Water and numerical numbers) and EMS 1 to EMS 23 (Estuarine Mangrove Sediment and numerical numbers). Totally 12 bacterium showed urease positive results in which 4 and 8 bacterium were isolated from mangrove water and sediment samples. Among them, the most potential urease activity was recorded in EMS 17 bacterium with positive activity in urea agar slant, 56 mm zone formation in urea agar well diffusion plate assay and 2.98 U/ml urease activity in biochemical enzyme assay.

Molecular identification using 16S rRNA partial sequence and the evolutionary distances computed using the maximum-composite-likelihood method revealed that the most potential bacterium, EMS 17 was identified as *Proteus vulgaris* and submitted to NCBI gene bank with the accession number, MH729054. *P. vulgaris* showed no urease activity in cell pellet and 2.99 Units/ml activity in cell free supernatant which represented that the production was extracellular. *P. vulgaris* revealed growth dependant urease production which was maximum at stationary growth phase of culture between 72 – 108 hrs incubation period with 3.12 ± 0.07 units/ml urease activity and 8.9 ± 0.39 g/L dry weight of cell biomass. *P. vulgaris* respond to all the different environmental and nutritional factors studied in this investigation and showed enhanced urease production with the following conditions viz., pH 7 (3.23 ± 0.12 units/ml), 35°C temperature (3.3 ± 0.13 units/ml), 20ppt salinity (3.38 ± 0.14 units/ml), 300rpm agitation (3.45 ± 0.14 units/ml), 2% inoculum size (3.49 ± 0.13 units/ml), 2% maltose as carbon source (3.67 ± 0.14 units/ml), 0.5% yeast extract as nitrogen source (3.71 ± 0.14 units/ml), 0.6% urea content (3.8 ± 0.15 units/ml), 0.02% $MgSO_4$ (3.84 ± 0.15 units/ml), 0.01% $FeCl_3$ (3.88 ± 0.14 units/ml) and 0.03% K_2HPO_4 (3.9 ± 0.14 units/ml) as mineral supplements, respectively. Fig 1 & Fig 2 shows the isolation of estuarine bacteria from estuary mangrove sed-

iment sample using 50% sea water prepared Nutrient agar plate at dilution 10^{-5} and 10^{-3}.

Using different concentration of ammonium sulphate, maximum urease enzyme precipitation was achieved at 80% NH_3SO_4 with 7.78 ± 0.25 units/mg and 1.38 ± 0.09 g/L dry weight of crude urease extract. Purification of urease enzyme was carried out using RP-HPLC column chromatography; purified enzyme was eluted at sixth fraction with 9.23 ± 0.16 units/mg and revealed 7.9% of the total crude urease extract. Application of urease in urea determination and reduction was estimated with the known volume of urea content. An excellent correlation was found between test and standard urease which evidenced the possibilities of its industrial applications as urea diagnosis and reduction.

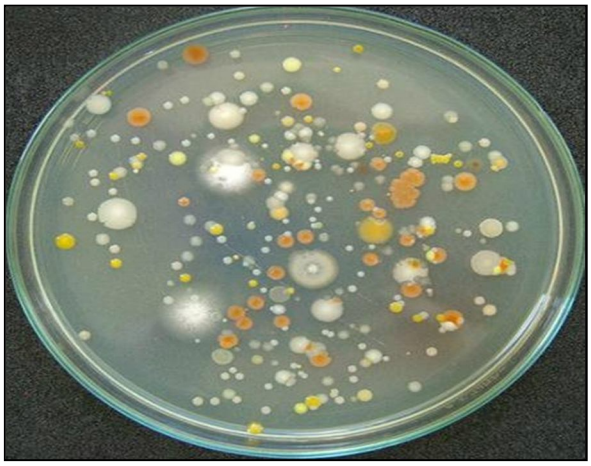

Figure 1: Isolation of estuarine bacteria from estuary mangrove sediment sample using 50% sea water prepared Nutrient agar plate at dilution 10^{-5}.

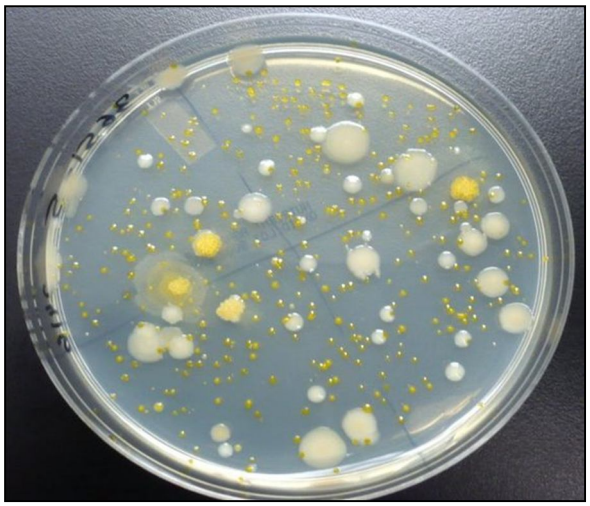

Figure 2: Isolation of estuarine bacteria from estuary mangrove water sample using 50% sea water prepared Nutrient agar plate at dilution 10^{-3}.

CONCLUSION: Urease is an enzyme studied for a long time. Its structure, synthesis and biochemical activity are known. There are also many studies concerning urease toxic effect on human tissues. However, its role in long-lasting autoimmune diseases is still controversial. Nevertheless, the presence of molecular mimicry between bacterial ureases and human proteins has been suggested. Based on the results obtained from the standard screening, bioprocess evaluation, chemical and functional characterization and its stability criteria, this study strongly suggesting the urease isolated from estuarine bacterium, *Proteus vulgaris* is an ideal product for the industrial production and various applications like determining and reducing urea contaminants. Moreover, this study explored a new commercially important strain to the enzyme industries.

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