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Screening and Characterization of *Maecescens Serretia and Proteus Myxofaciens* from Soil Sample

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ABSTRACT:
The aim of current study was to identify, characterize and isolate protease producing bacteria from soil sample. Commencing from the isolation by spreading technique on nutrient agar media, at 35^0C for 20 hrs. Followed by screening and characterization based on temperature, pH, nitrogen, and carbon sources. Two colonies U-2 and U-4 were selected and identified as Maecescens Serretia and Proteus Myxofaciens, the highest urease producing bacteria among the four that were taken forward for the study. Different Biochemical tests and microscopic identification tests were done for the screening of the bacteria. The U-2 bacteria showed maximum growth at temperature 35^0C, pH needed 3, potassium nitrate as nitrogen source and d-mannitol as carbon sources. Whereas, temperature requirement for the highest growth for U-4 was 25^0C, dextrose as carbon source but pH and nitrogen source were like the U-2 bacteria. At last, enzyme activity assay was performed to know the urease activity using well diffusion method.

Keywords: Characterization, Maecescens Serretia, Proteus Myxofaciens, diffusion, enzyme activity.

1. INTRODUCTION

The enzyme urease occurs in a wide variety of tissues in humans, as well as in bacteria, yeast, plants, and invertebrates. Ureases are found in numerous bacteria, fungi, algae, plants, and some invertebrates, as well as in soils, as a soil enzyme (Mekonnen et al., 2021). Microbial ureases hydrolyze urea to ammonia and carbon dioxide. There are many microbial sources for this enzyme including bacteria such as lactobacillus fermentum and lactobacillus reuteri and Klebsiella aerogenes and fungi such as Rhizopus Oryzae. Ureases functionally, belong to the superfamily of amidohydrolases and phospho-triesterases. They are nickel-containing metalloenzymes of high molecular weight.
These enzymes catalyse the hydrolysis of urea into carbon dioxide and ammonia:

$$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$$

The hydrolysis of urea occurs in two stages. In the first stage, ammonia and carbamate are produced. The carbamate spontaneously and rapidly hydrolyses to ammonia and carbonic acid. Urease activity increase the pH of its environment as it produces ammonia, which is basic.
Urease is also found in mammals and humans, the presence of urease is considered to be very harmful to mammals due to the production of the toxic ammonia product. However, mammalian cells do not produce urease, in fact, the (Lepus europaeus), a class of Mammalia, was discovered to have high urease activity in its large intestine, a part of gastrointestinal tract (Patra et al., 2018). In human kidneys, urea is present

in order for everyday functions and is estimated that per day, a healthy adult excretes about 10 to 30 g of urea. Other than urea being found in urine, it is also present in sweat, blood serum and stomach. Urease is also an immunogenic protein and is recognized by antibodies present in human sera (Konieczna et al., 2012).

Bacterial ureases are composed of three distinct subunits, one large (α 60–76kDa) and two small (β 8–21 kDa, γ 6–14 kDa) commonly forming (αβγ)3 trimers (Benini et al., 2013) stoichiometry with a 2-fold symmetric structure, they are cysteine-rich enzymes, resulting in the enzyme molar masses between 190 and 300kDa. An exceptional urease is obtained from Helicobacter sp. These are composed of two subunits, α (26–31 kDa) – β (61–66 kDa). These subunits form a supramolecular dodecameric complex (Ha NC et.al. 2001) of repeating α-β subunits, each coupled pair of subunits has an active site, for a total of 12 active site. All bacterial ureases are solely cytoplasmic, except for those in *Helicobacter pylori*, which along with its cytoplasmic activity, has external activity with host cells. In contrast, all plant ureases are cytoplasmic.

It is important to note, that although composed of different types of subunits, ureases from different sources extending from bacteria to plants and fungi exhibit high homology of amino acid sequences.

Urease enzymes effective as anticancer agent such as DOS is an enzyme called urease derived from the jack bean. By inducing the catabolism of urea in the interstitial medium surrounding cancer cells, urease action results in the production of metabolites: ammonia and hydroxide ions. It has been estimated that these metabolic products of urease activity stress cancer cells by direct toxicity and by the induction of alkaline effects. Cucumis melo is the plant source of urease enzyme and shows the diuretic effects in herbal medicines used in hypertension. Urease plays an important role in the nitrogen metabolism of ruminants such as cattle, sheep, and other animals that contain a forestomach. It has been shown that, urease functions to recycle nitrogen bound in urea that accumulates during early seedling development. It has also been studied that the plant enzyme was used as a vaccine based on the inhibition of catalytic activity to explain the mechanism of protection against infection by H. pylori which cause gastritis ulceration disease (H L Mobley, 1996) and possibly gastric cancer. Currently, there is significant concern regarding the occurrence of ethyl carbamate (carcinogen) in wine, that is formed from ethanol and urea (if present), during storage of wine (Shalamitskiy et al., 2023).

In current study, soil sample was collected from near by sector park of Noida, Haryana, India. And further tests were performed for the screening and characterization of bacteria under specific conditions (Damini et al., 2023).

2. MATERIALS AND METHODS

2.1 Sample Collection:

The bacteria used for study were isolated from the soil sample collected from outside of the house in Noida. The soil sample was collected in the plastic bags and transported to the laboratory.

2.2 Isolation of Urease bacteria:

Initially, 6 test tubes were taken for the serial dilution (Jackie Reynolds, 2005) of soil sample by adding 200µl. From the 6^{th} test tube, spreading was performed on the nutrient agar medium plate and incubated at 35^0C for 18-20 hours. Then, 4 different colonies were selected having distinct morphology on appearance. These colonies were taken further to screen the urease producing bacteria and urease medium were used for the screening. From those, 2 were selected as final for the additional research. These two colonies were

U-2 and U-4. To preserve as pure line, quadrant streak and nutrient broth were prepared. Based on the morphological and biochemical tests the isolated bacteria were identified and physiological characters were also studied for more information about favourable conditions required for maximum growth of the urease bacteria.

3. IDENTIFICATION OF BACTERIA

For identification of urease bacteria myriads of morphological tests that include gram staining and motility test. Along with this, many biochemical tests were also performed i.e., casein test, catalase test, protease test, starch test, carbohydrate utilization test, MR and VP test, citrate test, nitrate reduction test, hydrogen sulphide test. Moreover, different physiological factors such as pH, temperature, nitrogen, and the carbon sources were also included to characterized the isolated urease bacteria (Rupali Dalal., 2015). And took absorbance at 600nm using spectrophotometer Plotted the graph between physiological factors and absorbance using spectrophotometer (Pokhrel et al., 2014).

3.1 Testing for optimum temperature: To examine the optimum temperature at which an enzyme shows its maximum activity, the substrate with crude enzyme was exposed to different temperature range i.e., 4°C, 25°C, 35°C and 45°C. Observed the growth of bacteria after 24 hrs. and read absorbance.

3.2 Testing for optimum pH: Four different range of pH like 3, 5, 7, 9 were adjusted of the culture media containing 100µl bacteria in four separated test tubes. Incubated the test tube to the 35°C and 25°C (maximum growth at this temp.) for 18-20hrs. and the absorbance at 600nm.

3.3 Testing for optimum Nitrogen: Different nitrogen sources such ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrite. Each nitrogen source with 1% concentration was used. 100µl bacteria was inoculated in each test tube. Finally, the test tubes were incubated at 35°C and 25°C for 18-20hrs and the absorbance at as same.

3.4 Testing for optimum Carbon source: Prepared the nutrient broth and transfer the media with 1% of each carbon source that were dextrose, sucrose, D-mannitol, lactose in different test tube. Incubated the test tubes at 35°C and 25°C for 18-20 hrs. Read OD and plotted graph.

3.5 Crude Enzyme Preparation: The urease producing bacteria were inoculated in fermented media and incubated at 121°C at 15psi for 15 min. Incubated period was of 2 days for fermentation process. The culture medium was filtered aseptically in LAF. The filtered culture medium was directed to centrifugation at 10,00rpm for 10 minutes to remove the undesired particles. The supernatant was used as crude enzyme preparation for further research (Padmapriya et al., 2012).

3.6 Enzyme Activity Assay: To examine the ureolytic activity, the above-mentioned supernatant was used as urease enzyme source. Well diffusion method was used (Vijayaraghavan et al., 2013) and considering all necessary physiological factors for obtaining maximum results, where the urease media plate with 25µl centrifuged fermentation medium poured in all four wells was incubated at 35°C for 18-20 mins. Activity of urease enzyme would be confirmed by the Zone of inhibition around the wells.

4 RESULT AND DISCUSSION

4.1 Screening of proteolytic activity: Different isolates were screened and characterized for enzyme activity on protease media plates. Protease enzyme activity was observed through the zone of hydrolysis on nutrient agar media as displayed in figure 1. For further research the strain showing largest zone of hydrolysis was considered and coded as M-4 and was maintained by sub- culturing (Jain et al., 2020).

4.2 Optimization of Growth Conditions:

4.2.1 Testing for optimum temperature on urease activity: Increase in temperature will increase the activity of enzyme, but if the temperature rises, enzyme activity will diminish and the protein will denature. Finally, the maximum activity of urease bacteria U-2 at 35^0C and U-4 at 25^0C.

4.2.2 Testing for optimum pH on urease activity: To study the effect of pH, four different range of pH such as 3, 5, 7, 9 was adjusted in four separated test tubes containing culture media with 100µl of selected bacteria. Incubate the test tube at the 35°C for 18-20hrs and the maximum activity of urease of both the bacteria were at pH 3.

4.2.3 Testing for optimum Nitrogen on urease activity: To determine the effect of different nitrogen sources such ammonium chloride, ammonium sulphate, sodium nitrate, potassium nitrite. Each nitrogen source with 1% concentration was used. Both bacteria showed maximum enzyme activity in case of potassium nitrate medium.

4.2.4 Testing for optimum Carbon source on urease activity: To study the effect of carbon sources media was prepared with 1% of four different carbon sources such as dextrose, sucrose, D-mannitol, and lactose in different test tubes, later test tubes were incubated at 35°C for 18-20 hrs. In case of U-2, d-mannitol and for U-4, dextrose medium showed the maximum enzyme activity.

4.2.5 Testing for optimum fermented media on urease activity: Zone of inhibition signified the amount of urea degradation. In which both B well of U-2 and U-4 represented the characterized bacteria that showed the maximum zone of inhibition whereas the A well had small zone of inhibition that was of uncharacterized culture.

Table 1. Results of morphological test of U-2 and U-4

S.No.	Morphological Test	Result of U-2 bacteria	Result of U-4 bacteria
1.	Gram Staining	negative (rod)	negative (rod)
2.	Motality	positive	Positive

Table.2 Biochemical Characterization:

S.No.	Biochemical Test	Result of U-2 bacteria	Result of U-4 bacteria
1.	Urease	Positive	Negative
2.	Starch	Positive	Positive
3.	Protease	Positive	Positive
4.	Methyl Red	Positive	Positive
5.	Voges Proskauer	Positive	Positive
6.	Citrate	Positive	negative
7.	Nitrate	Positive	negative
8.	Catalase	Positive	positive
9.	H_2S	negative	Positive
10.	Dextrose	positive	Positive
11.	Lactose	negative	negative

12.	D-mannitol	positive	positive
13.	Sucrose	positive	positive
14.	**Identifed Bacteria**	***Marcescens serretia***	***Proteus myxofaciens***

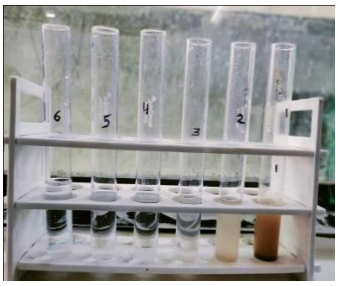


Figure 1 Serial dilution

Figure 2 Prime screening of urease bacteria.

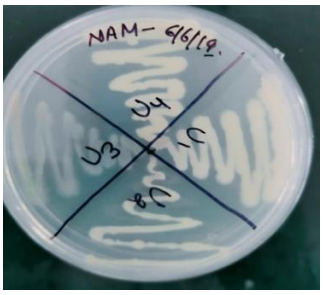
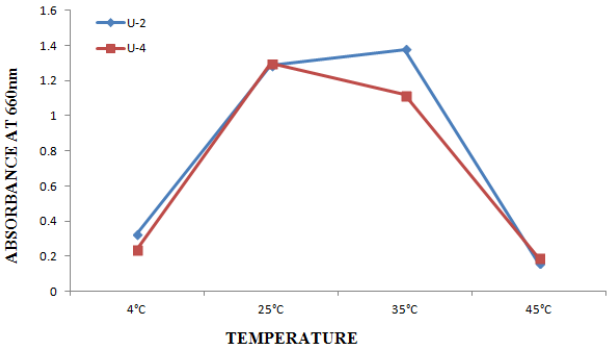


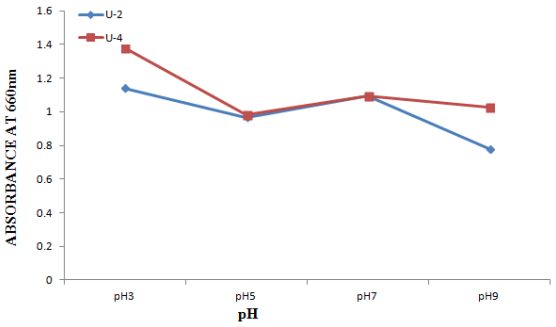
Figure 3 Zone of Inhibition on Nutrient Agar

Figure 4 Isolated colony of urease bacteria

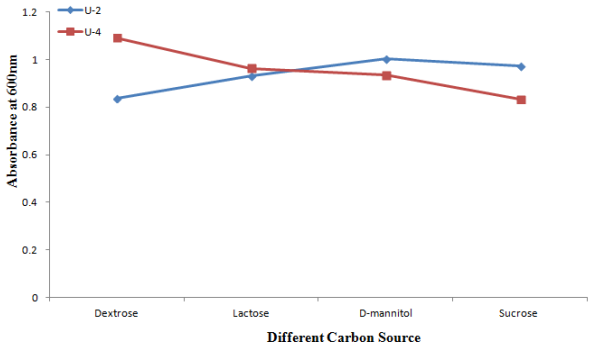
Figure 5 Urease activity- Well Diffusion Method



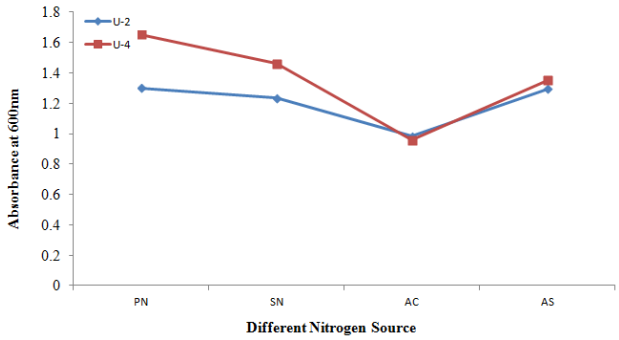
Graph 1. Bacterial growth at different temperature.



Graph 2. Bacterial growth at different pH



Graph 3. Bacterial growths in different carbon source



Graph 4. Bacterial growths at different nitrogen source

Coclusion:

By doing this project we can categories urease producing bacteria from non-producing. Some of the biochemical test such as urease test, citrate test, carbohydrates utilization test etc. helped in the recognition of urease producing bacteria. We characterized bacteria at different temperature, pH, nitrogen, and carbon sources to understand the optimum growth factors. So, it was concluded that the motive of doing the project was to identified the bacteria is successfully completed. The names of the identified bacteria were *Marcescens Serretia, Proteus Myxofaciens.* This study was revealed that urease producing bacteria can be locally isolated from soil. It is worthy to note that microbial urease enzymes are more stable and have properties which are more diverse than other urease enzymes derived from plants and animals.

this enzyme continued to be the focus of researchers around the world, in the fields of genetics, biochemistry and physiology . Strategies based on urease inhibition are considered as a promising mean to treat the diseases caused by bacteria producing urease, as well as a mean to diminish nitrogen loss from urea used as fertilizer. The future perspective seems to relay on better understanding of binding preferences of the enzymes from different sources and on the application of computer-aided prediction of potentially active compounds.

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