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Urease Test to Detect Ammonia Producing Microorganisms

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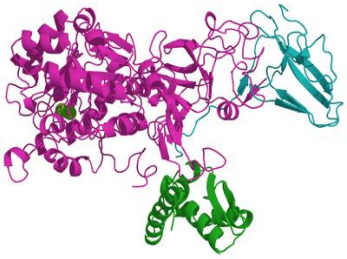
SUMMARY

Urease test is one of the oldest and well-known method to detect the ammonia producing microbes from soil or bacterial cultures. Many soil dwelling bacteria have a capacity to degrade the urea in soil. In taxonomic studies urease test is one of the common tests to differentiate the non-ammonia producing bacteria from ammonia producing bacteria. This test also narrows down the taxonomic burdens by providing clues. Many microbes degrade urea in the presence of urease enzyme produces the ammonia in this process the colour of media where bacteria incubated usually change from orange-yellow colour to bright pink indicates the urease production and ammonia utilization by microorganism.

INTRODUCTION

Urea is one of the common metabolite output from amino acid decarboxylation pathway, the Urease enzyme produced from organisms degrade this urea into ammonia and CO_2.In 1875Reoch hypothesized that production of ammonia is due to alkaline fermentation of urea done by microorganisms. Subsequently many microbes (fungi and bacteria) which have urease activity identified and isolated. Stuart first formulated the Proteus species specific media to identify urease producing *Proteus* species from other non-lactose fermenting members of Enterobacteriaceae *family*. Subsequently, Christensen improved this method by formulating a media which allow other members of Enterobacteriaceae which cannot utilise only ammonia as nitrogen source for growth. Christensen's urea agar has a decreased buffering capacity which allows the detection of the organisms which have delayed or lowered quantities of urease activity and smaller quantities of alkali produced from the urea degradation.



Schematic representation of the *K. aerogenes* urease structure (R. P Hausinger and P.A karplus 2011)

Principle of Urease Test: Christensen urea agar media contains urea as a nitrogen source and phenol red as pH indicator when this media is provided to microbes as a source of nutrition the microbe which capable of producing urease enzyme can split the urea into two, ammonia and CO2 molecules in the presence of water this ammonia molecule again reacts with CO2 and water and combines and yields ammonium carbonate. This ammonium carbonate is responsible for change in pH of medium into alkaline it turns indicator colour from orange-yellow colour to bright pink (yellow (pH 6.8) to bright pink (pH 8.2)).

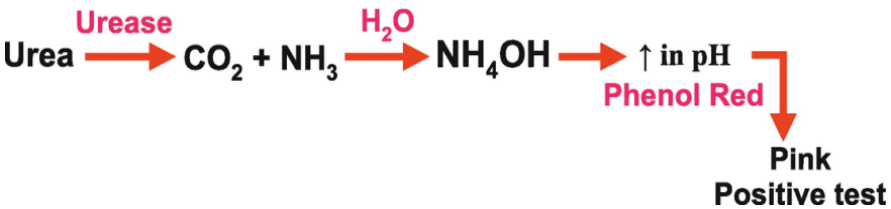


Fig 2: Chemical reaction of ammonia production from urea

Common subjective microbes for urease test

This test is performed as part of the identification of several genera and species of the *Enterobacteriaceae* family, including *Klebsiella, Proteus,* andsome *Citrobacter* and *Yersinia* species, as well as some *Corynebacterium* species. The test is also useful to identify *Cryptococcus, Brucella, Helicobacter pylori*, and many other bacteria that produce the urease enzyme.

Composition: Christensen's Urea Agar is commonly preferred media to differentiate and to detect urease activity in a variety of microorganisms.

Ingredient Amount: Yeast extract (0.1 g), Potassium phosphate, monobasic (9.1 g), Potassium phosphate, dibasic (9.5 g), Urea (20 g), Phenol red (0.01 g).

Media preparation: To prepare Christensen's Urea Agar media we have to dissolve all above 6 ingredients into distilled water. Dissolve agar in 900 ml water and boil it to dissolve completely. Autoclave at 121℃and 15 psi for 15 minutes. After that Cool the agar to 50 to 55℃, Now add 100 ml of first 6 ingredients (filter sterilized solution) into cooled agar solution and add into each test tube and place in slant position, after preparation of slant, store it for a while until bacterial culture get ready. After obtaining full grown bacterial colonies of desired organism take a full-grown colony with help of inoculum loop spread and streak it on slant surface. Incubate itat 35℃ in BOD incubator observe for colour change in media up to 6 days.

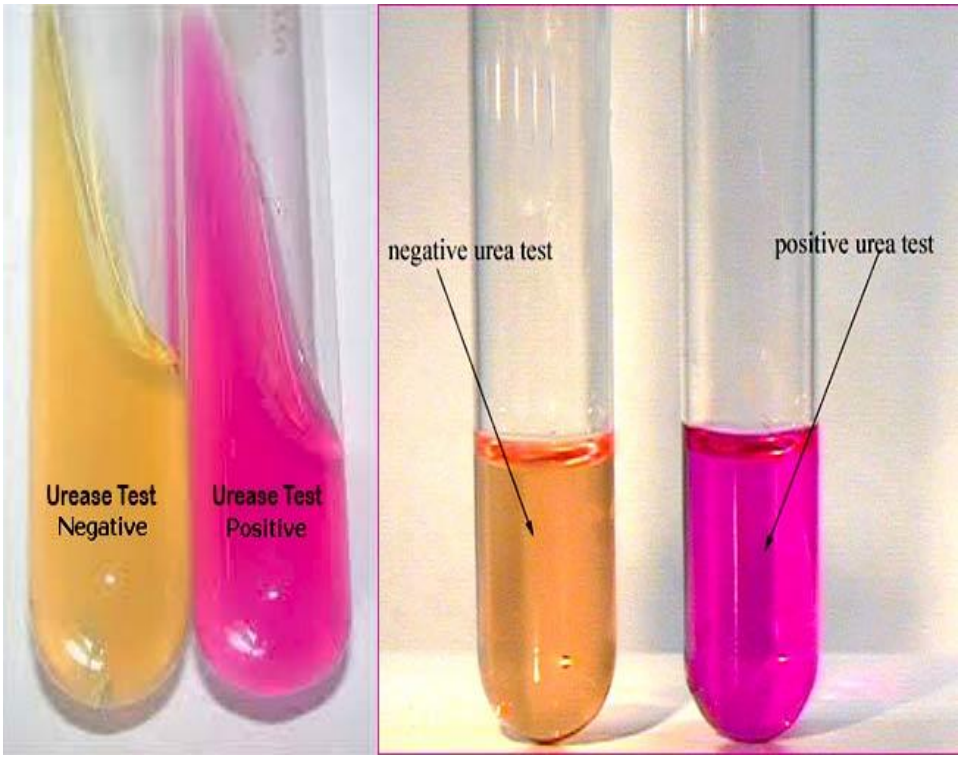


Fig3-Urea agar test (a) uninoculated, (b) *Proteus mirabilis* (rapidly urease positive), (c) *Klebsiella pneumoniae* (delayed urease positive), (d) *Escherichia coli* (urease negative). (Benita A. Brink, Adams State College, Alamosa, CO)

Results interpretation: Any small change in colour will be considered as positive result. Colour of media usually change from orange-yellow colour to bright pink, this indicates the urease production and ammonia utilization by microorganism.

CONCLUSION

Urease test is one of the well-known methods to differentiate and to detect ammonia producing microbes, although few microbes' catalyses rapidly and few slowly, as urease is sensitive to light it should be kept 2 to 8°C in the dark to prevent degradation to make it more sensitive should not forget to make it buffered by using this

method we can detect the load of urea producing microbes in soil or on culture plate colonies to narrows the taxonomy of the bacteria.

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