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Research article

Evaluation the Uricase Produced from Different Clinical isolates of *Pseudomonas aeruginosa* by Plate Assay Methods

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ABSTRACT

Out of 150 clinical samples, 43 isolates of *P. aeruginosa* were identified based on biochemical tests, API 20 NE and VITEK 2 system. The results showed that 38 (88.37%) isolates were uricase producers while only 5 (11.62%) isolates were uricase non producers, based on uricolytic activity on agar medium using two methods, qualitative method by using agar plate medium which contain many ingredients (uric acid, dextrose, yeast extract and NaCl), the results obtained by this method indicated by the presence of clear zone around streaking line on an agar plate assay. The another method is semi quantitative, in which agar plate medium contain 0.5%w/v uric acid as the only nutrition source and showed diameter of clear zone around well on an agar plate. The diameter of clear zone around wells ranged between 12-26 mm. In conclusion , both methods gave same results for screening uricase producers, but semi quantitative method gave more accurate results because its medium contain only uric acid as a source of carbon or nitrogen or both which make this method more specific and sensitive than the other one.

Keywords: *Pseudomonas aeruginosa*, **Semi quantitative method, Uricase, Plate assay methods.**

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INTRODUCTION

P. aeruginosa is a species of genus *Pseudomonas*. It is Gram negative rods, motile by pollar flagella, oxidase and catalase positive. It is single bacteria, in pairs, and occasionally in short chains. It has ability to form biofilm on different surfaces and grows at 37-42 ^{o}C, growth of *P. aeruginosa* at 42 ^{o}C is very important to identify the

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species*, P. aeruginosa* [1,2]. Many enzymes and virulence factors can be produced by *P. aeruginosa*, one of them is uricase (urate oxidase) enzyme that produced by various members of the genus of *Pseudomonas*, this bacteria can grow on purines as nitrogen source or as carbon and nitrogen sources, where xanthine, hypoxanthine, guanine, adenine serve as a nitrogen source for the bacterial growth [3].

Uricase enzyme is an oxidoreductases enzymes class (EC 1.7.3.3), it is a peroxisomal enzyme that catalyzes the oxidation of uric acid into soluble products [4].

This enzyme conserved in many species ranging from microorganisms to mammals; but it is absent in primates as a result of evolutionary gene mutations [5, 6]. This enzyme is therapeutic interest enzyme belonging to the purine degradation pathway, which catalyzes the hydroxylation of uric acid in presence of molecular oxygen to a primary intermediate that is identified as 5-hydroxyisourate (5-HIU) .

There are different microbial sources for enzyme production, such as Bacillus fastidious spores [7], *Chlamydomonas reinhardtii* [8], *Candida* sp. [9], *Rhizopus oryzae* [10], *Arthrobacter globiformis* [11], *B. subtilis* [12], *B. fastidious* [13], *Aspergillus flavus* [14], *P. aeruginosa* [15], and *P. putida* [16].

The present aimed to screen *P. aeruginosa* that produces the uricase, moreover, the comparison between two methods was investigated to find which method is the best for evaluation of uricase producer bacteria.

MATERIALS AND METHODS

Bacterial Isolation

One hundred and fifty clinical specimens were collected from different clinical sources (burn, urine, blood, wound swab, ear swab, sputum and cerebrospinal fluid). The isolates were identified according to the [17], API 20 NE system and VITEK 2 system were used for precise and accurate identification of the isolates at generic and species level. The project was conducted following approval from ethical committee of University of Baghdad.

Screening the bacterial ability to produce uricase

The ability of *P. aeruginosa* isolates to produce uricase enzyme was tested on solid media by two methods; qualitative method by plate assay, and semi quantitative method by wells diffusion on an agar plate medium.

Qualitative screening

The standard method of Amirthanathan and Subramaniyan, (2012) was followed. Briefly, bacterial samples were streaked on media containing 10.0 g dextrose (BDH, England); 2.0 g yeast extract (BDH, England); 3.0 g uric acid (BDH, England), and 5.0 g NaCl (BDH, England). The materials were dissolved in 1000 ml distilled water and adjusted the pH to 7 and incubated at 37 ^{o}C for 24 h. The bacteria that produce uricase were isolated based on uricolytic activity on agar plate, which was identified as clear zone around the colonies [18].

Semi-quantitative screening

Semi-quantitative was used to screen the microorganisms that produced uricase. Medium for this assay and procedure was described previously [19]. Briefly, clinical isolates were cultured on the Luria Bertani nutrient broth (Himedia, India) medium (pH 7.5) at 37 ^{o}C. 1.25 ml of culture medium was added to 23.5 ml sterile LB broth and incubated at 37 ^{o}C for 48 h. Then 50 µl of growth culture was put in a well that made by cork borer in an agar plate medium containing uric acid, agar-agar and safranin, this medium was prepared by previous method [19]. Control well filled with sterilized nutrient broth. The plates were incubated at 37 ^{o}C for 24 h. Positive result indicated by appearing clear zone around the well.

RESELTS

Isolation and identification of bacteria

One hundred and fifty clinical specimens were collected from different hospitals in Baghdad, 43 isolates were successfully diagnosed as *P. aeruginosa* (28.66%). Diagnosis based on standard biochemical tests besides the API 20E, API 20NE system assay and VITEK 2 GN diagnostic system.

Screening uricase production

Qualitative screening method

Fig 1. shows the qualitative method of uricase production on plate (plate method). The results showed that

Fig.1 quanlitative assay of uricase production by *P. aeruginosa* isolates using plate assay method.

from 43 isolates of *P. aeruginosa* only 38 isolates (88.37%) were produced uricase while, the other 5 isolates (11.62%) were did not produce uricase. The results were appeared as a clear zone around the uricase produced colonies on the solid medium.

Semi quantitative Screening Method:

Semi-quantitative method (wells diffusion assay) was used to identified the level of uricase that produce by several isolates of P. aeruginosa. **Fig 2** showed the diameter of clear zone that appeared around uricase produced bacteria (colonies). The present study showed that the diameter of clear zone was ranged between 1.2-2.6 cm (**table 1**). The maximum production of uricase was found in P. aeruginosa (PA 7), while the lowest level of uricase production was found in case of *P. aeruginosa* (PA35).

Fig. 2 Semi quantitative production of uricase by *P. aeruginosa* isolates using wells diffusion assay method.

Table 1 shows semi quantitative method for detection the ability of different clinical isolates of *P. aeruginosa* to produce uricase. The results were performed by clear zone around uricase produced bacteria (colony). The diameter was measured by millimeter.

DISCUSSION

In the present study, the uricase-producing bacterium was isolated from clinical samples (burn, urine, blood, wound swab, ear swab, sputum and cerebrospinal fluid) and identified as *P. aeruginosa* according to bacteriological and standard biochemical tests, besides the API 20E system, API 20NE system assay and VITEK 2 GN diagnostic system. The results showed that the uricase producing *P. aeruginosa* gave the clear zones with diameter ranged between 12-26 mm. Regarding to qualitative method, results of this study agreed with previous studies [19, 20].

Table 1. Diameter of clear zones that represents the production of uricase by *P. aeruginosa* isolates using wells diffusion assay method.

No. of isolates	Clear zone diameter in mm
PA7	26
PA9	19
PA10	24
PA15	16
PA17	20
PA18	20
PA19	14
PA29	14
PA35	12
PA36	22
PA37	20
PA38	25
PA40	19
PA42	21
PA49	26
PA51	14
PA52	19
PA53	25
PA61	13
PA62	20
PA66	26
PA67	23
PA68	20
PA69	15
PA70	14
PA71	18
PA72	20
PA74	20
PA75	21
PA76	20
PA77	15
PA80	25
PA81	24
PA82	24
PA83	21
PA24	21
PA84	18
PA85	23

It was found that uricase producing strain of *Bacillus cereus* isolated from marine sediments and *P. aeruginosa* give 19 mm and 16 mm zone of clearance around the bacterial colony, respectively. One of the previous studies was proved that the *P. aeruginosa* produce extracellular inducible uricase enzyme on nutrient agar media that contained uric acid and was identified by several biochemical tests [15]. Previous study of a strain of P. putida was tested and checked for its potential of uricase production, which showed a zone

of clearance of 14 mm on uric acid agar plate by using well assay method [16].

The reason of Inability of isolates of *P. aeruginosa* to produce uricase is lacking of regulatory gene of uricase induction [21]. The semi-qualitative method is a simple, not expansive, and effective in screening of uricase production, as compared with using the kit for detecting the uricase assay. When compared with other methods, semi-quantitative method is sensitive method for detection of low concentration of uricase (0.5 mU/ml) and higher concentration [17]. The presence of difference in ability of bacteria for uricase production also uricase induction was dependent on the nutrients. The nutrients supplemented to media affected differently on induction of uricase and this was reflected in the variation rate of the oxidation reaction. Uric acid supplement showed the highest inducer for uricase production [22]. From present study it can be concluded that the semi-quantitative method is sensitive, specific and more efficient method for screening of uricase production as compare with qualitative method and gave more accurate results because its medium contain only uric acid as a source of carbon or nitrogen or both, which made this method more specific for uricase production.

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Conflict of interest

The authors declare that they have no conflict of interests.

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