Effect of tannic acid on urease and protease produced from *Proteus mirabilis*

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Abstract

Back ground: *P.mirabilis* is a gram negative bacterium, motile with its peritrichous flagella .Widely distributed in environment, especially in contaminated water and soil, Many virulence factors like LPS, urease, protease, hemolysin and biofilm formation play an important roles in the pathogenicity of *P. mirabilis*. Urease is a Nickel containing enzyme causes elevation of urine pH after hydrolyzing urea to ammonia and CO_2 forming stones that blocks the urinary track.

Aims: The effect of tannic acid on the production of urease and protease.

Material and methods: Twenty one isolates of *Proteus* were collected from different sources, Clinical and animal sources all isolates were cultured on MacConkey and blood agar and identification of *P. mirabilis* by, Vitek -2 compact system. Determine the effect of tannic acid on the production of urease and protease.

Results: Twenty one isolates of *Proteus* were identified depending on Vitek-2 compact system, after identification, it turns out that only 18 isolate were *P. mirabilis*. All isolates were 100% able to produce urease and 72.2% isolate were able to produce protease. The addition of tannic acid showed an inhibitory effect on urease and protease production.

Conclusion: The effect of tannic acid on urease and protease depending on concentration, type of strain, incubation period, number of isolates and truculence of isolate.

Keywords: *Proteus mirabilis*, Urease, protease, Tannic acid.

Introduction

The term "*Proteus*" was used for the first time by Hauser and its refers to the ability to changing in form, after isolating *Proteus* from the putrefied meet and depending on the liquefy of gelatin, Hauser classify *Proteus* bacteria to three species *mirabilis*, *vulgaris* and *zenkeri* (1). *Proteus* then classified according to biochemical test to five species *mirabilis*, *vulgaris*, *penneri*, *hauseri* and *myxofaciens* (2). The genetic analysis of *16S rRNA* proves that *Proteus mirabilis* belongs to the family of Gammaproteobacteria (3). *P.mirabilis* is a gram negative bacterium, chemotrophic organism gain its energy from fermentation and respiration, motile with its peritrichous flagella forming concentric zones on the solidified agar (3).Widely distributed in environment (4), especially in contaminated water and soil, also its part of human colon normal flora (5), as well as contaminated catheters (6). Many virulence factors like LPS, urease, protease, hemolysin and biofilm formation play an important roles in the pathogenicity of *P. mirabilis* (7). Urease is a Nickel containing enzyme causes elevation of urine pH after hydrolyzing urea to ammonia and Co_2 forming stones that blocks the urinary track. Crystalline assembly is one of the main reasons that lead to the formation of stones which in turn contribute in the protection of bacteria from the effect of antibiotics (8). *P. mirabilis* produce protease enzyme with 50- kilodalton (kDa), protease cleaves immunoglobulin A1,A2, and G, the cleavage of IgA1,2 occurs outside the hinge region, while cleaving of IgG occurs in the hinge region (9). Many substances inhibit the production of urease and protease enzymes produced *P. mirabilis*, like Curcumin (10), NAC (8), Ammonium salts (11), and leaves of *Coccinia grandis* (12). This study aimed to detect the effect of tannic acid on urease and protease production by *Proteus mirabilis.*

Materials and methods

Isolation and identification of *Proteus*

Twenty one isolates of *Proteus* were collected from different sources. Clinical isolates were 7 from urine (U), 3 for both wound swabs (W) and Sputum (S) and 4 from hospital environment (H.en), while isolates of animal sources were 2 from cat rectum (CR) and one for both chicken feces (CF) and dog rectum (DR). All isolates were cultured on MacConkey and blood agar media provided by (Oxiod). Biochemical tests (Catalase, oxidase, Methyl red, indole, voges - proskauer and citrate utilization) were used for identification of *P. mirabilis* (2), Vitek -2 compact system was used to confirm ours identification.

Urease and Protease production test

The ability of urease production by *P. mirabilis* was investigated as mentioned in (13), while for protease production, a bacterial suspension for each isolate with a concentration of ($1.5x10^8$) cell per ml was prepared by inoculation of bacterial colonies into the normal saline, then 200 µl of the suspension were carried to the wells that already done by using(8 mm) cork borer in the skim milk agar, the media was incubated at 37ºC for 24 hours and the diameter of hydrolysis zones were calculated (14).modified.

Tannic acid effect on urease

To determine the effect of tannic acid(BDH/England) on the production of urease enzyme in *P. mirabilis*, three concentrations (0.001, 0.01 and 0.1) % of tannic acid were added urea agar and the isolates of *P. mirabilis* were cultured by streaking the slant of urea agar, and then the media aerobically at 37º C for 24 hours were incubated.

Tannic acid effect on protease

Three concentrations (0.001, 0.01 and 0.1) % of tannic acid were mixed with skim milk agar before autoclaving and then 200µl of each bacterial suspension was carried to the bores that already done by using (8 mm) cork borer in the skim milk agar, the media was incubated at 37ºc for 24 hours and the diameter of hydrolysis zones were calculated.

Results and Discussion

Morphology diagnosis was done by using macConkey and blood agar media showed that all isolates belong to genus *Proteus* (5). Biochemical tests indicate that only 18 isolates were *P. mirabilis.* All the bacterial isolates were giving a positive result for catalase and methyl red, while indol, oxidase and vogues -proskaur were negative and citrate utilization was variable (2). Vitek -2 compact system confirmed the diagnosis with percentage of 93-99%.

Urease and protease production

The results of urease production revealed that all *P. mirabilis* isolates the clinical ones and animal sources isolates were urease positive as shown in Table (1).

Table (1): Urease production by *P. mirabilis* isolates

Bacterial isolates(source)	Urease production(number positive)	Percentage
U	+	
W	+	
S	+	
CF	+	100%
DR	+	
CR	+	
H.en	+	

U: Urine, W: Wounds, S: Sputum, CF: chicken feces, DR: Dog rectum, CR: Cat rectum

Our results consistent with (15)(16)(17)(18)(19)(20),whom indicate that the rate of urease production was (100%) from *P.mirabilis* bacteria isolated from urine and wound swabs.

Concerning protease, as shown in Table (2), 13/18 (72.2%) isolates were able to produce protease enzyme, the proportion of isolates producing the enzyme was distributed as follows: 5/7 from urine , 2/2 for both wound swaps and sputum, 1/1 from dog rectum , 1/2 from cat rectum and 2/3 from hospital environment, while the isolate from chicken feces was unable to produce the enzyme.

Table (2): Protease production by *P. mirabilis* isolates

Bacterial isolates(source)	Number of bacterial isolates(total)	Number of isolates produced protease(positive)	Percentage (%)
U	7	5	72.2
W	2	2	100
S	2	2	100
CF	1	0	0
DR	1	1	100
CR	2	1	50
H.en	3	2	66.6

U: Urine, W: Wounds, S: Sputum, CF: chicken feces, DR: Dog rectum, CR: Cat rectum

The results of current study showed that the rate of protease production is differ from one isolate to another depending on number and source of isolates, as noted *P. mirabilis* isolated from clinical samples are not very different from isolates of animal sources in their ability to produce protease and this may depends on the strain. Al-Dawah (20) pointed that the rate of urease production from *P. mirabilis* isolated from urine was (40%), while Ali and Jasim (21) pointed out that (100%) of *P. mirabilis* urine isolates were able to produce the enzyme. Al-Azawy (18) indicates that *P. mirabilis* isolated from wounds unable to produce protease, while Hussain (22) found that (100%) of urine, cat rectum, wounds and dog rectum isolates were able to produce the enzyme and (90%) of chicken feces isolates were able to produce protease. The variance between isolates may be due to the different in number of isolates, type of strain, source of isolate and the truculence of bacteria.

Tannic acid effect on Urease

Tannic acid effect was used to inhibit the production of urease; Table (3) shows the ability of *P. mirabilis* isolates to produce urease and the effect of tannic acid in urease production. The results showed that the effect of tannic acid is vary depending on source of isolate and concentration, as noted the concentration (0.001%) unable to inhibit urease production in all isolates after 5 and 24 hours of incubation except sputum (S) isolate, after 5 hours of incubation the concentration (0.01%) was unable to inhibit urease in clinical and animal isolate, but after completion of incubation for 24 hours no result has appear for wounds, sputum and chicken feces isolates, while in urine and dog rectum isolates tannic acid gives a low inhibition, as for (0.1%) concentration, it was successful to inhibit urease production in both clinical and animal sources isolates.

Table (3): Tannic acid effect on urease production by *P. mirabilis* isolates

Bacterial isolates	Control 5h	Control 24h	Concentration (%)					
			0.001 5h	0.001 24h	0.01 5h	0.01 24h	0.1 5h	0.1 24h
U	±	+	±	+	±	-	-	-
W	±	+	±	+	±	-	-	-
S	±	+	±	±	±	-	-	-
CF	±	+	±	+	±	-	-	-
DR	±	+	±	+	±	-	-	-
CR	±	+	±	+	±	-	-	-

U: Urine, W: Wounds, S: Sputum, CF: chicken feces, DR: Dog rectum,CR: Cat rectum, h:Hours, ±(50%positive+50%negative) +:100%positive ,-:100%negative

Tannic acid gives a clear effect to inhibit urease activity and this may be due the binding with active site of the enzyme causing changing in the structure of the enzyme, or the acid effect may inhibit quorum sensing systems (QS) leading to inhibition of virulence factors including urease enzyme, and in the same time tannic acid caused an inhibitory effect on swimming and swarming motility of *P. mirabilis* which composition is synchronized with virulence factors. Also the inhibitory effect of tannic acid may be attributed to its effect on many genes like *Rsb*A

that related to swarming phenomenon and virulence factors (23), while Ranjbar-Omid (24) point out that some substances have the ability to inhibit proton pump (proton pump inhibitors) that inhibit urease production.

Tannic acid effect on Protease

The inhibitory efficacy of tannic acid on protease production was estimated as shown in figure (1), both concentrations (0.01, and 0.1)% were able to inhibit protease production in both clinical and animal sources isolates, while the concentration (0.001%) was able to inhibit protease production in all isolates except wound swaps (W) and dog rectum (DR) isolates. Tannic acid show an inhibitory effect even in low concentration, and the inhibition of enzyme may be due to the same reasons of urease enzyme inhibition, tannic acid bind with active site of protease enzyme which leads to changing in the structure of enzyme causing its inhibition. Or the inhibitory effect may be on chemical signals (QS) which lead to inhibit the virulence factors including protease. In the same time tannic acid may effect on swimming and swarming motility of *P. mirabilis* bacteria and its virulence factors or it may effect on some genes like *Rsb*A (23).

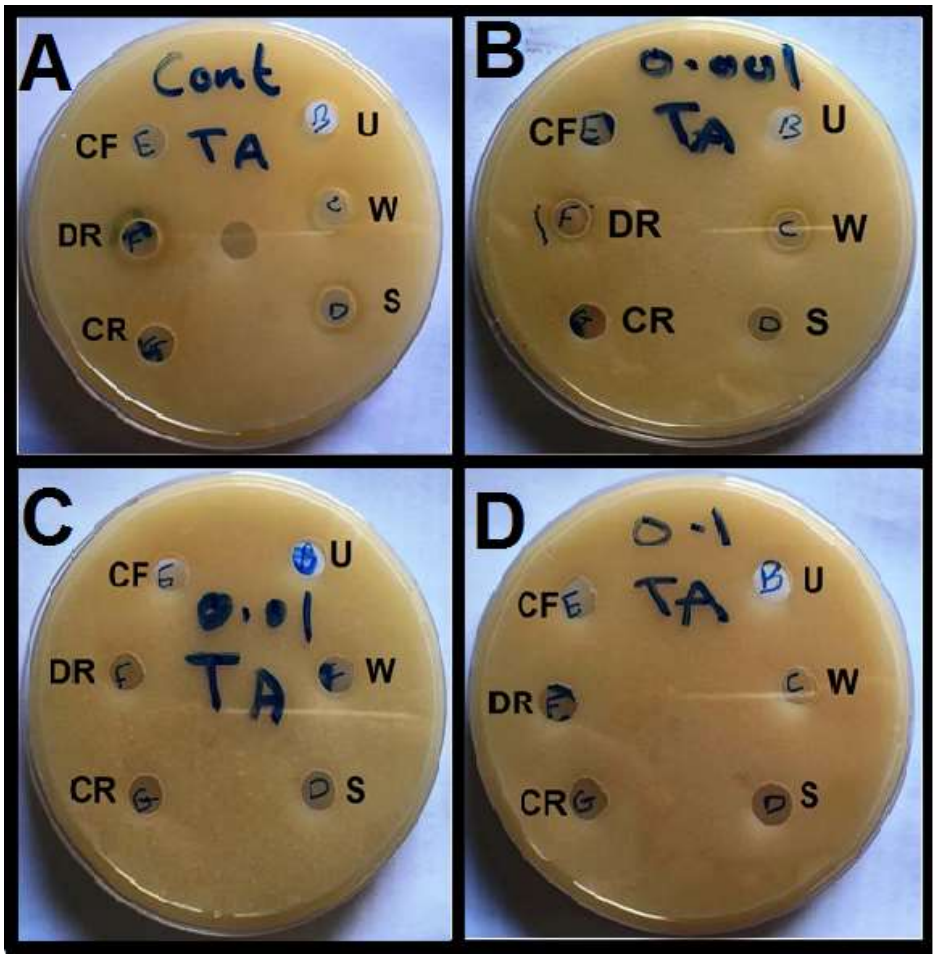

Figure (1): Effect of tannic acid on protease production in *P. mirabilis* isolates
A: Milk agar (control), B: Milk agar+0.001%tannic acid, C: Milk agar+0.01%tannic acid, D: Milk agar+0.01%tannic acid

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