The Effect of Tannic Acid on Swimming and Swarming Motility of *Proteus mirabilis*

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Abstract

This study was aimed to detect the effect of tannic acid on *Proteus mirabilis* swimming and swarming motility. Twenty-one isolates of *Proteus* bacteria were collected from different clinical samples and animal's sources, for the period from October 2017 to November 2017. All isolates were identified depending on microscopic characterization, biochemical tests and Vitec- 2 compact system. The results revealed that eighteen isolates were belonging to *P. mirabilis*. All *P. mirabilis* isolates were able to swim and swarm over semi-solid media. The addition of tannic acid at concentrations of 0.001, 0.01 and 0.1% was able to inhibit swimming and swarming motility. By using PCR technique, *Wos*A a gene responsible of swarming regulation was detected in all isolates and that *P. mirabilis* isolates were possessed this gene and the levels of fold change of *Wos*A gene were 0.19 and 0.12 for swimmer and swarmer cell, respectively. The levels of fold change of *Wos*A gene were 0.27 for the concentration (0.001%) of tannic acid when compared with controls (1-fold change).

Keywords: *Proteus mirabilis*, Swimming, Swarming, Tannic acid

تأثير مادة حامض التانيك في حركتي السباحة و الانثيال في بكتيريا *Proteus mirabilis*

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الخلاصة

هدفت الدراسة الحالية الى الكشف عن تأثير حامض التانيك على حركة السباحة والانثيال في بكتيريا *P.mirabilis*. تم جمع 21 عزلة من بكتيريا *Proteus* من مصادر سريرية وحيوانية مختلفة خلال المدة من تشرين الاول 2017 الى تشرين الثاني 2017 . بالاعتماد على الكشف المجهري والاختبارات الكيموحيوية و نظام الفايتك -2 تبين ان 18 عزلة فقط تعود للنوع *P. mirabilis* . امتلكت جميع عزلات بكتيريا *P. mirabilis* القدرة على السباحة والانثيال في وسطي السباحة والانثيال. ان اضافة حامض التانيك بتركيز (0.001، 0.01، 0.1) % الى الاوساط الزرعية يثبط كل من حركتي السباحة والانثيال. تم الكشف عن الجين المسؤول عن تنظيم ظاهرة الانثيال باستخدام تفاعل البلمرة المتسلسل وقد اظهرت النتائج امتلاك جميع العزلات لهذا الجين اذ بلغ مستوى التعبير الجيني 0.19 و 0.12 fold change في الخلايا السابحة والانثيالية على التوالي . مستوى التعبير الجيني عن جين *WosA* بلغ 0.27 fold change عند استعمال حامض التانيك مقارنة بالسيطرة الذي بلغ 1 fold change .

الكلمات المفتاحية : *Proteus mirabilis*، Swimming، Swarming، Tannic acid

Introduction

In 1885, Hauser discovered for the first time the genus *Proteus* when he isolated it from the putrefied meat, he described three species and they were *P. mirabilis*, *P. vulgaris* and *P. zenkeri* [1]. *Proteus mirabilis* is a gram-negative rod (0.4-0.8 x 1-3µm), moving by peritrichous flagella forming concentric regions known as Bull's Eye and the optimum temperature for the growth is 37C^{o} [2].

Proteus mirabilis distributed in many environments like soil and they are part of human colon normal flora or they may be natural, parasites or commensals in many animals [3-4-5]. Many virulence factors play role in the pathogenicity of *P. mirabilis* like LPS, biofilm, urease and hemolysin [6]. Urease is an enzyme containing nickel group and causes elevation of urine pH

when hydrolyzing urea to ammonia and Co_2 and forms apatite, struvite and carbonate crystals and consequently causes blocking of urinary tract [7].

Swarming phenomenon is the rapid migration of bacterial groups to invade and colonize host tissues, this require physical or chemical signals [8]. *P. mirabilis* is a dimorphic bacterial cell, depending on the culture media so they may be short rods with (6-8) flagella in broth culture known as 'swimmer cells' but as soon as they transmitted to solid culture they become elongated, hyperflagellated cells known as 'swarmer cells' [6]. Swarmer cells move quickly (1-2) hours at 37C^{o} forming concentric zones and enter to consolidation state to dedifferentiate to swimmer cells and this process multiple repeated many times forming a Bull's eye colony on the surface of solid media [9]. Rather [10] pointed that large numbers of loci play role in the swimmer cells differentiation and these genes are involved in cell division, peptidoglycan synthesis and ATP production. It's possible to inhibit swarming phenomenon by many substances like Fatty Acids [11], adding agar at a concentration more than 3% in culture media [12], PNG [13], NAC and Dipropyl disulphide [7].

This study aimed to determine the effect of tannic acid on swimming and swarming motility of *P. mirabilis* phenotypically and genetically.

Materials and Methods

Isolation of *Proteus* bacteria and identification

Twenty-one isolates of *Proteus* bacteria were collected from different clinical and animal sources. The clinical isolates were collected from 7 urine samples (U), 3 from wound swabs (W), 3 from Sputum samples (S) and 4 from hospital environment (H. en), while the isolates from animal sources were 1 from chicken feces (CF), 2 from cat rectum (CR) and 1 from dog rectum (DR). All isolates were cultured on MacConkey and blood agar media, provided by (Oxiod) company, then the isolates were incubated for 24 hours at 37 C^{o}. Used biochemical test (Catalase, oxidase, Methyl red, indole, voges - Proskauer and citrate utilization) for identification of *P. mirabilis* [14]. Our identification was confirmed by Vitec -2 system.

Swimming and Swarming test

Full loop of each isolate was cultured on the center of swimming and swarming media [15].

Effect of tannic acid on swimming and swarming

Three concentrations (0.1, 0.01 and 0.001%) of tannic acid were used to estimate their effect on swimming and swarming activities. After filtration of tannic acid solution by 0.22 um Millipore filters, the three concentrations were added separately to swimming (1% tryptone, 0.5% NaCl and 0.3% agar) and swarming (0.5% agar, 0.8% nutrient broth and 0.5% glucose) media [15]. A full loop of each isolate was cultured on the center of the media described above and incubated at 37 C^{o} for 24 hours.

Detection of genes and gene expression

According to manufacturer's instruction of Promega (USA), a mini kit was used to extract DNA from all isolates. By using the F- GCCCCTTATGCTGTCATGAA and R-GCCATTCAAAATCTGGTCACG of *Wos*A gene. The gene was amplified by the polymerase chain reaction (PCR) technique. PCR thermocycler Biorad (USA) conditions were "initial denaturation" at 95c^{o} for 5 minutes, followed by 35 cycle "denaturation, annealing and extension" 95c^{o} for 30 seconds, 60 c^{o} for 30 seconds, 72 c^{o} for 30 second with "final extension" at 72c^{o} for 7 minutes [16]. The PCR product then electrophoresed on 1% Agarose gel and stained with 5µg/ml of Ethidium bromide at 100 volts for 80 minutes and then the gel was visualized by UV Transilluminator. For RNA extraction a mini kit provided by Promega (USA) was used. *16SrRNA* with F-CCGAAGGTTAAGCTACCTAC and R-CCATGTGTAGCGGTGAAATG used as a house keeping gene [16]. This experiment was done by using RT- PCR with modified conditions [16]. Real time PCR profile require three steps, the first one was "hold steps" which divided to two steps "activation" at 37c^{o} for 10 minutes and "hold" at 95c^{o} for 15 minutes, the second step "cycling" which is divided to three cycles at (95c^{o}, 60c^{o} and 72c^{o}) respectively for (20, 20 and 45) seconds respectively, the third step was "melt on green" for 0.3 seconds at 72-95c^{o}.

Results and discussion

The first diagnosis which was done by using Macconkey and blood agar media showed that all isolates belong to the genus *Proteus* [4]. Biochemical test showed that only fifteen isolates were belonging to the species *P. mirabilis.* All the bacterial isolates were catalase and methyl red positive, oxidase, vogues proskaurs and indol negative but citrate utilization was variable [18]. Vitec -2 compact system confirmed the diagnosis with a percentage of 93-99%.

The result of this study showed that all isolates of *P. mirabilis* can swim with a diameter ranging from (25-80) mm and swarm with a diameter ranging from (10-35) mm as shown in Figure (1).

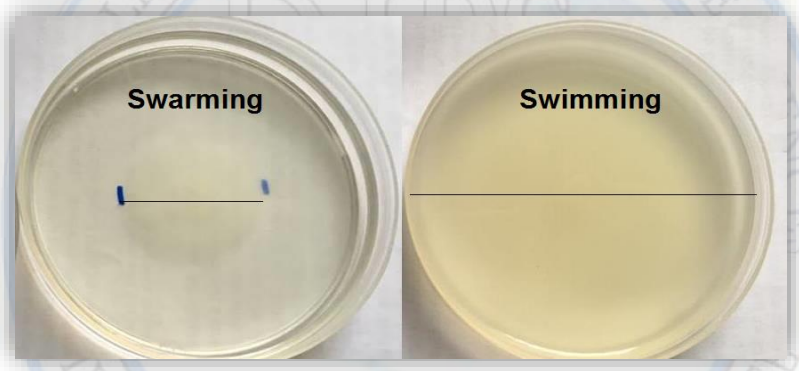

Figure 1: Swarming and Swimming motility in *P. mirabilis*.

The results of this study exhibited the ability of tannic acid to inhibit both swimming and swarming of *P. mirabilis*.as shown in Figure (2). The three concentrations (0.001, 0.01 and 0.1%) of tannic acid were capable to inhibit *P. mirabilis* swimming motility. At the concentration of 0.001%, the inhibition rates of clincal isolates (U1 , W1 , S1) were 56.1, 65.1and 75.6%, respectively while in animal isolates (CF1 , DR1 , CR1) the inhibition rates were 41, 35.7 and 56.3%, respectively . At the concentration of 0.01%, the inhibiton rate was approximately similar in all isolates and was ranging from 83.3 to 87.6%. At 0.1 % concentration, the (U1) isolate showed the highest inhibition ratio of 89% while the W1 and S1 isolates showed inhibition rates of 83.3 and 78.7%, respectively, while the animal isolates have shown an inhibtion rates ranging from 81.4 to 86.3%.

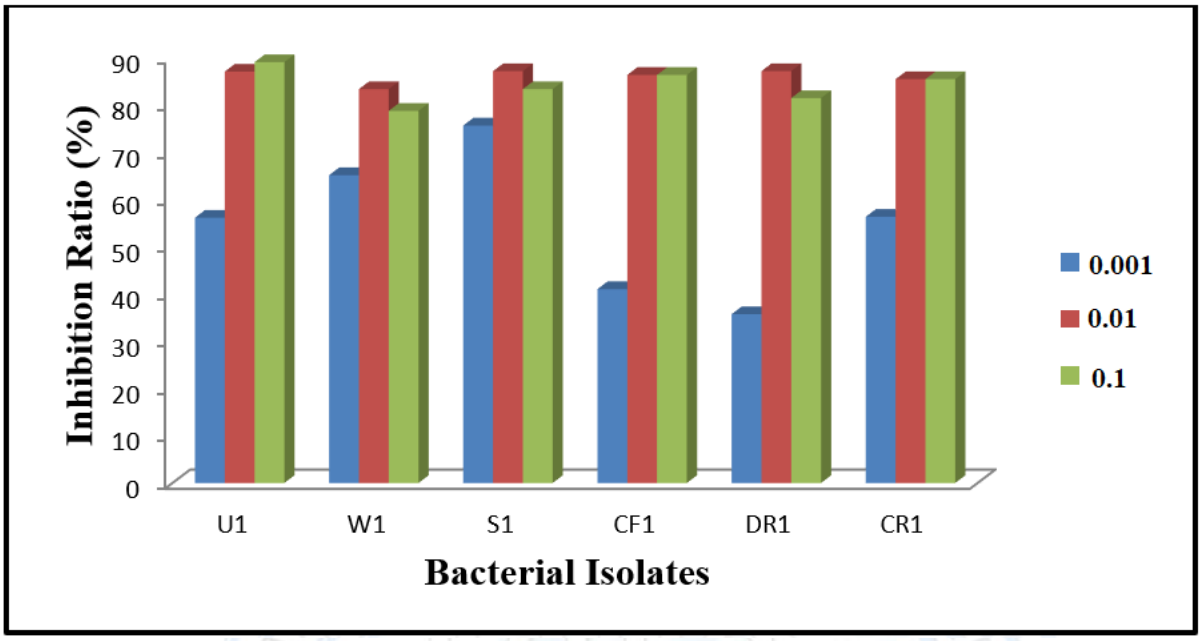

Figure 2: The effect of Tannic acid on Swimming motility of *P. mirabilis* Isolates

(U1: Urine, W1: Wound, S1: Sputum, CF1: Ckicken Feces, DR1: Dog Rectal, CR1: Cat Rectal).

Figure (3) shows that swarming motility was also inhibited but, none of the three concentrations were effective at inhibition of swarmer cells of CR1 isolate. The concentration of 0.001% was not capable to inhibit the swarmer cells of (U1) isolate while in W1, S1, CF1and DR1 isolates, the inhibition ratios were 48.7, 47.8, 61.5, 31.2% respectively. The maximum ratios of inhibition were in (W1) and (CF1) isolates at 0.01% concentration and were 85 % and 81.5 %, respectively. In U1 and S1 isolates, the inhibition ratios were 68.6 and 73.9%, respectively while the inhibition ratio of (DR1) isolate was 37.5%. The inhibition ratios of (U1, W1, S1, CF1) isolates were ranging from 72.5 to 82.5% at 0.1 % concentration, while in (DR1) isolate the inhibition ratio was 18.7%. These results showed that tannic acid was more effective in swimmer cells state than swarmer cell depending on the source of the isolate and the concentration. In addition, the swarmer cells of *P. mirabilis* bacteria isolated from animals have shown more resistance to the effect of tannic acid than the isolates obtained from other sources. Smith [19] pointed out that adding tannic acid at concentrations of 0.00001 to 0.1% to the culture media may block the swarming phenomenon as a result of binding tannic acid with the proteins or the phospholipids in the outer membrane of the cell

wall but did not affect the growth rate or it may affect on chemical signals in quorum sensing system which plays an important role in swimmer and swarmer cells differentiation.

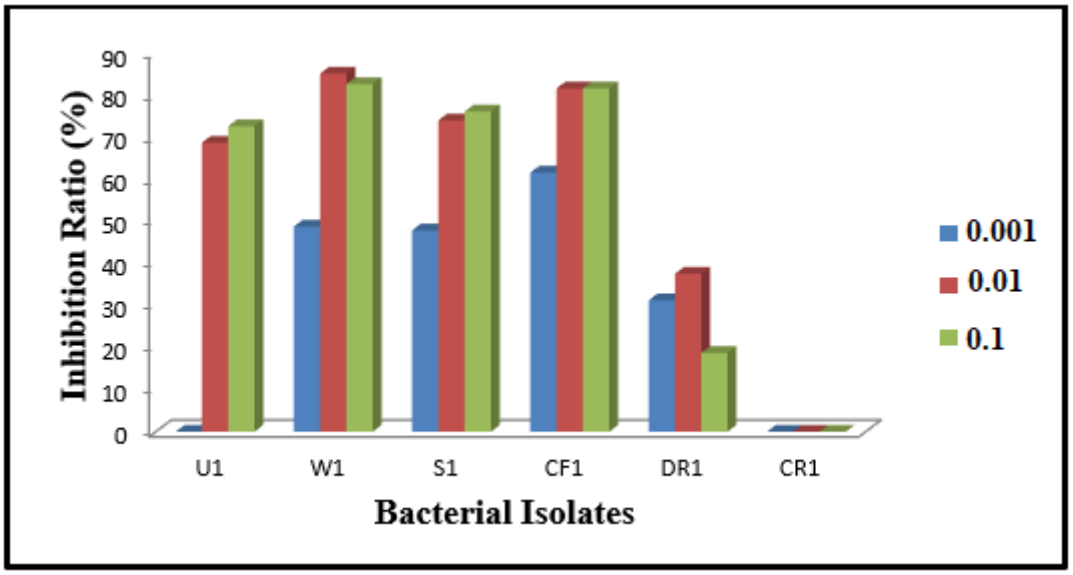

Figure 3: The effect of tannic acid on swarming motility of *P. mirabilis* isolates
(U1: Urine, W1: Wound, S1: Sputum, CF1: Ckicken Feces, DR1: Dog Rectal, CR1: Cat Rectal).

The DNA of each isolate was extracted. By using PCR technique, *Wos*A gene was detected in all *P. mirabilis* isolates and the results showed that *Wos*A amplicon with molecular weight of 183bp on agarose gel (Figure 4). Many genes including *Wos*A regulate swarming phenomenon and the over expression of *Wos*A responsible for *P. mirabilis* swarming on the culture media [20]. The over expression of *Wos*A increases the expression of *FlhDC* to varying levels depending on swarming cycle leading swimmer cell to differentiation into swarmer cell [9]. As shown in Figure (5), the fold change levels of *Wos*A gene were 0.19 and 0.12 for swimmer and swarmer cell, respectively. High levels of *Wos*A expression in liquid culture media result from the cells density [19], while in the semi-sold media, the expression was less. In the semi-solid culture media, swimmer cells express *Wos*A gene more than swarmer cells. A mutation in *Fla*A decreases the levels of *Wos*A expression and this can be observed in the solid culture media [9]. By using tannic acid at a concentration of 0.001% in broth culture media, the expression of *Wos*A was minimized to 0.27-fold change compared to

the control which was 1-fold change as shown in Figure (5). These results suggest that the effect of tannic acid may be due to the binding of the acid with the proteins or phospholipids in the outer membrane of the cell wall [19].

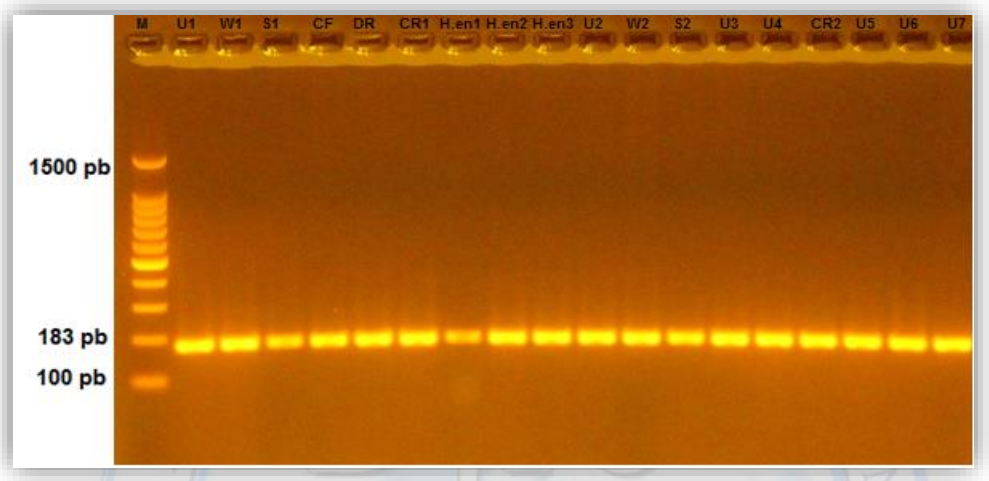

Figure 4: Electrophoresis of total *P. mirabilis* isolates *Wos*A gene on agarose gel 1% at 100 volts for 80 minutes, Lane M: DNA ladder, Lane (U: Urine, W: Wound, S: Sputum, CF: Ckicken Feces, CR: Cat Rectal, DR: Dog Rectal, H. en: Hospital environment): Amplicon of *Wos*A of *P. mirabilis*.

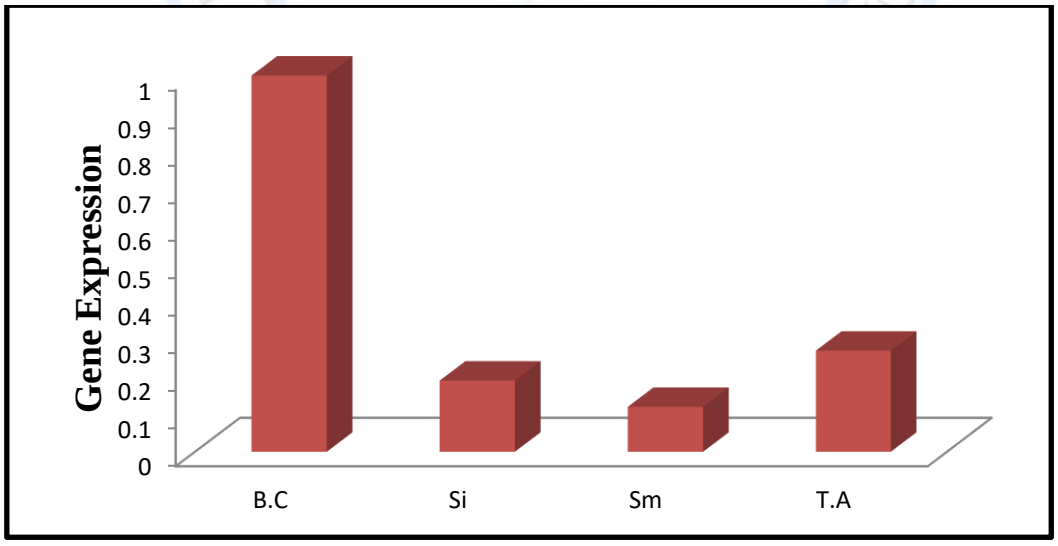

Figure 5: *Wos*A expression in broth culture media and solid media

B.C: Broth media, Si: swimmer cells in Semi-solid media (0.3% Agar), Sm : swarmer cells in Semi-solid media (0.5% Agar) , T.A :tannic Acid

Conclusions

Tannic acid has been found to have inhibitory effect in swimmer and swarmer cells. The inhibitory function varies according to the concentration of the acid and duration of incubation.

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