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RESEARCH ARTICLE

Phenotypic and Genotypic Study of Sodium Azide Effect on Proteus Mirabilis Swimming and Swarming Phenomenon

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Abstract

The present study intended to detect the effect of sodium azide on *P. mirabilis* swimming and swarming phenomenon. Twenty one isolates of *Proteus* bacteria were collected from different clinical and animals samples for the period from October 2017 to November 2017. All isolates were identification depending on microscopic characterization , biochemical tests and Vitek 2E compact system, it appears that eighteen isolates were belong to *P. mirabilis* . All *P. mirabilis* isolates were able to swim and swarm over semi-solid media. The addition of sodium azide with (0.001, 0.01, 0.1) % concentrations was able to inhibit swimming and swarming phenomenon. Using PCR technique, *WosA* a gene responsible of swarming regulation was detected in all isolates and its expression was measured using RT-PCR in normal and sodium azide conditions. The result of molecular detection of *WosA* gene showed that all of the *P. mirabilis* isolates were possessed this gene and the levels of fold change of wosA gene were 0.19 and 0.12 for swimmer and swarmer cell respectively. The levels of fold change of wosA gene were 0.4 for the concentration (0.001%) of sodium azide when compared with control 1 fold change.

Keywords: *Proteus mirabilis, Swimming, Swarming, Sodium azide.*

Introduction

The genus *Proteus* was discovered for the first time by Hauser in 1885 when he isolated it from putrefied meat he described three species included *P. mirabilis, P. vulgaris, P.zenkeri* depending on gelatin liquefying [1]. *P. mirabilis* is gram negative rods with (0.4-0.8) µm in length and (1-3) µm in width ,belong to the Enterobacteriaceae family, chemoorganotrophic and gain energy by fermentation or Respiration, and motile by Peritrichous flagella [2].

P. mirabilis can be isolated from different source as contaminated soil and water [3], and the part of human colon normal flora [4], they are also found in many animals and mammals depending on the nature of life, the may be Natural, Commensals and Parasitic [5]. *P. mirabilis* have many virulence factors as biofilm formation, Protease, Hemolysin, LPS and Urease [6], Urease is nickel enzymes capable of hydrolyzing the ammonia which increase the pH of urine and facilitate the forming struvite, carbonate and apatite crystals leading to urinary stone formation [7].

Swarming phenomenon is the rapid migration of bacterial groups which enhance the colonization of nutrient-rich environment or host tissues by the bacteria, a mechanism of swarming require a cell-cell signals may be physical or chemical and lead to physiological and morphological differentiation of swimmer cells to swarmer cells [8, 6]. Mention that the Urothropathogenic *P. mirabilis* is a dimorphic bacteria , its shape depend on the culture media , they are short rods with (1-2) µm in length and (6-8) peritrichously flagella called Vegetative cells 'Swimmer cells' when they are transmitted to solid media , they are changed into elongated rods (60-80)µm, non satiated and hyper flagellated cells called 'Swarmer cells' .

The migration of Swarmer cells occur quickly forming a concentric regions and after (1-2) hours at 37 C the swarmer cells enter to Consolidation state and dedifferentiate into swimmer cells, this process is multiple repeated forming a Bull's eye colony on the surface of the agar plate [9]. The genetic analysis showed that large number of loci

play role in the differentiation of swimmer cells to swarmer cells including genes involved in regulation, peptidoglycan synthesis, cell division and ATP production [10]. Swarming may be discouragement by many Natural extract and Chemical substances like P-Nitrophenyl Glycerol [11], N-Acetyl cysteine and Dipropyl disulphide [7], Fatty Acids [12]. Resveratrol [13], Agar with (1-6) % in culture media [14], also the swarming phenomenon can be initiated by Amino Acids for being serve as carbon and energy sources which increase the growth rate [15; 16].

Materials and Methods

Isolation and Identification of P. mirabilis

Twenty one isolates of *Proteus* bacteria as shown in Table (1) were collected from different clinical samples and animals Sources. All isolates were collected and cultured on MacConkey agar and Blood agar for the first isolation. Primary Identification of isolates was involved Morphological, Biochemical tests and Vitec -2E compact system was done.

Table 1: Number and Sources of *Proteus* isolates

Source	Abbreviation	Number of isolates
Urine	U	7
Wound Swap	W	3
Sputum	S	3
Chicken Feces	CF	1
Dog Rectal	DR	1
Cat Rectal	CR	2
Hospital Environment	H.en	4

Effect of Sodium azide on swimming and swarming

Three concentration (0.1, 0.01, .0.001) % of Sodium Azide were used to estimate there effect on swimming and swarming motility. NaN_3 were added to swimming and swarming media described by [17].

DNA Extraction and Gene Expression

A DNA extraction mini kit processed by Promega (USA) was used. PCR technique used to amplify the *WosA* gene using F-GCCCCTTATGCTGTCATGAA and R-GCCATTCAAAATCTGGTCACG [18]. PCR product of *WosA* gene was prepared by mixing 12.5µl of Go Taq Green master mix, 2µl of DNA template, 1µl of Forward primer, 1µl of Reverse primer and 8.5µl of Nuclease free water to reach 25µl of the final mixture. PCR thermocycler Biorad (USA) was used and the most proper conditions were explained in table (2) [18]. The PCR product then electrophoresed on Agarose gel 1% stained with 5µg/ml of ethidium bromide for 80 minutes at 100 volt and then the gel was visualized by UV Tran illuminator to take a picture.

For RNA extraction a mini kit provided by Promega (USA) was used and *16Sr RNA* with F-CCGAAGGTTAAGCTACCTAC and R-CCATGTGTAGCGGTGAAATG was used as a house keeping gene [19], this experiment was done by using Real time PCR with modified conditions list in Table (3) [18].

Table 2: PCR thermo cycle conditions

Gene Name	Series	Stages	Temperature	Time	Num. of Cycles
WosA	1	Initial denaturation	95 C°	5 minutes	1
	2	Denaturation	95 C°	30 seconds	35
	3	Annealing	60 C°	30 seconds	
	4	Extension	72 C°	30 seconds	
	5	Final extension	72 C°	7 minutes	1
	6	Hold	4 C°	Free	-

Table 3: Real time PCR conditions

Run Profile	Steps	Time	Temperature	Num. of Cycles
Hold steps	Activation	15 min	37 C°	-
	Hold	10 min	95 C°	-
Cycling	Cycle 1	20 seconds	95 C°	45
	Cycle 2	20 seconds	60 C°	
	Cycle 3	45 seconds	72 C°	
Melt on Green	-	0.3 seconds	72-95 C°	-

Results and Discussion

Identification of *P. mirabilis*

Twenty-one isolates of *Proteus* were identification depending on microscopic characterization and conformist biochemical

tests. The biochemical tests and Vitec -2 compact system showed that only Eighteen isolates were belong to the specie *P. mirabilis* with a percentage (93-99) %.

Swimming and Swarming Test

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

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

Effect of Sodium on Swimming and Swarming

The results of this study exhibit the ability of Sodium azide to inhibit both swimming and swarming motility of *P.mirabilis*.As shown in Figure (2) the 0.001 % concentration was not capable of inhibiting swimming of the clinical and animal isolates, at concentration 0.01% inhibition of swimming isolates was variable depends on the source, the Sputum, Chicken feces and Dog rectal isoltes reaches the highest inhibtion ratio (87.5,85,86.6) % respictivily, Wounds and Cat rectal isoltes were approximate (62.5,68.5) % respicitvily, while the Urine isolates showed the lowest inhibition ratio 50%. The 0.1% concentration was very effective to inhibit swimming and its inhibtion ratio was ranging from (81.4-88.7) %.

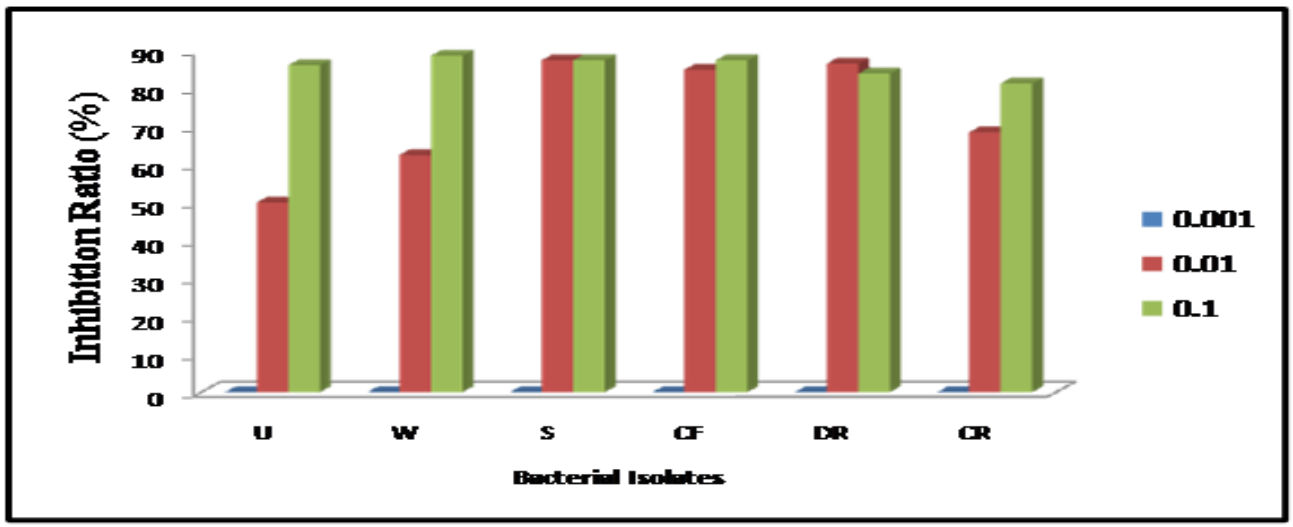

Figure 2: the effect of Sodium Azide on Swimming motility of *P.mirabilis* Isolates (U1: Urine, W1: Wound, S1: Sputum, CF: Ckicken Feces, DR: Dog Rectal CR1: Cat Rectal)

About the Swarming motility, the Figure (3) showed that effect of sodium azide is different between isolates depending on their source , the three concentraion were effective to inhibit swarming in Chicken feces isolate while non of them were capable of inhibitting the Cat Rectal isolate, 0.001 % concentration was little inhibit swarming in Sputum isolates with an inhibition ratio reach to 6.5 % and no inhibition was for the rest of the isolates. the 0.01 % concentration showed in clinical isolates (Urine, Wounds, sputum) an inhibtion ratio (11.7, 43.7, 2.1) % respictivily while in Dog Rectal isolate the inhibtion ratio was 12.5 % also the 0.1 % conecntration reach its maxium effect in Sputum and Chicken feces isolate (73.9,75.3) % while in urine , wounds, Dog Rectal was

(50.9,57.5,25) %. These results reveals that Sodium Azide is more effective to inhibit *P.mirabilis* swimmer cells more than swarmer cells depends on the source of isolate. A study done by [20] showed that sodium azide with concentration 0.005 % and 0.01 % block both swimming and swarming motility of *P.mirabilis* , also [21] indicate that adding 0.005 % of sodium azide to the culture media couses poisoning to the areobic respiration by effecting on the genes encodes to Tricarboxylic Acid cycle (TCA), enzymes like Fumerase and Argininosuccinate Lyase preventing *P. mirabilis* swarming. In other studies [22] pointed that using chemical substance can inhibit the swarming phenomenon by blocking the formation of flagella , effecting on the motility mechanism of bacterial cells or by effecting on the function of flagella while [23] suggest that the chemical substance discourage the differentiation of swimmer cells to swarmer cells and blocks the swarming.

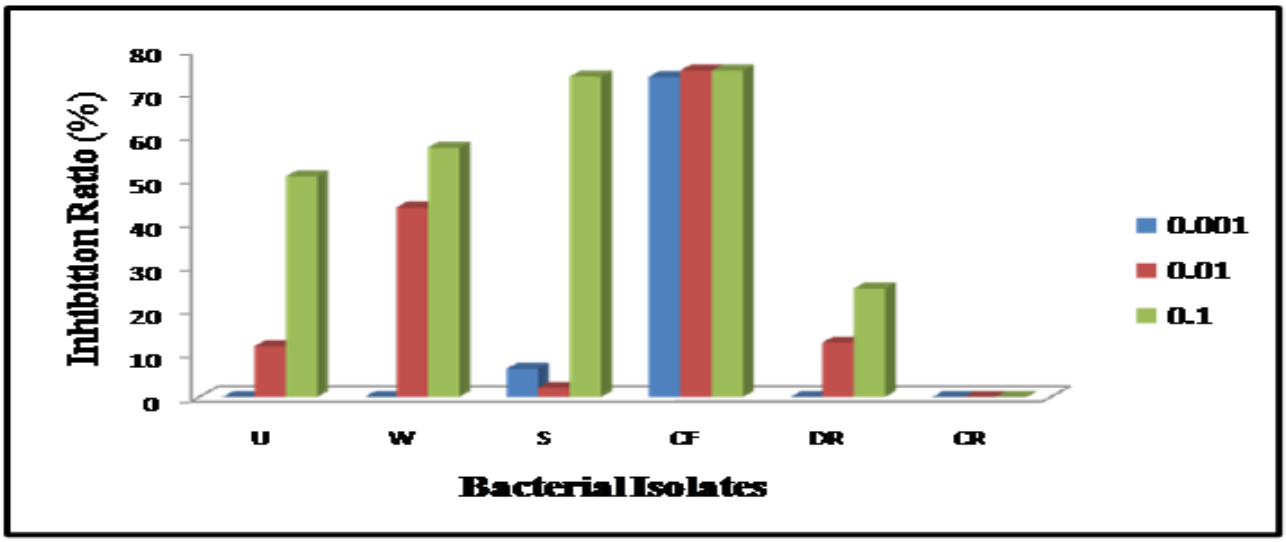

Figure 3: The effect of Sodium Azide on swarming motility of *P.mirabilis* Isolates (U1: Urine, W1: Wound, S1: Sputum, CF: Ckicken Feces, DR: Dog Rectal CR1: Cat Rectal)

DNA Extraction and Gene Expression

The total DNA of each isolate was extracted as shown in Figure (4). *WosA* gene was detected in all *P. mirabilis* isolates by using PCR technique and the results showed that *WosA* Amplicon with molecular weight 183bp on agarose gel Figure (5). [24] Showed that the over expression of *WosA* gene leads to *P. mirabils* swarming, many genes including *WosA* regulate swarming motility. The over expression of *WosA* gene leads to increase the expression of *FlhDC* varying levels depending on swarming cycle leading to differentiation of swimmer cell to swarmer cell [9].

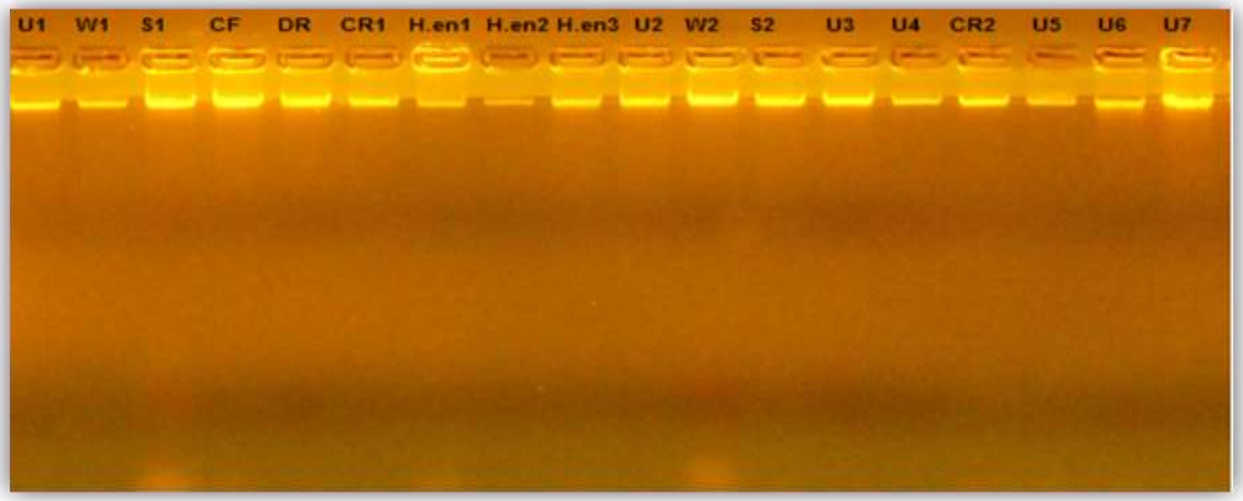

Figure 4: Elecetrophoresis of total *P. mirabilis* isolates DNA on agarose gel 1% at 100 volt for 80 minutes , Lane (U: Urine , W:Wound , S: Sputum , CF: Ckicken Feces , CR: Cat Rectal, DR: Dog Rectal , H. en: Hospital environment) : Total DNA of *P. mirabilis*

As shown in Figure (6) the levels of fold change of *WosA* gene were 0.19 and 0.12 for swimmer and swarmer cell respectively. The *WosA* expression is high in liquid culture media as a result from the cells density [24], while in the semi-sold media the expression in less. Swimmer cells shown more expression of *WosA* gene in the semi-solid media compared to swarmer cells. A mutation in *FlaA* gene decreases the levels of *WosA* gene expression and this can be noticed in the solid media [9]. The using of Sodium Azide with concentration 0.001 % in liquid culture media as Figure (7) shows, the levels of fold change of wosA gene were 0.4 for the concentration (0.001%) of sodium azide when compared with control 1 fold change, minimize the expression of *WosA,* this result suggest that Sodium Azide may be effect on TCA cycle [21], or may block the quorum sensing system between cells [23].

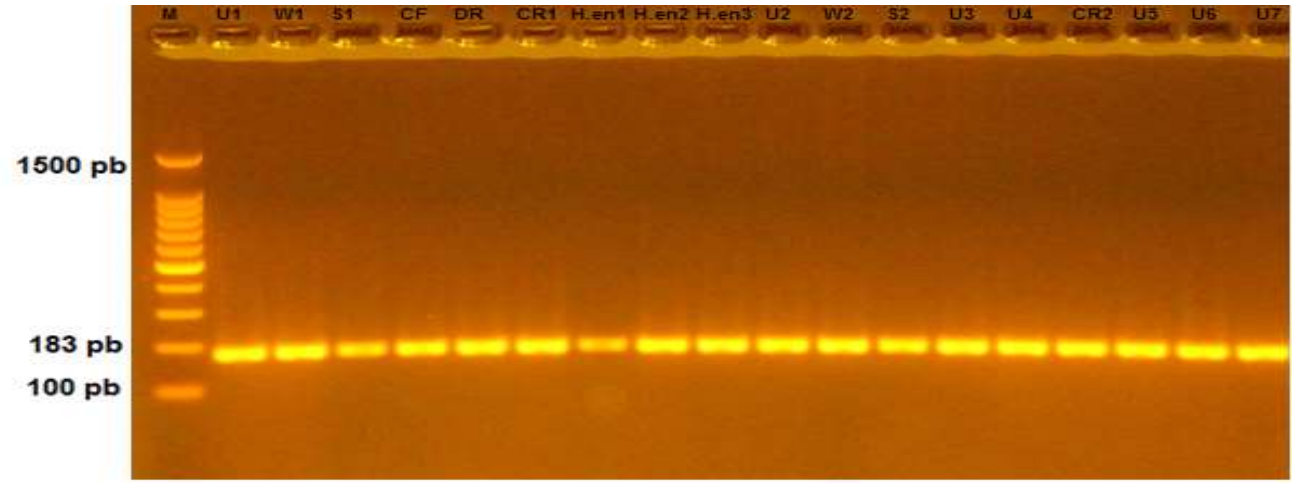

Figure 5: Electrophoresis of total *P. mirabilis* isolates *WosA* gene on agarose gel 1% at 100 volt for 80 minutes , Lane M : DNA ladder , Lane (U:Urine , W:Wound, S:Sputum , CF: Chicken Feces , CR: Cat Rectal , DR: Dog Rectal , H. en: Hospital environment): Amplicon of *WosA* of *P. mirabilis*

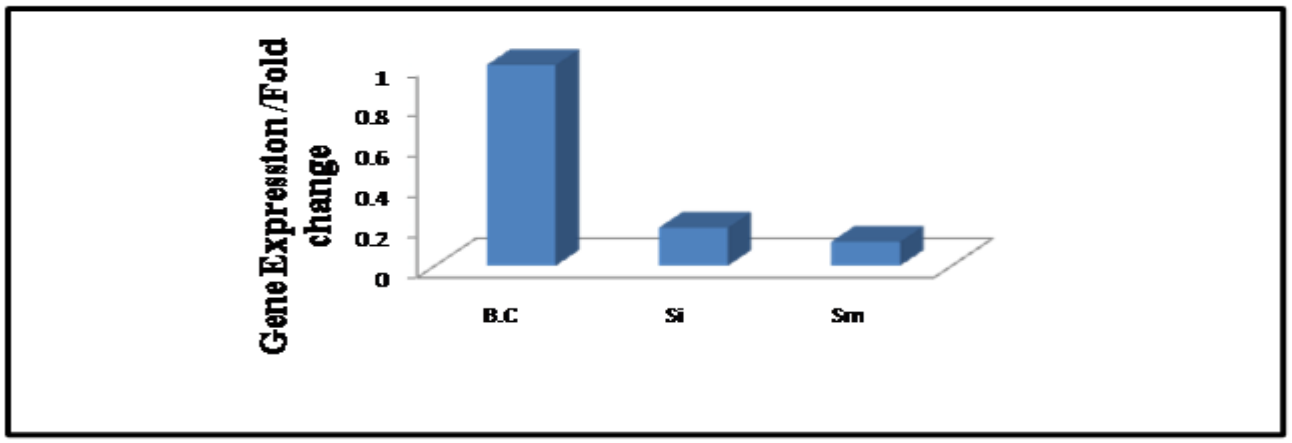

Figure 6: *WosA gene* expression of *p. mirabilis* (U1) , B.C = broth culture , Si = swimmer cells in semi – solid media (0.3 % Agar) , Sm = Swarmer cells in semi – solid media (0.5 % Agar)

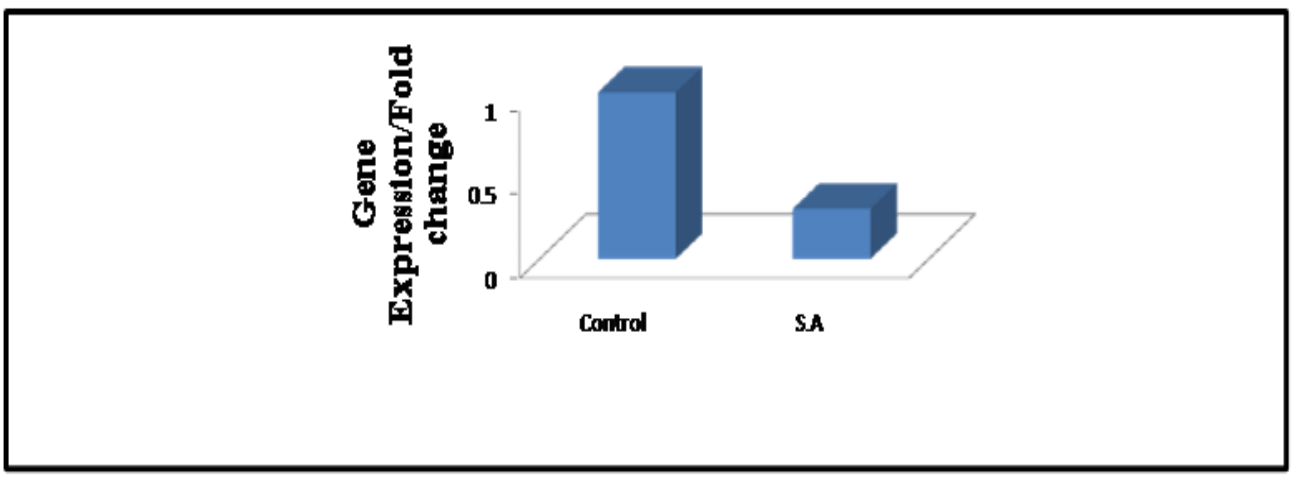

Figure 7: The effect of Sodium Azide on *WosA* gene expression in broth culture S.A = Sodium Azide

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