

# Validation of Horizontal Genes Transfer Using Selected Clinical Strain of *P. Mirabilis* for Swarming Activity

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## Abstract

**Introduction:** *Proteus mirabilis* expresses several virulence factor which enable them to colonize, survive and grow in the host organism. This study was to investigate whether *P. mirabilis* could deliver a spontaneous plasmids to competent strains, and to investigate whether *P. mirabilis* can be as a donor strains to delivery their virulent such as adhesion and swarming activity.

**Methodology:** PCR was used to detect the motility genes that encoded in *P. mirabilis*. Using also transformation assay to transfer these genes of *Proteus* to standard strain. PCR technique used again to determine the whether genes were transfer or not after transformation.

**The results:** the findings show that 9 (*filD*, *flhC*, *flhD*, *motA*, *motB*, *fliL*, *flaA*, *flgM*,) genes were encoded on chromosomal DNA of *P. mirabilis*. Interestingly data, only 3 (*flaA*, *flgM* and *filD*) genes were carried on different plasmids of *P. mirabilis*. The results also show that spontaneous plasmids of *P. mirabilis* can transfer to standard strains of *E. coli*. We found one gene transfer to *E. coli* S17λ *filD*. The gene(*filD*) has a role in the *P. mirabilis* swarming motility.

**Conclusions:** we demonstrated that these genes can carry not only on chromosomal DNA but also in Plasmidal DNA and transferring genes associated-characteristics, swarming motility

**Key words:** *filD*, *flhC*, *flhD*, *motA*, *motB*, *flaA*, *Proteus mirabilis*, *swarming motility*.

## Introduction

One of opportunistic pathogen is *Proteus mirabilis* which is can cause severe invasive diseases, in critically ill and patients who have immunocompromised [1]. It is an important source of hospital-acquired infections [2]. *P. mirabilis* is the main cause of urinary tract, respiratory tract and wounds infections, burns, digestive tract infections, ear infection and otitis [3]. *P. mirabilis* has also a wide virulence factors which enable them to colonize, survive and grow in the host organism such as swarming, fimbriae, urease, hemolysin, protease and Lipopolysaccharides (LPS) [4]. Furthermore, *P. mirabilis*

is well known for its ability to form biofilms, and thereby resists antibiotic treatment [5].

Swarming motility of the *Proteus* is made up of circular waves on solid media [6]. These waves include consolidation and swarming rings, during the consolidation phase, the cells are in short form, and in the swarming phase, they are in long form [6].

*P. mirabilis* have many virulence factors. Some of these virulence factors encoded in plasmids could be acquired from hospitals or environments. We try to demonstrate whether clinical strains have acquired some genes related with swarming activity and can transfer these genes to standard bacteria strains, which is *E. coli* S17λ.

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## Methodology

### Bacterial strains:

We isolated 40 samples of *P. mirabilis* from different site of infection, and we selected (P23) which has plasmids and a strongly swarming. *P. mirabilis* (P23) was grown in lysogeny agar. It was also grown an overnight culture and were cultured at 37 °C to reach OD<sub>600</sub> 0.4. It was equal 1x10<sup>8</sup>. *E. coli* S17λ was provided from Life technologies New England Biolabs®.

PCR detection of genes encoded in chromosomal or plasmidal DNA

### Isolation of total DNA

The Genomic DNA Extraction Kit was used for DNA extraction by manufacturer's protocol performed according to (Intron\ Korea).

### Isolation of total Plasmid

DNA plasmid were extracted from all bacteria isolates using QIAprep spin miniprep kit (Qiagen) according to the manufacturer's instructions.

### Gel electrophoresis

PCR product was separated based on their size, using gel electrophoresis. 2g/100mL of agarose from Fisher Scientific was dissolved in tris base, acetic acid and EDTA (TAE) buffer by boiling. The agarose solution was left in 25°C to allow cooling around 55 °C, and then 5µL of 0.5 mg/ml of ethidium bromide to stain the DNA fragments. Typically, 1 µL of 5X DNA gel loading dye (Thermo) and 5µL of samples were mixed before loading in the gel. The UV transilluminator was used and images was captured using an EDAS 290 imaging system (Kodak) to visualise the DNA pieces.

**Table 1 primers quotation**

Gene type		Primer Sequence (5'-3')	Amplicon size (bp)	Reference
flhD	F	AAGGCTTCCGCAATGTTTAGAC	190	[7]
	R	GTTGCAAATCATCCACTCTGGA		
mot A	F	GATGGTGACGGGGAATATGAA	215	[8]
	R	CCATTTCCCAGCAGGTCTA		
mot B	F	GCGTTACGTCCACATCTCAA	200	[9]
	R	ATGTCGCGCATATAGGGTTC		
fliL	F	GGTGATCGCCATTATTGCAG	220	[8]
	R	AGCGTAAACGTGATCCCTATG		
flhC	F	GCCAGTGAAAAAAGCATTGTCA	500	GenBank: O34202
	R	CCAACAGCCTGTACTCTCTGTTC		
flgM	F	CGCACAAATCCACTTATCCC	180	[8]
	R	GGGCAACTTTTTTCGACATTG		
flaA	F	TGCTGGTGCAACTTCATACG	200	[9]
	R	TTTGTGAGCACCTTCCAGTG		
fliD	F	GCCGCAAGTATTTTCATCGCTGGG	650	GenBank: P42274
	R	CCTTTGGAATTACTGTTGTTTTTC		

### Statistical Analyses

Statistical analyses are performed using GraphPad Prism.8. Significance was determined using ozone correlation test. Data are presented as mean ± standard error of mean.

### The Results and Discussion

#### Detection of swarming genes

Nine virulence genes *rpoA*, *flhC*, *flhD*, *motA*, *motB*, *fliL*, *flaA*, *flgM*, *flhD* that carried on *P. mirabilis* DNA were studied. These genes have a role to induce many infections [8] [9] [10]. The aim of this section was to quantify *P. mirabilis* genes whether were encoded in chromosomal and/or plasmid.

The *motA*, *motB* and *fliL* genes play a role in the rotary motor of flagella [11]. Two *motA* and *motB* genes

are required for swimming and twitching motility of *Proteus mirabilis*. The stator complexes are not only encoded in *P. mirabilis* but also in most pathogenic bacteria that need to swarm in over surfaces [12]. All isolates 40 (100%) have *motA* and *fliL* genes, while *motB* was detected in 37 isolates (92.5%), **Fig1**. These results were in consistent with result of [13] [14]. This findings were similar with other researchers [15] [16]. **As shown in Fig 2**, From 8 genes, which were encoded in DNA chromosome, only 3 genes were carrying on DNA plasmid of *P. mirabilis*. The percentage of genes *flhD*, *flaA* and *flgM*, were accounted in 3(60%),3(60%) and 2(40%) isolates respectively. These results in regards to swarming activity genes were similar with findings obtained by [17, 18]. [15] [16]. They demonstrated that the virulence genes are encoded in DNA plasmidial or chromosomal by using plasmid curing, which is clean the bacteria from any plasmid.



**Figure (1)** Agarose gel electrophoresis of PCR amplified products of chromosomal DNA genes using species-specific PCR primer sets interested in this study. Lanes of genes are examined *P. mirabilis* isolate NO.23 . Lane L, 1350 bp (ladder).

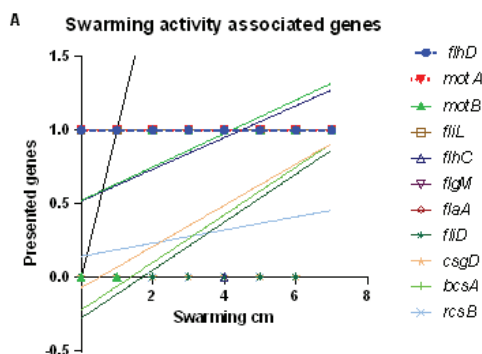


**Figure (2)** Agarose gel electrophoresis of PCR amplified products of plasmidial DNA genes using species-specific PCR primer sets interested in this study. Lanes of genes are examined *P. mirabilis* isolate NO.23 . Lane L, 1350 bp (ladder).

Correlation of *P. mirabilis* swarming activity and 8 genes

The relation between swarming activity and *flhC*, *flhD*, *motA*, *motB*, *fliL*, *flgM*, *flaA* & *fliD* genes were conducted in different isolates of *P. mirabilis*. The results showed there are a good correlation between *flhC*, *flhD*, *motA*, *motB*, *fliL*, *flgM*, *flaA* & *fliD* with

swarming activity. as demonstrated in Figs (3A and B). Many studies are investigated the correlations between virulence genes and swarming. For example, study has been shown that *fliF*, *motA*, *motB*, *flhC*, *fliG*, and *fliM* genes of *Salmonella enterica* were related with swarming [19]. However, not only these genes have a role in swarming, but also *wosA*, *flaA*, *flhD*, *flgM* and *fliL* genes have a role in this phenomenon [20]



Genes	R-square	P-value	
<i>flhD</i>	1	0.000	*****
<i>motA</i>	1	0.000	*****
<i>motB</i>	0.3552	0.0011	**
<i>fliL</i>	1	0.000	*****
<i>flhC</i>	0.2461	0.0089	**
<i>flaM</i>	1	0.000	*****
<i>flaA</i>	1	0.000	*****
<i>fliD</i>	0.2412	0.0004	**

Figure (3 A and B) Relationship between swarming activity and associated genes.

Swarming is totally positively correlated with *flhD*, *motA*, *flgM* and *flaA* (R-square 1, Kruskal–Wallis). Swarming is also positively correlated with *motB*, *flhC* and *fliD* genes (P values were between 0.001-0.01). The significant differences between all genes were tested by using ozone correlation test in Graphpad prism-8 program. Blue means direct role in swarming, green means indirect role in swarming activity.

Genes transferring

This step was established to detect which gene can transfer from *P. mirabilis* to competent bacterial cells using the horizontal gene transfer method,

transformation. It was accomplished using *E. coli* S17λ competent cells and isolates *P. mirabilis* donor cells. *E. coli* S17λ competent cells because one of them only used in transformation based on the gene profile of these competent bacterial cells. Briefly, for transformation, we used heat shock method based on antibiotics resistance marker. It was purified the *E. coli* S17λ based on whether successfully resisted to antibiotics and received the plasmid from *P. mirabilis*. The results appear that both methods were shown that only 1 gene are transferred to *E. coli* S17λ from the DNA plasmid extracted from *P. mirabilis*. In addition *P. mirabilis* gene can also transfer to *E. coli* S17λ during transformation process, which were included: *fliD*. Fig (4).

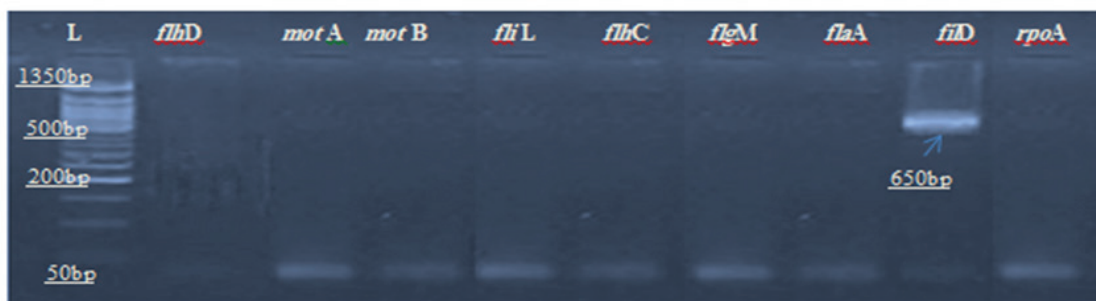


Figure (4) Agarose gel electrophoresis of PCR amplified products of plasmidial DNA gene using species-specific PCR primer sets interested in this study. Lanes of genes are examined *E. coli* S17λ competent strain after transformation. Lane L, 1350 bp (ladder).

## Conclusions

*P. mirabilis* can form swarming and attach to Uroepithelial cells strongly. From 8 different genes, which have a role in swarming, only 1 gene could be expressed in plasmid profile, and transfer to standard strain.

**Declaration:** The authors have no conflict of interest to declare.

**Ethical statement:** This article does not contain any studies with human participants or animals performed by any of the authors.

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## References

1. Torzewska, A. and A. Róźalski, Various intensity of *Proteus mirabilis*-induced crystallization resulting from the changes in the mineral composition of urine. *Acta Biochimica Polonica*, 2015. 62(1).
2. Feneley, R.C., I.B. Hopley, and P.N. Wells, Urinary catheters: history, current status, adverse events and research agenda. *Journal of medical engineering & technology*, 2015. 39(8): p. 459-470.
3. Jamal, M., et al., Bacterial biofilm and associated infections. *Journal of the Chinese Medical Association*, 2018. 81(1): p. 7-11.
4. Seo, S.-U., et al., Distinct commensals induce interleukin-1 $\beta$  via NLRP3 inflammasome in inflammatory monocytes to promote intestinal inflammation in response to injury. *Immunity*, 2015. 42(4): p. 744-755.
5. Al-Mayahi, F.S.A., Phenotypic and Molecular detection of Virulence factors in *Proteus mirabilis* isolated from different clinical sources. *Bas. J. Vet. Res*, 2017. 16(1).
6. Armbruster, C.E. and H.L. Mobley, Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nature Reviews Microbiology*, 2012. 10(11): p. 743-754.
7. Lee, C. and C. Park, Mutations upregulating the flhDC operon of *Escherichia coli* K-12. *Journal of Microbiology*, 2013. 51(1): p. 140-144.
8. Cusick, K., et al., Perturbation of FliL interferes with *Proteus mirabilis* swarmer cell gene expression and differentiation. *Journal of bacteriology*, 2012. 194(2): p. 437-447.
9. Maisuria, V.B., Z. Hosseinidoust, and N. Tufenkji, Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. *Applied and environmental microbiology*, 2015. 81(11): p. 3782-3792.
10. Pesavento, C., et al., Inverse regulatory coordination of motility and curli-mediated adhesion in *Escherichia coli*. *Genes & development*, 2008. 22(17): p. 2434-2446.
11. Che, Y.S., et al., Load-sensitive coupling of proton translocation and torque generation in the bacterial flagellar motor. *Molecular microbiology*, 2014. 91(1): p. 175-184.
12. Belas, R., Biofilms, flagella, and mechanosensing of surfaces by bacteria. *Trends in microbiology*, 2014. 22(9): p. 517-527.
13. Lenahan, M., et al., Transcriptomic analysis of triclosan-susceptible and-tolerant *Escherichia coli* O157: H19 in response to triclosan exposure. *Microbial Drug Resistance*, 2014. 20(2): p. 91-103.
14. Heo, M., Functional dynamics of the bacterial flagellar motor driven by fluorescent protein tagged stators and by evolutionary modified foreign stators. 2016.
15. El-Hamid, M.I., et al., Genetic Diversity of *Campylobacter jejuni* Isolated From Avian and Human Sources in Egypt. *Frontiers in microbiology*, 2019. 10: p. 2353.
16. Stevenson, L.G. and P.N. Rather, A novel gene involved in regulating the flagellar gene cascade in *Proteus mirabilis*. *Journal of bacteriology*, 2006. 188(22): p. 7830-7839.
17. El-Baghdady, K.Z., et al., Plasmid mediated virulence factors of some *Proteus* isolates. *Egyptian Academic Journal of Biological Sciences, G. Microbiology*, 2009. 1(1): p. 7-22.
18. Kojima, S. and D.F. Blair, The bacterial flagellar motor: structure and function of a complex molecular machine. *International review of*

- cytology, 2004. 233: p. 93-135.
19. Komatsu, H., et al., Genetic analysis of revertants isolated from the rod-fragile *fliF* mutant of *Salmonella*. 2016. 13: p. 13-25.
  20. Verstraeten, N., et al., *Living on a surface: swarming and biofilm formation*. 2008. **16**(10): p. 496-506.