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Original article

Prevalence of twitching motility in *Pseudomonas aeruginosa* isolated from medical facilities

—— Prevalence of TM in *P. aeruginosa* isolated from medical facilities ——

Yuji NAKADA *Associate professor, Department of Nursing, Faculty of Nursing and Rehabilitation, Aino University*

Tomoyo MATSUSHITA *Graduate student of Osaka Medical College*

Abstract

Pseudomonas aeruginosa is an opportunistic bacterial pathogen, the infection system of which includes the possession of type IV pili. Type IV pili-dependent twitching motility (TM) is important for attachment to the epithelium and biofilm formation. Accordingly, there is a close relationship between the expression of pili and infection through intermediate biofilm formation. However, not all isolated strains of *P. aeruginosa* carry the pili-motility function. In this study, we isolated *P. aeruginosa* strains from various medical facilities and determined the TM prevalence. A total of 27 strains were isolated from bathrooms and nursing station sinks, 52% of which possessed clear TM ability. The strength of the TM ability differed by strain, with 15% indicating a marked ability. These results support the possibility that some of *P. aeruginosa* strains isolated from medical facilities can act as causative organisms of infection.

Key words : *Pseudomonas aeruginosa*, pili, twitching motility

INTRODUCTION

Pseudomonads are prevalent opportunistic human pathogens that cause acute pneumonia, cystic fibrosis, and septicemia in immunocompromised individuals (Govan *et al.*, 1996). In addition, their natural low-level susceptibility to antimicrobial agents, accumulated resistance after exposure to various antibiotics, and the cross-resistance between agents have resulted in multi-drug resistant strains (Aloush *et al.*, 2006). In the *Pseudomonas* genus, *P. aeruginosa* (Stover CV *et al.*, 2000) is a well-known cause of pneumonia, with high rates of fatality in senior and long-term inpatients. *P. aeruginosa* is a gram-negative bacterium living in soil and aqueous environments, where it survives due to its environmental adaptations, such as it being able to use a variety of nutrients and its ability to form a biofilm through highly developed gene expression and regulation systems (Nishijyo *et al.*, 2001).

Biofilms are composed of surface-associated communities of sessile bacteria embedded in a polysaccharide matrix (Hall-Stoodley *et al.*, 2004), and their characteristic resistance to antibiotics and host immune responses compromise infection control (Drenkard E, 2003). Biofilm development has been well characterized in *P. aeruginosa*, which comprises surface attachment, microcolony formation, and differentiation into a mature antibiotic-resistant population encased in an extracellular polymeric matrix (Costerton *et al.*, 1995). In the early and multiple steps of biofilm formation, flagellar-based swimming and type IV pili-dependent twitching motility (TM) are important for attachment to abiotic surfaces and the epithelium (O'Toole *et al.*, 1998; Dorr *et al.*, 1998). As a result, there is a close relationship between pili expression and infection through intermediate biofilm formation. However, not all isolated strains of *P. aeruginosa* carry the pili-motility function, and

the distribution of TM among different strains has yet to be examined. In the present study, we performed the screening of *P. aeruginosa* in medical facilities populated by a large number of long-term inpatients, and assessed the prevalence of TM in the isolated strains.

MATERIALS AND METHODS

Bacterial strains and growth conditions

The standard strains and plasmids used are listed in Table 1. *Escherichia coli* and *P. aeruginosa* strains were respectively cultured in Luria-Bertani (LB) and nutrient yeast broth (NYB) supplemented with antibiotics when appropriate (Hass *et al.*, 1977; Sambrook *et al.*, 1989). The standard strains of *P. aeruginosa* (PAO1, ATCC33348, ATCC33350, ATCC 33351, ATCC33353, ATCC33354, ATCC 33356, ATCC33357, ATCC33358, ATCC 33359, and ATCC33360) were isolated from patients.

DNA techniques

DNA purification, restriction enzyme analysis, and other DNA manipulations were conducted as previously described (Itoh 1997; Sambrook *et al.*, 1989). PCR was performed with KOD DNA polymerase (Toyobo Biochemicals, Japan) under the reaction conditions recommended by the supplier, and nucleotides were sequenced using an ABI3130 DNA sequencer and Big-Dye Terminator sequencing kit (Applied Biosystems, USA), as described previously (Nakada & Itoh, 2002).

Isolation of sample strains from medical facilities

Strains were collected from core hospitals with a large number of senior long-term inpatients (1,300 beds) in the Kansai region of Japan in 2007–2008. The isolated samples were obtained from nursing station sinks and bathrooms using pseudomonad-selective NAC agar plates for environmental microorganism sampling (Eiken Kagaku, Japan). After a 24-hr incubation at 37℃, the strains which formed colonies of more than 1 mm in diameter were selected for the following experiments.

Identification of isolated pseudomonads

Isolates grown on the NAC agar plates included a few other species in addition to the genus *Pseudomonas*. Therefore, amplification and detection using *oprI* and *oprL* lipoprotein genes were conducted to identify *Pseudomonas* and *P. aeruginosa* (De VD *et al.*, 1993, 1997). Positive amplification of the *oprI* gene indicated the genus *Pseudomonas*, while *oprI* and *oprL* genes identified *P. aeruginosa*. For further confirmation and species identification, the analysis of 16SrRNA gene sequences was performed. The primers used for PCR are presented in Table 2.

Sequence determination of 16SrRNA gene sequences

The nearly complete sequence (corre-

Table 1 Strains and the plasmid used in this study

Strains & plasmid	Relevant characteristics	Reference or source
Strains		
P. aeruginosa		
PAO1	wild type	Stover CV *et al.* 2000
ATCC33348	wild type	Veron M *et al.* 1961
ATCC33350	wild type	Veron M *et al.* 1961
ATCC33351	wild type	Veron M *et al.* 1961
ATCC33353	wild type	Veron M *et al.* 1961
ATCC33354	wild type	Veron M *et al.* 1961
ATCC33356	wild type	Veron M *et al.* 1961
ATCC33357	wild type	Veron M *et al.* 1961
ATCC33358	wild type	Veron M *et al.* 1961
ATCC33359	wild type	Veron M *et al.* 1961
ATCC33360	wild type	Veron M *et al.* 1961
E. coli		
DH5α	*F-/endA1 hsdR17 (rk−, mk+) supE44 thi-1 recA1 gyrA relA1 D(lacIZYA-argF)U169 deoR(F80dlacD(lacZ)M15)*	Bethesda Research Laboratories
Plasmid		
pUC118	ApR, ColE1 replicon	Vieira et al. 1987

Table 2 PCR primers, their target genes and annealing temperatures

Primer	Oligonucleotide sequence (5'-3')	Annealing temperature (℃)	Target and size of the expected product (bp)
SP1 SP2	ATGAACAACGTTCTGAAATTCTCTGCT CTTGCGGCTGGCTTTTTCCAG	57	*oprI*, 249
PAL1 PAL2	ATGGAAATGCTGAAATTCGGC CTTCTTCAGCTCGACGCGACG	56	*oprL*, 504
UFPL URPL	AGTTTGATCCTGGCTCAG GGTTACCTTGTTACGACTT	56	16SrRNA gene, 1490

Table 3 TM ability of isolated and standard strains of *P. aeruginosa*

TM zone (mm)	Number of isolated strains	Number of standard strains
0～5	13	3
6～20	10	2
21～	4	6

sponding to positions 9 to 1,500 in the *E. coli* numbering system) of the 16SrRNA gene of pseudomonads was amplified by employing PCR with the conserved primers UFPL (AGTTTGATCCTGGCTCAG) and URPL (GGTTACCTTGTTACGACTT) (Table 2). The amplified fragment was cloned into plasmid pUC118 at the *Hinc*II site to verify the nucleotides by sequencing. The sequencing primers used were UFPL, URPL, 16SF1 (5'-GCCTTCGGGTTGTAAAGCAC-3'), 16SF2 (5'-CCTTACCTACCCTTGACA- 3'), 16 SB 1 (5'-GCGCTCGTTGCGGGACT-3'), and 16SB2 (5'-GTATTACCGCGGCTGCTG- 3') (Lixia Liu, 2002). Sequence assembly was performed using ATGC Ver. 5 (Genetyx Corporation, USA).

TM assay

The isolated and standard strains of *P. aeruginosa* colonies were stab-inoculated in 2 -mm-thick LB agar on an underlying Petri dish using a sterilized toothpick (Alm RA *et al.*, 1995). After incubation at 37℃ for 24 hr, the zone of motility at the agar/Petri dish interface (TM zone) was measured (Fig. 1).

Results

Bacterial isolation and identification of pseudomonads

A total of 83 strains were obtained from nursing station sinks and bathrooms in the examined medical facilities. Through the *oprI* target PCR assay, 73 isolates were identified as *Pseudomonas*, while a further *oprL* target PCR assay revealed 27 isolates of *P. aeruginosa*. The isolated *Pseudomonas* strains were also examined using 16SrRNA gene sequencing, revealing three typical strains: *P. aeruginosa, P. alcaligenes*, and *P. mendocina*.

TM prevalence in the isolated *P. aeruginosa* strains

We examined the TM ability of all strains identified as *P. aeruginosa*. As shown in Table 3, 14 strains showed a clear TM zone (more than 6 mm), with the overall TM rate being 52%.

TM prevalence in standard strains of *P. aeruginosa*

The TM ability of 11 standard strains was then determined. As shown in Table 3, 8 strains showed a clear TM zone, with the overall TM rate being 73%.

DISCUSSION

In this investigation, we surveyed nursing station sinks and bathrooms in medical facilities populated by a large number of senior long-term inpatients for strains of the genus *Pseudomonas*. The dominant isolated strains were identified as *P. aeruginosa, P. mendocina*, and *P. alcaligenes*, all of which are known to be opportunistic pathogens. Due to their source, they are also believed to be causative organisms of nosocomial infection. The main investigations in the past involved detection and antibiotic sensitivity tests of bacteria found on inpatients, healthcare workers, visitors, and in medical facilities. However, few close pathogenic factor investigations of resident environmental flora in medical facilities have been reported.

The isolated and identified *Pseudomonas* strains (especially *P. aeruginosa*) this time are all causative bacteria of opportunistic infections, and, thus, analysis of the pathogenic factors is particularly significant. One pathogenic factor of *P. aeruginosa* is the possession of pili, which are involved in biofilm formation and are thought to play an important role in human infection (George *et al.*, 1998). The TM ability resulting from type IV pili varies by strain, and the biogenesis and function of type IV pili are controlled by a large number of genes, almost 40 of which have been identified in *P. aeruginosa* (Jhon SM, 2002). Strains with defective pili or a lack of expression do not show apparent TM. Moreover, during colonization and multiplication on human tissue, strains showing high TM ability are more likely to cause infection (George *et al.*, 1998; Maria-Cristina *et al.*, 1994).

In the present study, we therefore focused on pili motility, revealing that about half of the medical facility-derived *P. aeruginosa* strains possessed clear TM ability. Three-quarters of the standard *P. aeruginosa* strains isolated from patients possessed clear TM ability. Furthermore, approximately 15% of the medical facility-derived strains and more than half of the standard strains isolated from patients showed a particularly marked TM ability (more than a 21-mm TM zone). These results support the possibility that the strains exhibiting high TM ability are more likely to cause infection (George *et al.*, 1998; Maria-Cristina *et al.*, 1994). Although TM ability is only related to attachment to the epithelium, we should be aware that a marked TM ability is showing by strains in medical facilities. In the future, we plan to investigate the relationship between pili expression in *P. aeruginosa* and the presence of long-term resident in medical facilities.

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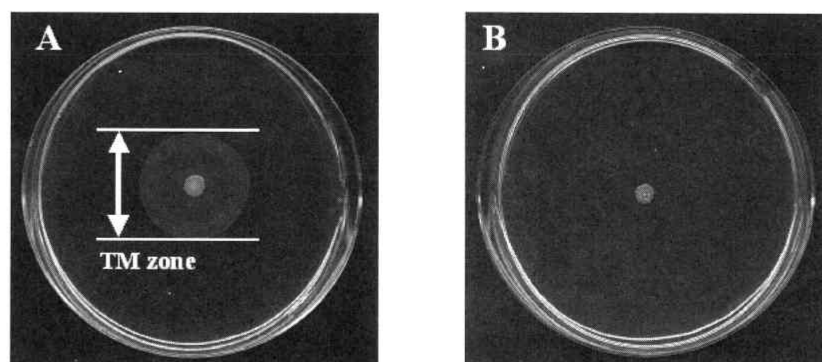

Fig. 1 Results of the TM assay with isolated and standard *P. aeruginosa* strains
A: TM assay of ATCC33358. TM zone=29mm.
B: TM assay of ATCC33356. TM zone=0mm.

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