

Phenotypic and genetic characterization of successive *Pseudomonas aeruginosa* isolates obtained from the same cystic fibrosis patient

C. Lozano¹, J. M. Azcona-Gutiérrez², F. Van Bambeke³, Y. Saénz¹

¹Centro de Investigación Biomédica de La Rioja (CIBIR), Logroño, Spain; ²Departamento de Diagnóstico Biomédico. Laboratorio de Microbiología, Hospital San Pedro, Logroño, Spain;

³Pharmacologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium.

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BACKGROUND

Pseudomonas aeruginosa (PA) is the major causing agent of infections in cystic fibrosis (CF) patients. Different adapted morphotypes are found in chronic infections.

Objective: to characterize PA successively isolated from the same CF patient over a 4-years period (2012-2015).

MATERIAL/METHODS

- **17 PA isolates:** 5 small colony variant (SCV) and 12 mucoid.
- **Molecular typing:** PFGE and MLST^{1,2}.
- **Antimicrobial susceptibility:** to 15 antibiotics was performed by disk-diffusion; AmpC hyperproduction by phenotypic test, and efflux activity was investigated using PAβN²⁻⁴.
- **Antimicrobial resistance mechanisms:** alterations in porin OprD and integron structures were determined by PCR and sequencing; the expression of *oprD* and *ampC* genes by RT-qPCR^{2,5}.
- **Virulence genes:** the presence and expression of virulence genes were studied by PCR and RT-qPCR^{2,9}.
- **Phenotypic assays:** generation times were determined by growth curves; capacity to form biofilms by CV staining (biomass) and FDA assay (metabolic activity); elastase activity, pyocyanin/pyorubin production and motility were also determined¹⁰⁻¹³.

RESULTS

- ✓ All isolates had closely related PFGE patterns and belonged to ST412.
- ✓ Antimicrobial resistance and molecular characterization of porin OprD are shown in Table 1. AmpC hyperproduction was detected in all isolates. PAβN increased susceptibility to ciprofloxacin in all isolates, and to imipenem only among SCVs. Two class 1 integrons were detected (Fig 1).
- ✓ All isolates amplified *exoS*, *exoY*, *exoT*, *exoA*, *lasA*, *lasB*, *aprA*, *rhlAB*, *rhlI*, *rhlR*, *lasI*, and *lasR* genes.
- ✓ Growth and phenotypic assay results are shown in Fig 2 and Fig 3, and expression of studied genes in Table 2.

Table 1. Antimicrobial resistance and characterization of OprD

Isolates (No.)	Resistance phenotype ^a	OprD size	OprD pattern ^b
SCV 5	IMP, DOR, CAZ ^I , PIP ^I , FEP ^I , TIC ^I , TZP ^I , NET, GEN ^I	189	A
Mucoid 9	susceptible	441	B
2	IMP ^I	441	C
1	IMP ^I , CAZ ^I , PIP ^I , FEP ^I , TIC ^I , TZP ^I	326	D

^aIMP:imipenem;DOR:doripenem;CAZ:ceftazidime;PIP:piperacillin;FEP:cefepime;TIC:ticarcillin; TZP:piperacillin-tazobactam;NET:netilmicin;GEN:gentamicin, I: intermediated resistance.

^bA:D43N, S57E, S59R (Deletion of 11 bp at codon 130; B:D43N, S57E, S59R, E202Q, I210A, E230K, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L (L7 short); C:L11Q, D43N, S57E, S59R, E202Q, I210A, E230K, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G, V359L (L7 short);D:D43N, S57E, S59R, E202Q, I210A, E230K, S240T, N262T, A267S, A281G, K296Q, Q301E, R310G

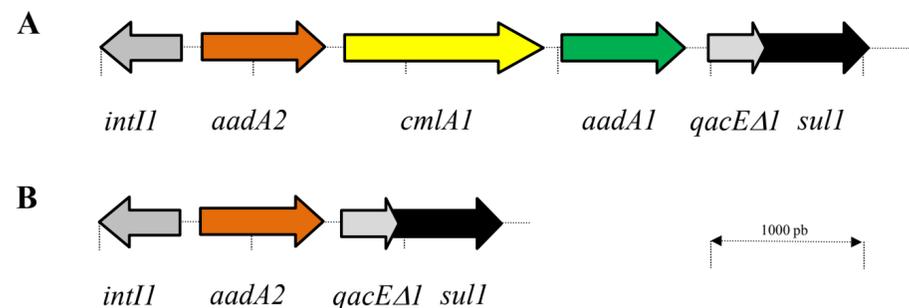


Fig 1. A) new integron (In1342) found in 7 isolates; B) integron found in all but one isolate.

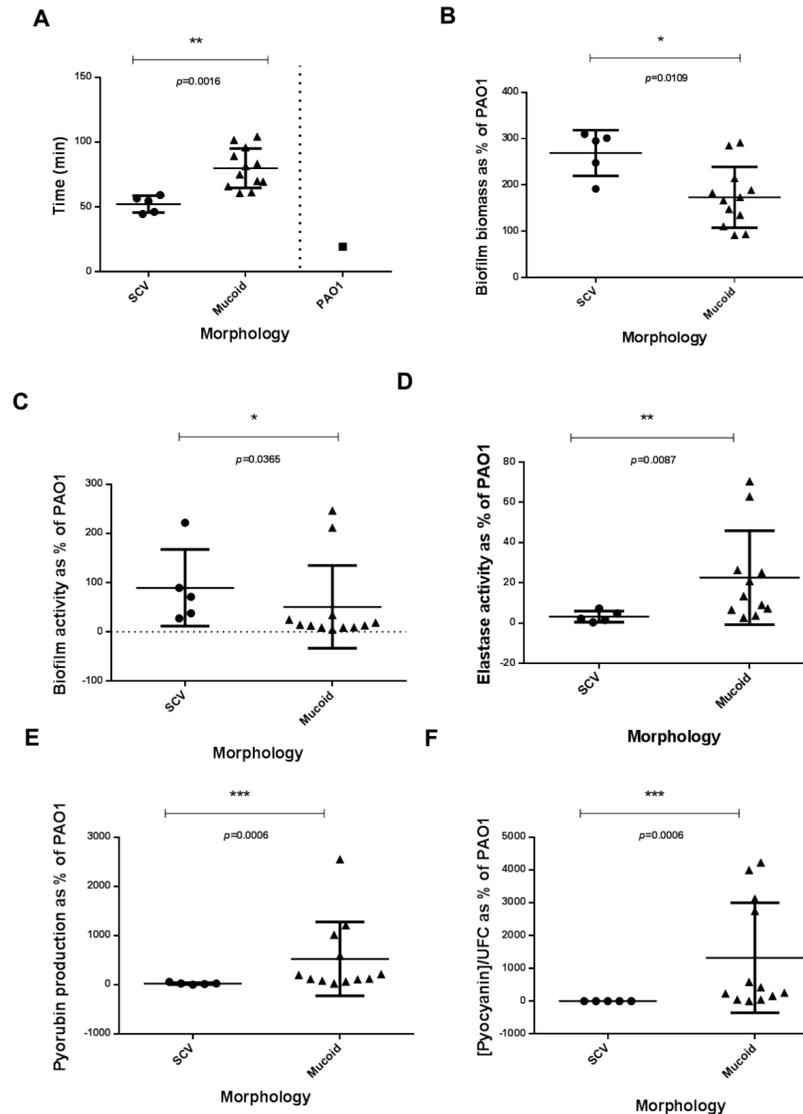


Fig 2. A) Generation times; B) Biofilm biomass; C) Metabolic activity of biofilm; D) Elastase assay; E) Pyorubin assay; F) Pyocyanin assay.

CONCLUSIONS

- All isolates showed the same ST and closely related PFGE patterns; however important phenotypic and genotypic differences were found among them.
- Two main groups (SCV and mucoid) were identified.
- The adaptation and persistence of PA during chronic infections result in numerous variants which can complicate the treatment and diagnosis of CF patients.

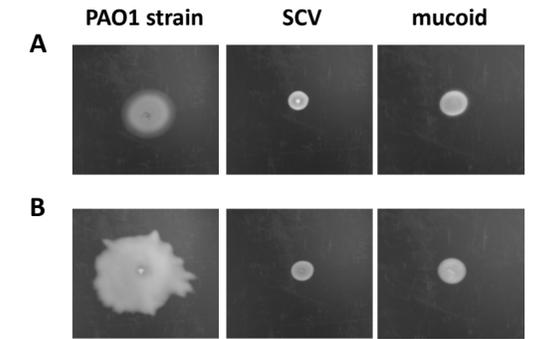


Fig 3. A) Swimming motility; B) Swarming motility.

Table 2. mRNA expression ($2^{-\Delta\Delta Ct}$)

Gene	SCV	Mucoid
<i>ampC</i>	702.4±394.3	1170.9±1220.4
<i>oprD</i>	0.5±0.2	1.8±0.7
<i>algD</i>	3.3±1	11029.7±5790.3
<i>rhlR</i>	0.9±0.5	1.6±0.6
<i>lasR</i>	5.9±2.9	7.2±4
<i>lasB</i>	0.4±0.2	0.7±0.3
<i>pslA</i>	2.4±1.7	2.3±0.8
<i>pelA</i>	1.9±0.8	1.9±1.7
<i>exoS</i>	1.7±0.5	2±0.6
<i>exoT</i>	4.6±1.6	6.5±2.4
<i>pcrV</i>	0.5±0.1	0.6±0.2
<i>popB</i>	0.3±0.1	0.4±0.1
<i>popD</i>	0.8±0.5	1±0.3
<i>fliC</i>	2.3±1.6	0.2±0.3

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