Incorporation of *Pseudomonas aeruginosa* into drinking-water biofilms on elastomeric material

Bressler, D., Balzer, M., Dannehl, A., Flemming, H.-C., Wingender, J.

Biofilm Centre, Aquatic Microbiology
University of Duisburg-Essen, Germany

Introduction

- Biofilms in water distribution systems are supposed to be possible reservoirs for the opportunistic pathogen *Pseudomonas aeruginosa*.

- The aim of the study was to investigate the potential of *P. aeruginosa* to become incorporated into drinking-water biofilms on ethylene propylene diene monomer (EPDM), which represents an elastomeric plumbing material with a tendency to support biofilm formation.

Conclusions

- *P. aeruginosa* can colonize established mixed-population drinking-water biofilms and persist there for at least 7 days under stagnant and 4 weeks under flow conditions, respectively.

- From a health perspective, these biofilms can provide a reservoir for *P. aeruginosa* and represent a source of contamination in water distribution systems.

Methods

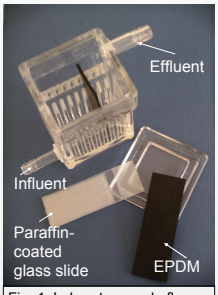

Fig. 1: Laboratory-scale flow-through reactor

Characterization of water and biofilms:
- total cell count: microscopic enumeration of DAPI-staind bacteria
- heterotrophic plate count: colony-forming units (cfu) on R2A medium, 20°C, 7 d
- *P. aeruginosa*: cfu on CN or CFC ager, 36°C, 2d
- Biofilm architecture: confocal laser-scanning microscopy (CLSM)
- Bacterial diversity: 16S rDNA-directed polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE)

Results

Drinking-water biofilms on EPDM and paraffin (material known to support biofilm growth) were in a quasi-stationary state after 14 d under flow conditions

Characterisation of these biofilms:

- Heterogenous architecture visualized by CLSM (Fig. 2)
- Total cell counts: in the range of 10^8 to 10^9 cells/cm^2
- Viable cell count (HPC): approximately 1 log unit lower than total cell counts
- Uniformity of coupon populations determined by PCR-DGGE: Almost identical in biofilms on EPDM couponds at different positions of the same reactor run, and an average similarity of 71 % in EPDM biofilms of separate reactor runs (Fig. 3)

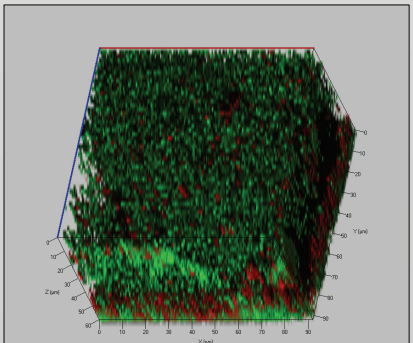

Fig. 2: 3D-CLSM image of 14 d-old biofilm on EPDM (1000x). Biofilms were staind using SYTO 9 (green) and propidium iodide (red). [Live/Dead-Kit, Molecular Probes]

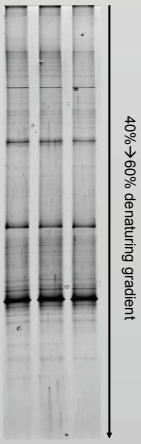

Fig. 3: DGGE-profiles of PCR-amplified 16S rDNA fragments derived from three 14 d-old biofilms on EPDM couponds at different positions in a flow-through reactor.

Number of bands: 68

Results

- Under flow conditions, *P. aeruginosa* became incorporated into the biofilms both on EPDM (Fig. 4) and paraffin surfaces (Fig. 5), persisted for 4 weeks (termination of experiments) in the biofilms with a slow decline in culturable numbers and could be detected in the water phase during this period.

- Under stagnant conditions, *P. aeruginosa* became incorporated into the biofilms on EPDM and persisted for 7 days (termination of experiments) at high culturable levels in the biofilms (Fig. 6).

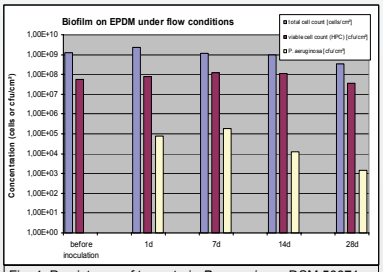

Fig. 4: Persistence of type strain *P. aeruginosa* DSM 50071 in drinking-water biofilms on EPDM surfaces under flow conditions

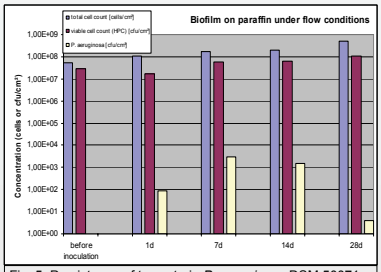

Fig. 5: Persistence of type strain *P. aeruginosa* DSM 50071 in drinking-water biofilms on paraffin-coated surfaces under flow conditions

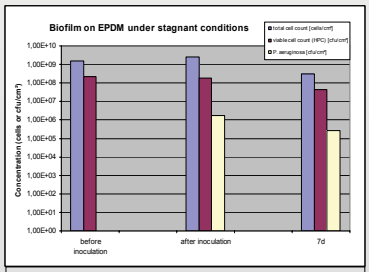

Fig. 6: Persistence of type strain *P. aeruginosa* DSM 50071 in drinking-water biofilms on EPDM surfaces under stagnant conditions

- Incorporation and persistence was observed, independent of the genotype or phenotype of several *P. aeruginosa* strains: mucoid/non-mucoid, deficient in lectins LecA and LecB supposed to be involved in biofilm formation (Table).

Table: Persistence of different *P. aeruginosa* strains in drinking-water biofilms; n. d., not determined

Strain	Genotype/phenotype	Persistence under stagnant conditions (7 d)	Persistence under flow conditions (28 d)
DSM 50071	Type strain	+	+
SG81	Mucoid (alginate-producing) environmental strain	+	+
SG81/R1	Non-mucoid revertant, derived from SG81	+	n. d.
FRD1	Mucoid clinical strain	+	n. d.
PAO1	Wild-type	+	n. d.
PATI2	LecB mutant derived from PAO1	+	n. d.
PATI5	Lec AB mutant drived from PAO1	+	n. d.