Supplementary Material

Parallel evolution of enhanced biofilm formation and phage-resistance in *Pseudomonas aeruginosa* during adaptation process in spatially heterogeneous environments

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Supplementary Figure S2 (Yamamoto et al.)



Supplementary figure S2. Transition of colony morphology of PA in the course of evolution experiment under spatiallyhomogeneous conditions. The evolution experiment of PA pure culture was conducted in shaken test tube using same culture conditions as the experiment under static conditions except for continuous agitation (200 rpm). The culture broth at 1st, 5th, 10th, and 15th round of passages were streaked on TSB plates and then incubated at 30°C. Colony morphology was checked after 18~36h incubation. N = 20. Colony of replicate No. 1 and 11 are shown as representatives.

Replicate No.

11



Supplementary Figure S3 (Yamamoto et al.)

Supplementary figure S3. Detection of replication-form DNA of Pf4. **a)** Position of Pf4 in the PAO1 genome. Green and brown bars indicate regions used for sequence alignment. **b)** PCR analysis was performed with primer sets (arrows in a) specifically amplifying replication-form DNA of Pf4: forward (5'-AGCAGCGCGATGAAGCAAT-3') & reverse (5'-TAGAGGCCATTTGTGACTGGA-3') (Webb et al., 2004. J. Bacteriol. 186, 8066–8073). The expected PCR product size was 839-bp. Templates were the heat-treated culture filtrates (0.22um; 100°C, 15 min). **c)** Sequence alignment of the PCR products and Pf4 region of PAO1 genome. Pf4-2-12, Pf4-10-4, and Pf4-12-22 corresponds to P-7-12, PN-10-4, and PM-9-22 in b, respectively. Pf4RFa and PF4RFb correspond to the regions indicated in a. Sequencing primer for the PCR products was the forward primer used for PCR amplification. Sequence alignment was performed with CLC Main Workbench (Qiagen).

Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	GCCAGTACGC GCCAGTACGC GCCAGTACGC	AGAAGGCAAG AGAAGGCAAG AGAAGGCAAG AGAAGGCAAG	CATCGCTCTG CATCGCTCTG CATCGCTCTG CATCGCTCTG	TTGATGATTT TTGATGATTT TTGATGATTT TTGATGATTT	GAAAGCTCGC GAAAGCTCGC GAAAGCTCGC GAAAGCTCGC	CTTTCCAGGA CTTTCCAGGA CTTTCCAGGA CTTTCCAGGA	GGTTCGCTCA GGTTCGCTCA GGTTCGCTCA GGTTCGCTCA	GCCAGAATAA GCCAGAATAA GCCAGAATAA GCCAGAATAA	30 30 30 71
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	GGAGGTTTAA GGAGGTTTAA GGAGGTTTAA GGAGGTTTAA	TGTCCCCGGT TGTCCCCGGT TGTCCCCGGT TGTCCCCGGT	CGTCATTCGT CGTCATTCGT CGTCATTCGT CGTCATTCGT	TTTACTGATA TTTACTGATA TTTACTGATA TTTACTGATA	CCGCAGAGCA CCGCAGAGCA CCGCAGAGCA CCGCAGAGCA	AAGCATCGAA AAGCATCGAA AAGCATCGAA AAGCATCGAA	GACCAAGTCC GACCAAGTCC GACCAAGTCC GACCAAGTCC	ACCACTTGGC 1 ACCACTTGGC 1 ACCACTTGGC 1 ACCACTTGGC 1	160 160 160 151
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	TCCATTCCAA TCCATTCCAA TCCATTCCAA TCCATTCCAA	GGTGAACAGG GGTGAACAGG GGTGAACAGG GGTGAACAGG	CTGCACTCCA CTGCACTCCA CTGCACTCCA CTGCACTCCA	GTCAGTACTG GTCAGTACTG GTCAGTACTG GTCAGTACTG	AGCCTTTTGG AGCCTTTTGG AGCCTTTTGG AGCCTTTTGG	ATGAGATTGA ATGAGATTGA ATGAGATTGA ATGAGATTGA	AGAGAAGATT AGAGAAGATT AGAGAAGATT AGAGAAGATT	TCACTTGCAC 2 TCACTTGCAC 2 TCACTTGCAC 2 TCACTTGCAC 2	240 240 240 231
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	CTAAAGGTTA CTAAAGGTTA CTAAAGGTTA CTAAAGGTTA	CCCAGTCAGC CCCAGTCAGC CCCAGTCAGC CCCAGTCAGC	CAGCAGGCGA CAGCAGGCGA CAGCAGGCGA CAGCAGGCGA	GTCTTCTGGG GTCTTCTGGG GTCTTCTGGG GTCTTCTGGG	GGTGCTGAGC GGTGCTGAGC GGTGCTGAGC GGTGCTGAGC	TATCGCGAGC TATCGCGAGC TATCGCGAGC TATCGCGAGC	TTAATACCGG TTAATACCGG TTAATACCGG TTAATACCGG	CCCCTATCGT 3 CCCCTATCGT 3 CCCCTATCGT 3 CCCCTATCGT 3	320 320 320 311
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	GTTTTTTACG GTTTTTTACG GTTTTTTACG GTTTTTTACG	AATTCCACGA AATTCCACGA AATTCCACGA AATTCCACGA	AGAGCAAGGC AGAGCAAGGC AGAGCAAGGC AGAGCAAGGC	GAGGTGGCAG GAGGTGGCAG GAGGTGGCAG GAGGTGGCAG	TGATCTTGGT TGATCTTGGT TGATCTTGGT TGATCTTGGT	TTTGCGACAG TTTGCGACAG TTTGCGACAG TTTGCGACAG	AAGCAGAGCG AAGCAGAGCG AAGCAGAGCG AAGCAGAGCG	TTGAGCAGCA 4 TTGAGCAGCA 4 TTGAGCAGCA 4 TTGAGCAGCA 3	100 100 100 391
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	ATTGATCCGC ATTGATCCGC ATTGATCCGC ATTGATCCGC	TACTGCTTGG TACTGCTTGG TACTGCTTGG TACTGCTTGG	TGGGGCCAAT TGGGGCCAAT TGGGGCCAAT TGGGGCCAAT	CGAGTGATGG CGAGTGATGG CGAGTGATGG CGAGTGATGG	CTTTCTACTC CTTTCTACTC CTTTCTACTC CTTTCTACTC	CTGAGCATGT CTGAGCATGT CTGAGCATGT CTGAGCATGT	AGCGCTGAAT AGCGCTGAAT AGCGCTGAAT AGCGCTGAAT	GCGCCTCGAC 4 GCGCCTCGAC 4 GCGCCTCGAC 4 GCGCCTCGAC 4	180 180 180 171
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	ACTTCTTCGA ACTTCTTCGA ACTTCTTCGA ACTTCTTCGA	CACCTTTCCT CACCTTTCCT CACCTTTCCT CACCTTTCCT	TCCCCCAAAA TCCCCCAAAA TCCCCCAAAA TCCCCCAAAA	AGCAAAGCCC AGCAAAGCCC AGCAAAGCCC AGCAAAGCCC	CCGAAACGCT CCGAAACGCT CCGAAACGCT CCGAAACGCT	AGGCATTTCA AGGCATTTCA AGGCATTTCA AGGCATTTCA	GGGGCTTG-G GGGGCTTG-G GGGGCTTG-G GGGGCTTTG-G GGGGCTTTTG	CAGGGTGATT CAGGGTGATT CAGGGTGATT CAGGGTGATT CATTGGGATC	359 559 559 550 23
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	TGGAGCGGGC TGGAGCGGGC TGGAGCGGGC TGGAGCGGGC	GAAGGGAATC GAAGGGAATC GAAGGGAATC GAAGGGAATC	GAACCCTTTA GAACCCTTTA GAACCCTTTA GAACCCTTTA	AAATAGGGTT AAATAGGGTT AAATAGGGTT	TTGAAGCGTT TTGAAGCGTT TTGAAGCGTT	CCTATACATT CCTATACATT CCTATACATT	CTAATGCCAC CTAATGCCAC CTAATGCCAC	TGCCTTCGAT TGCCTTCGAT TGCCTTCGAT TGCCTTCGAT	339 339 339 577 103
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFa	TTTTAGGCGT TTTTAGGCGT TTTTAGGCGT	CTTACTCCGT CTTACTCCGT CTTACTCCGT	AGCTCTTCGT AGCTCTTCGT AGCTCTTCGT	GTCATTGATT GTCATTGATT GTCATTGATT	TCGACACATT TCGACACATT TCGACACATT	TTCGACAGGT TTCGACAGGT TTCGACAGGT	CCTGCATGAG CCTGCATGAG CCTGCATGAG	GATTGCATTT 7 GATTGCATTT 7 GATTGCATTT 7	/19 719 719 577
Pf4-2-12 Pf4-10-4 Pf4-12-22 Pf4RFa Pf4RFb	TTATCCTGGT TTATCCTGGT TTATCCTGGT	TGCTTTATGA TGCTNTATGA TGCTNNATGA	TCACCTTATG TCACCTTATG TCACCTTATG	CCAAGACTAG CCAAGACTAG CCAAGACTAG	GAGCAGCTCC GAGCAGCTCC GAGCAGCTCC	TGT 772 769 769 577 233		NAT INVALUE	100



Supplementary figure S4. Transition of diversity. Three diversity index (Margalef's species richness, Shannon's index, and Simpson's index) were calculated in all time points using population structure data. Transition of diversity was separately presented by two independent experiments. Margalef's species richness: d = (S-1)/Log(N); S, the number of species in each sample; N, the number of individuals in each sample. Shannon's index: $H' = SUM(p_i * Log_e(p_i)); p_i$, the proportion of individuals belonging to the ith species in the dataset. Simpson's index: $1-\lambda = 1-SUM(p_i^2)$. Values were median of 3-5 replicates. Calculation was performed with PRIMER 6 software. * time point having statistically significant difference (t-test, p<0.05) with the pure culture lines (P). There was no time point having statistically significant difference among all three culture series (ANOVA, p<0.05).

No. of transfer

Supplementary Figure S5 (Yamamoto et al.)



Supplementary figure S5. Phenotypic features of non-smooth CMVs predominated after Sm dominance. **a**) Susceptibility to temperate phage. Phage solution and its dilution series were spotted onto a lawn of strains indicated. **b**) Twitching motility (48 h). Values are expressed as means for two independent experiments (n=3-6). Error bars indicate SD. N.D., Not detected.