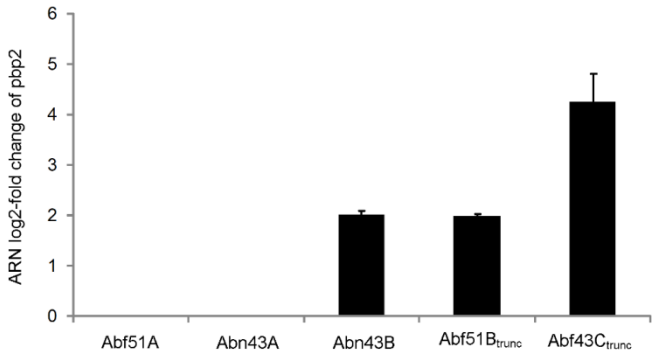

Figure 7: Gene expression analysis. mRNA level from Abf51A, Abn43A; Abn43B, Abf51B$_{trunc}$ and Abf43C$_{trunc}$ in *E. coli*. was quantified by RT-qPCR and normalized to PbP2 mRNA levels.

Material and Method

Analysis of mRNA levels by quantitative RT-qPCR analysis of the G12 metagenomic clone transcripts. The G12 metagenomic clone was cultivated overnight in 10 ml Luria-Bertani medium, supplemented with 12.5 µg/ml chloramphenicol at 37°C under agitation (600 rpm). At the end of the culture, the cells were recovered by centrifugation (5000 rpm, 10 min, 4°C) and frozen at -20°C overnight. After, the frozen cell pellet was suspended in 700 µl RLT buffer (Qiagen) (with β-mercaptoethanol) and transferred to 2 ml Precellys tubes containing Ø 100 µm ceramic beads and lysed two times at 6000 rpm for 30 s using the Precellys 24 instrument (Bertin Technologies). Following lysis, the mixture was centrifuged at 13000 rpm for 2 minutes to remove beads and debris. The supernatant was transferred to fresh 2 ml tubes and an equal volume of 70% ethanol was added. Total RNA was extracted using the RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. A qualitative analysis of RNA was performed by measuring the ratio of absorbance at 260/280 nm and 260/230 nm using a Nanodrop 2000 (Thermo Scientific). To perform reverse transcription of

the RNA, a High Capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, California) was employed, following the manufacturer's instructions and using 1 µg of RNA (in 20 µl final volume) and a cycle of 10 min at 25°C and then 2 h at 37°C. Quantitative real-time PCR reactions (qPCRs) were performed on a StepOne instrument (Applied Biosystems). Primers were validated by testing PCR efficiency using standard curves (95% efficiency 105%) as described previously (1). The primers (5'→3') used were : Abf51AFo TGGTAAGTGCGACCGCTTCAA ; Abf51Are TCGAAATGTGGATCCTGCCGT ; Abn43AFo CCGTACCCAAAGTGCCCATCA ; Abn43AFo GGCGGAAAGTGTCATGGTGGA ; Abn43BFo AGGAACCTGCGTATGAACGCC ; Abn43BRe ATGTTCCGGATCTCTCCAGCG, Abf51B_{trunc}Fo CCGCATCTTCCTCACCTCGAA ; Abf51B_{trunc}Re AGCCTGTGCGAGGTCTTTACG ; Abf43C_{trunc}Fo GTTATGCAATGAGCCGCAGCA ; Abf43C_{trunc}Re TCGACGATCGCCTGATGGTTC. Gene expression was quantified using the comparative CT method, using the penicillin-binding protein 2 (pbp2)-encoding gene, *pbp*A as the reference. This reference was selected among many gene candidates using the software application Normfinder (1) operated according to the developers' manual. Results are expressed as the mean ± standard deviation. Statistical significance was assessed by Student's *t* test. Results with $p < 0.05$ were considered statistically significant.

Reference

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