Supplementary Information

Water disinfection byproducts increase natural transformation rates of environmental DNA in *Acinetobacter baylyi* ADP1

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1. Biomass collection for BAA toxico-genomics and RNA extraction

Biomass sampling points were determined based on the temporal mutagenic response of *Salmonella* TA100 to BAA. After 5 min of exposure to BAA, the number of revertants in the 200 μM BAA treatment raised above the background levels in the 0 μM control (Supplementary Figure 1a). As the mutagenic response in *Salmonella* TA 100 seams to plateau at 60 min, the biomass sampling points 0, 10, 30 and 60 min were selected.

Based on the dose-response transformation stimulation experiments, the linear phase of transformation enhancement occurs in the range of 100 – 200 μM BAA. BAA cytotoxicity was detected at doses higher than 200 μM (Figure 1b). The transformation enhancement phenotype is linked to the mutagenic potential of BAA; therefore, in order to avoid capturing the cytotoxic response to BAA, the transcriptome analysis was restricted to the 0-200 μM concentration range, where no increased cytotoxicity was detected (Figure 1, Supplementary Figure 1b).

As the natural transformation phenotype of *A. baylyi* is a growth dependent trait, the peak transformation potential of the reporter strain was determined as a function of growth. For this, 100 mL of fresh LB Miller broth was inoculated with 100 μL of an overnight *A. baylyi* cell culture and incubated at 37° C x 200 rpm. At each sampling point, OD_{660} was recorded and a 10 mL aliquot was reserved and immediately processed. 100 μL of the cell cultures were used for cell count determination by plating techniques, while the remaining of the sample was employed for transformation frequency determination. The remaining 9.9 mL of sample were centrifuged at 7000 rpm x 10 min at 4° C. Cell pellets where resuspended in 1 mL of PPB buffer containing 2 μg mL^{-1} of donor DNA, and incubated at room temperature for 15 min. Total cell counts and transformants were determined by plating. Transformation frequencies were also defined as previously described. *A. baylyi* transformation frequencies peaked at the early exponential

phase, at an initial cell density of 3 x 10^7 cells mL^{-1} (OD_{660} = 0.1, 75 min after inoculation, Figure 3a). Based on the biomass sampling points previously determined, the experimental window frame would correspond to 75 – 135 min after inoculation (1:1000 inoculum ratio).

As the 1 h window frame selected for the transcriptomic analysis greatly differs from the 24 h BAA exposure time in the concentration-response transformation stimulation experiments, it was important to determine whether the BAA transformation enhancement phenotype would be expressed in the 1 h exposure condition. Following the conditions determined for the transcriptomic experiment, at an OD_{660} = 0.1 *A. baylyi* cell cultures where exposed to 2 μg mL^{-1} of donor DNA and 100 and 200 μM BAA, or mock spiked with and equal volume of carrier (DMSO). After 1 h of incubation at 37° C x 200 rpm, cell cultures were immediately placed on an ethanol ice bath and transformation frequency was determined as previously described. After 1 h of exposure to BAA the stimulation phenotype was observed (Supplementary Figure 1c).

In accordance to these experimental data, the conditions for the biomass collection were chosen. A diagram representing the number of conditions is depicted in Supplementary Figure 2.

Table S1. List of primers for RT-qPCR. All amplification efficiencies were within the 90-110% range. *rpoB* was used to normalize expression data. Cycling conditions were: 50 °C x 2 min; 95 °C x 20 s; 40 cycles of 95 °C x 1 s and 60 °C x 20 s.

Gene	*Froward Primer*	*Reverse Primer*
rpoB	5’-CGCTGAAGGTGGCGTAAAAC-3’	5’-CTCACCTGGACGCATGACTT-3’
pilX	5’-GCGTCAGGTCGTAATCA-3’	5’-AGCGTAACCGCACACTACTT-3’
comA	5’-ATTCTGCTGCTGTAACGCCT-3’	5’-TAAGGCAGGTTGCTGAGGTG-3’
recA	5’-GCGTCAGGTGGTCGTAATCA-3’	5’-AGCGTAACCGCACACTACTT-3’
uvrB	5’-CGTGTGAACGACAACCAACC-3’	5’-TTACCCGAACCTGTAACGCC-3’

Table S2. Composition of the simplified disinfection byproduct (DBP) cocktail used to evaluate transformation induction in *A. baylyi*. The cocktail consisted of five classes of DBPs. For each class, reported values for lower and upper environmental DBP levels in effluent wastewater are shown in columns 2 and 3. For the simplified DBP cocktail, one compound per class was selected and dosed to the transformation assays at a final concentration matching that of the upper environmental limit. Due to the high toxicity of N-nitrosodimethylamine in comparison to other compound of its class, this was dosed to only half of the upper environmental limit reported for nitrosamines.

	Environmental concentration					
Class	*Lower limit*	*Upper limit*	*Units*	*Selected compound*	*Final concentration on transformation assay tubes*	*Ref.*
Trihalomethanes	4	164	μg L^{-1}	Trichloromethane	164 μg L^{-1} (1.37 μM)	1-3
Haloacetic acids	99	262	μg L^{-1}	Bromoacetic acid	262 μg L^{-1} (1.89 μM)	2
Aldehydes	21	114	μg L^{-1}	Formaldehyde	114 μg L^{-1} (3.8 μM)	2, 3
Nitrosamines	20	100	ng L^{-1}	N-nitrosodimethylamine	50 ng L^{-1} (0.67 nM)	3, 4
Haloacetamides	1.4	7.4	μg L^{-1}	Tribromoacetamide	7.4 μg L^{-1} (0.03 μM)	1

Table S3. Quality control of transcriptome samples.

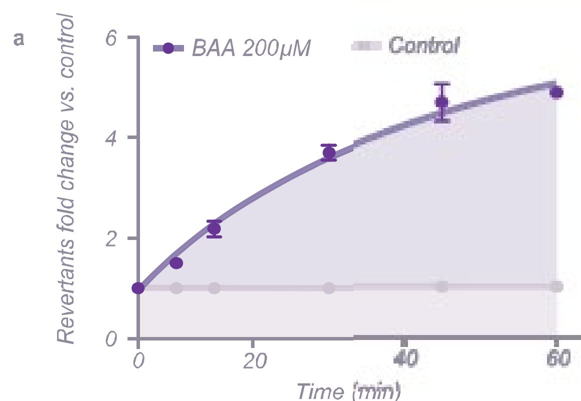
Sample	*Raw read*	*Trimmed reads*	*% Reads mapped*
R1-0-0-T0	11,967,851	9,935,127	71.1
R1-0-0-T10	12,967,023	11,038,167	72.5
R1-0-0-T30	11,942,769	10,035,720	83.1
R1-0-0-T60	13,910,554	11,797,829	84.6
R1-0uM-T0	12,636,715	10,739,997	72.0
R1-0uM-T10	12,537,060	10,591,212	72.9
R1-0uM-T30	12,331,033	10,337,736	82.9
R1-0uM-T60	12,894,668	10,987,217	84.6
R1-100uM-T10	15,963,844	13,275,626	72.4
R1-100uM-T30	14,397,651	11,980,097	82.7
R1-100uM-T60	14,461,985	12,015,001	85.0
R1-150uM-T10	14,165,676	11,922,150	70.8
R1-150uM-T30	14,538,272	12,163,817	82.8
R1-150uM-T60	14,179,271	11,846,414	84.3
R1-200uM-T10	16,224,261	13,428,133	72.2
R1-200uM-T30	13,879,690	11,427,143	82.1
R1-200uM-T60	15,296,164	12,687,880	83.1
R2-0-0-T0	14,483,690	12,193,323	82.8
R2-0-0-T10	12,471,734	10,205,628	80.9
R2-0-0-T30	13,162,804	10,538,287	82.7
R2-0-0-T60	11,736,891	9,463,992	83.0
R2-0uM-T0	12,699,450	10,549,879	81.3
R2-0uM-T10	13,725,600	11,093,386	80.8
R2-0uM-T30	11,195,745	9,006,965	82.6
R2-0uM-T60	12,557,537	10,360,759	83.6
R2-100uM-T10	14,137,531	11,960,392	80.5
R2-100uM-T30	12,628,360	10,304,023	83.3
R2-100uM-T60	14,027,615	11,683,543	84.2
R2-150uM-T10	15,917,901	12,836,986	80.6
R2-150uM-T30	14,105,366	11,511,289	82.0
R2-150uM-T60	14,146,819	11,408,188	83.3
R2-200uM-T10	12,667,237	10,738,388	79.2
R2-200uM-T30	12,947,499	10,914,815	82.7
R2-200uM-T60	12,634,900	10,801,288	83.4
R3-0-0-T0	11,515,976	9,950,362	72.9
R3-0-0-T10	11,463,802	9,956,149	79.2

Continuation Table S1. Quality control of transcriptome samples.

Sample	*Raw read*	*Trimmed reads*	*% Reads mapped*
R3-0-0-T30	11,072,127	9,484,298	82.3
R3-0-0-T60	11,729,195	10,103,257	80.1
R3-0uM-T0	10,814,005	9,277,893	70.6
R3-0uM-T10	11,533,113	9,718,842	78.2
R3-0uM-T30	14,680,260	12,257,494	83.1
R3-0uM-T60	9,706,682	7,990,602	80.8
R3-100uM-T10	11,750,514	9,926,781	79.8
R3-100uM-T30	11,301,492	9,474,360	82.9
R3-100uM-T60	12,039,663	9,907,142	81.8
R3-150uM-T10	12,105,870	9,836,191	78.2
R3-150uM-T30	13,594,341	11,405,652	81.4
R3-150uM-T60	13,113,201	10,430,240	80.2
R3-200uM-T10	13,066,168	10,479,067	77.9
R3-200uM-T30	13,383,943	10,988,217	81.9
R3-200uM-T60	13,511,926	10,736,576	81.8

Table S4. Selected differentially expressed genes following a dose-response behavior relative to BAA concentration.

Gene	*Annotation*	*Up/down regulation*	*p-value*
Antioxidant defense and detoxification			
ACIAD0436	Alkyl hydroperoxide reductase	↑	$4.4x10^{-4}$
ACIAD1232	Glutaredoxin	↑	$1.3x10^{-4}$
ACIAD1234	Alkyl hydroperoxide reductase	↑	$5.6x10^{-5}$
ACIAD2250	Alkyl hydroperoxide reductase	↑	$7.6x10^{-5}$
ACIAD3128	Aldo-keto-reductase	↑	$6.1x10^{-8}$
ACIAD3437	Putative methionine sulfoxide reductase A	↑	$1.2x10^{-4}$
ACIAD3518	Glutathione Synthase	↑	$1.2x10^{-4}$
ACIAD0682	S-formylglutathione hydrolase	↑	$2.3x10^{-3}$
ACIAD1879	Glutathione-dependent formaldehyde dehydrogenase	↑	$2.5x10^{-5}$
Multidrug efflux pumps (MEP) and efflux pump regulators			
ACIAD1154	Drug/metabolite transporter superfamily	↑	$9.9x10^{-20}$
ACIAD2333	Putative transcriptional repressor of MEP	↓	$4.2x10^{-3}$
ACIAD2650	Multidrug transport protein	↑	$1.1x10^{-3}$
Metabolic remodeling			
ACIAD2565	Glyceraldehyde-3-phosphate dehydrogenase	↑	$2.7x10^{-7}$
ACIAD0542	Phosphogluconate dehydratase	↓	$9.0x10^{-10}$
ACIAD0543	2-keto-3-deoxygluconate-6-phosphate aldolase	↓	$1.7x10^{-6}$
ACIAD0544	Gluconate transporter	↓	$5.8x10^{-6}$
ACIAD0730	NADH dehydrogenase chain A	↑	$2.7x10^{-3}$
ACIAD0733	NADH dehydrogenase chain C	↑	$2.0x10^{-3}$
ACIAD0734	NADH dehydrogenase chain E	↑	$9.1x10^{-4}$
ACIAD0735	NADH dehydrogenase chain F	↑	$1.5x10^{-4}$
ACIAD0738	NADH dehydrogenase chain I	↑	$2.5x10^{-3}$
ACIAD0742	NADH dehydrogenase chain M	↑	$2.4x10^{-3}$
ACIAD0743	NADH dehydrogenase chain N	↑	$3.2x10^{-3}$
SOS-response			
ACIAD3040	Integration host factor *himA*	↑	$3.4x10^{-3}$



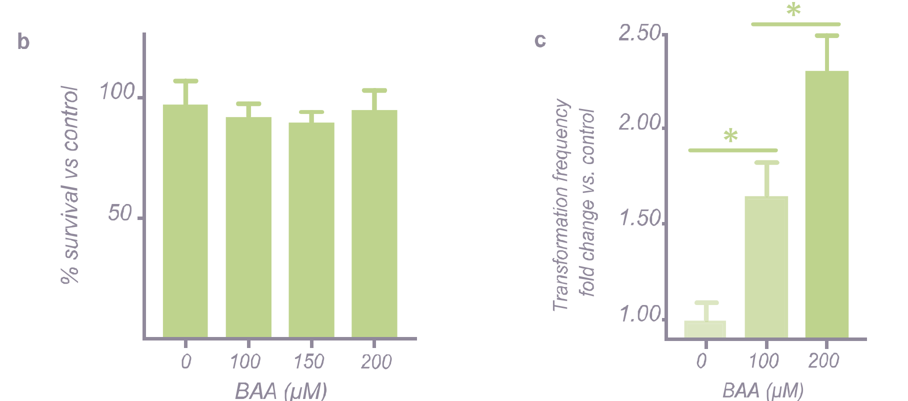


Figure S1. BAA exposure time selection and BAA cytotoxicity for toxico-genomic experiment. a) Time-dependent mutagenic response to BAA in *S. enterica* serovar Typhimurium TA100 in reversion assays. Five minutes after exposure, the number of revertants in BAA treatments increased above background levels. **b)** *A. baylyi* endpoint cytotoxic response to BAA in the 0-200 µM range after 1 h exposure. No increased cytotoxicity was detected at the selected concentrations. **c)** Transformation assays with *A. baylyi* cell cultures at early exponential phase (indicated by the experimental window in Figure 3a) exposed to 0-200 µM BAA for 1 h. * denotes $P < 0.001$.

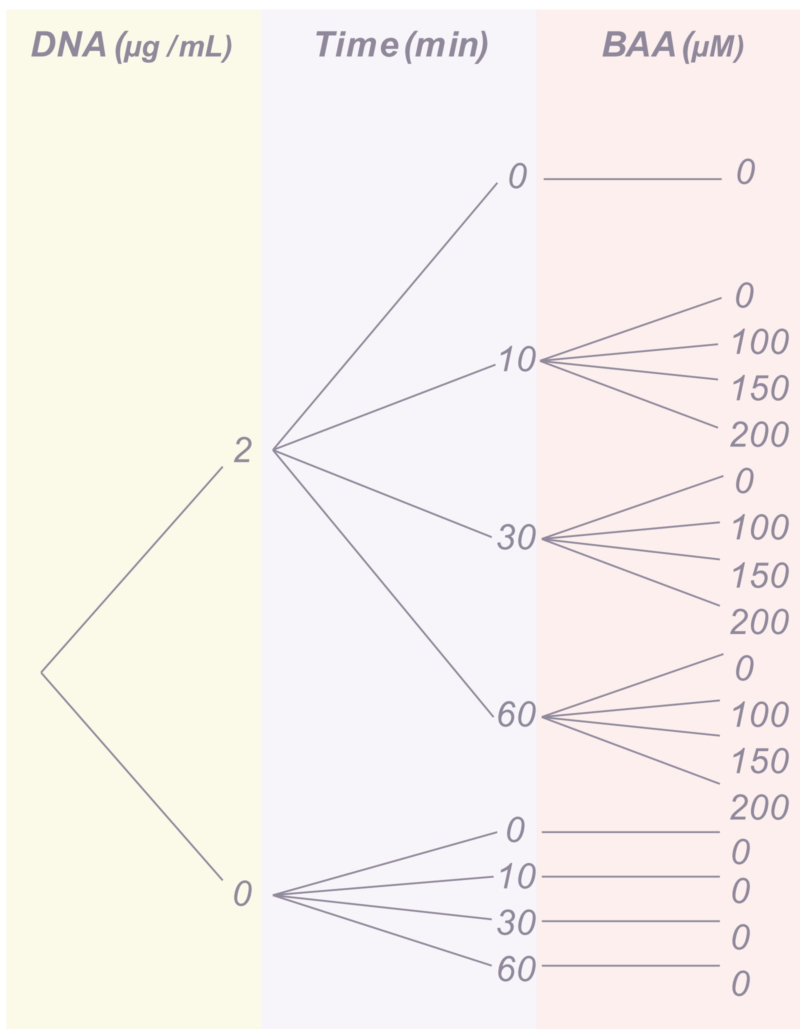


Figure S2. Toxico-genomic experimental design. Figure represents the condition per replicate run. Transcriptomic data derived from 3 independent replicate runs (n = 51).

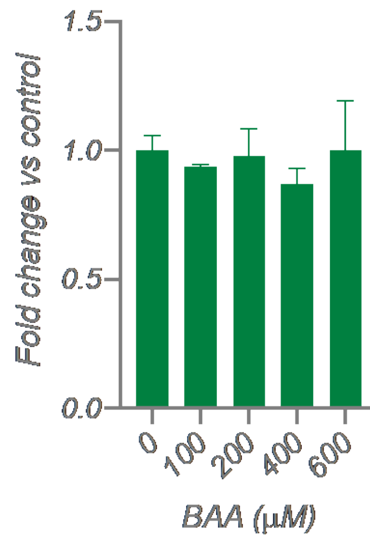


Figure S3. Fold changes in natural transformation rates when donor DNA was first exposed to BAA prior to natural transformation experiments in *A. baylyi* ADP1.

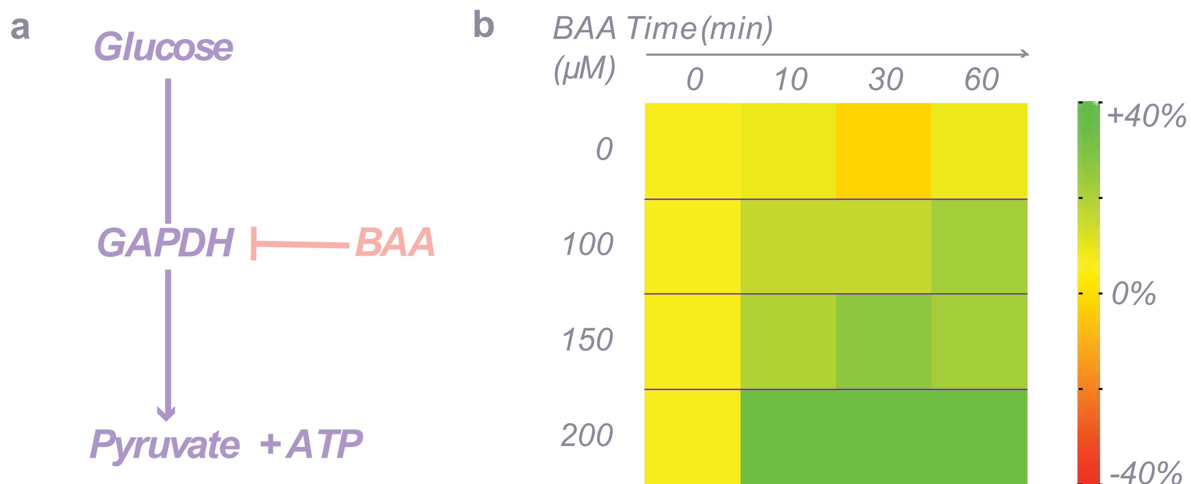


Figure S4. Verification of transcriptomic data using housekeeping gene with known response to BAA. **a)** Schematic representing the inhibition of glycolysis by the interaction of BAA with GAPDH. **b)** Concentration-response changes in transcripts per million (TPM) of the GAPDH gene.

References

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