Electronic Supplementary Material (ESI) for Molecular BioSystems.

Genome-scale reconstruction of the metabolic network in *Pseudomonas stutzeri* A1501

Parizad Babaei, Sayed-Amir Marashi, Sedigheh Asad

Supplementary file S3: Details of the *in silico* simulations

M9 medium simulation

In order to simulate chemically defined M9 medium, the constraints in Table S1 were set on exchange reaction.

Table S1. The metabolites represented in the table are the components of M9 minimal medium. These metabolites were allowed to enter the system and simulate aerobic growth on M9 medium using glucose as the only carbon source.

Metabolite	Lower bound	Upper bound
Cl^-[e] <==>	-1000	1000
CO_2[e] <==>	-1000	1000
Co^{2+}[e] <==>	-1000	1000
Cu^{2+}[e] <==>	-1000	1000
Fe^{3+}[e] <==>	-1000	1000
D-Glucose[e] <==>	-10	0
H^+[e] <==>	-1000	1000
H_2O[e] <==>	-1000	1000
K^+[e] <==>	-1000	1000
Mg^{2+}[e] <==>	-1000	1000
Mn^{2+}[e] <==>	-1000	1000
Na^+[e] <==>	-1000	1000
NH_4^+[e] <==>	-1000	1000
O_2[e] <==>	-1000	1000
Pi[e] <==>	-1000	1000
SO_4^{2-}[e] <==>	-1000	1000
Mo^{2+}[e] <==>	-1000	1000
Zn^{2+}[e] <==>	-1000	1000

Anaerobic growth simulation

Anaerobic conditions in M9 minimal medium were simulated by blocking oxygen from entering the systems and instead letting nitrate enter the system and serve as the final electron acceptor Additional conditions are described in Table S2.

Table S2. The constraints on the lower and the upper bounds of the reactions in the table were used to simulate anaerobic growth.

Reaction ID	Reaction name	Lower bound	Upper bound
IR10379	(S)-Dihydroorotate:fumarate oxidoreductase	-1000	1000
EX_EC0007	Oxygen exchange	0	1000
EX_EC0201	Nitrate exchange	-1000	1000
IR10372	Aerobic biomass	0	0
IR10380	Anaerobic biomass	0	1000

narG mutant construction and analysis

In order to simulate the knock-out strain we eliminated the reaction associated to *narG* gene by setting the lower and the upper bound of the aforementioned reaction to zero. Then, we ran FBA to see the effect of *narG* gene deletion on computational growth rate. Table S3 represents quantitative computational results.

Table S3. Experimental observations and computational results (h^{-1}) concerning anaerobic growth of the wild type and *narG* mutant strain of *Pseudomonas stutzeri* A1501 in the presence of nitrate and nitrite. The qualitative consistency is observable in all four cases.

	Nitrate-containing medium		Nitrite-containing medium	
	Experimental	Computational	Experimental	Computational
Wild type strain	0.453	0.399	0.416	0.399
NarG mutant strain	0	0	0.355	0.399

The values represented in the experimental column are estimated according to the results of [Ref. [1]].

Denitrification activity

The denitrification pathway flux is represented in Table S4.

Table S4. FBA results reflecting the activity of the denitrification pathway in $mmol/g_{DW}h$.

	Nitrate-containing medium	Nitrite-containing medium
Wild type strain	33.0	55.0
NarG mutant strain	0	55.0

Nitrate reductase activity

Whole-cell nitrate reductase activity (NRA) of *P. stutzeri* was measured under different oxygen-limiting conditions [1]. The mutant strain showed no NRA under neither anaerobic nor micro-aerobic conditions. Only a slight NRA (~61.2 $nmol_{nitrite}/min\ mg_{protein}$) has been observed for the mutant strain. The wild type strain showed nitrate reductase activity under all three oxygen-limiting conditions.

Table S5. Nitrate reductase activity in wild type and *narG* mutant strain of *P. stutzeri.* The experimental results are represented in $nmol_{nitrite}/min\ mg_{protein}$ (estimated from Figure 3 of Ref. [1]) . The computational results are shown in values representing the flux of nitrate uptake reaction ($mmol/g_{DW}h$). In cases where the minimum and the maximum nitrate uptake flux rates were not identical, both values are shown in the table.

	Anaerobic		Micro-aerobic		Aerobic	
	Experimental	Computational	Experimental	Computational	Experimental	Computational
Wild type strain	439 ± 142	33.0	663 ± 51.0	32.3	193 ± 173	6×10^{-5}
narG mutant strain	0	0	0	0.75–1	51 ± 10.2	4×10^{-5}

References:

1. H. Rediers, J. Vanderleyden and R. De Mot, *Microbiological Research*, 2009, 164, 461-468.