Metabolic flux analysis of *Shewanella spp* central carbon metabolism reveals evolutionary, genetic and environmental robustness

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Abstract

Metabolite fluxes link genes, proteins and metabolites to macroscopic biological functions. In spite of its importance, only a few, not thoroughly tested, general principles have been proposed to predict and understand the flux configuration of an organism. Among those general principles, robustness of central metabolism to genetic perturbation has been reported. Here we show that the relative metabolic flux distributions are very similar for phylogenetically and environmentally diverse members of the *Shewanella* genus. This phylogenetic robustness suggests understanding microbial fluxomics in terms of metabolic types (or metabotypes), as opposed to phylotypes. In addition to phylogenetic, environmental, and genetic robustness our data shows flexibility in the relative flux profiles when adapting to different carbon sources.

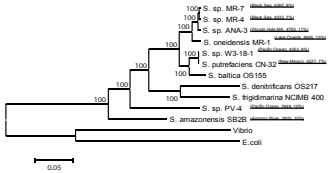

Figure 1

Phylogenetic relatedness of sequenced *Shewanella* genomes. *E. coli* and *Vibrio cholerae* were used as the outgroups. Eight *Shewanella* species were studied and the parenthesis includes the source of species, number of genes, and percent of unique genes not found by sequence homology in the other *Shewanella* genomes.

Materials and Methods

All *Shewanella* strains (including mutants) were cultured in the modified MR-1 defined medium in shaking glass tubes (12 mL) at 30°C. The carbon source was [3-13C] sodium L-lactate (98%, Cambridge Isotope, USA). The isotopomer in proteinogenic amino acids were measured by GC-MS. For each species and strain, the ^{13}C based flux analysis was performed through genetic algorithms.

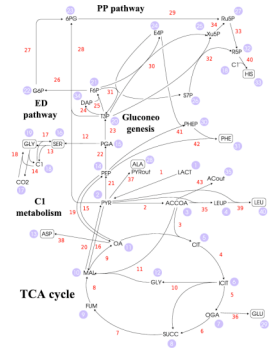

Figure 2

Pathways in *S. oneidensis* MR-1. The amino acids used for isotopomer models are boxed. Numbers denote the arbitrary flux indices used in modeling the pathways and circled numbers denote metabolite numbers.

Abbreviations: 6PG, 6-phosphogluconate; ACCOA, acetyl-coenzyme A; ACout, acetate outside the cell; ALA, alanine; ASP, aspartic acid; CIT, citrate; DAP, dihydroxyacetone phosphate;E4P, erythrose-4-phosphate; C1, 5,10-Me-THF; F6P, fructose-6-phosphate; FUM, fumarate; G6P, glucose-6-phosphate; GLU, glutamate; GLY, glycine; GLYC, glycogen; HIS, histidine; ICIT, isocitrate; LACT, lactate; LEU, leucine; LEUP, leucine precursor; MAL, malate; OA, oxaloacetate; OGA, 2-oxoglutarate; PEP, phosphoenolpyruvate; PGA, 3-phosphoglycerate; PHEP, phenylalanine precursor; PHE, phenyalanine; PYR, pyruvate; R5P, ribose-5-phosphate; Ru5P, ribulose-5-phosphate; Xu5P, xylulose-5-phosphate; S7P, sedoheptulose-7-phosphate; SER, serine; SUCC, succinate; T3P, triose-3-phosphate; PYR, pyruvate.

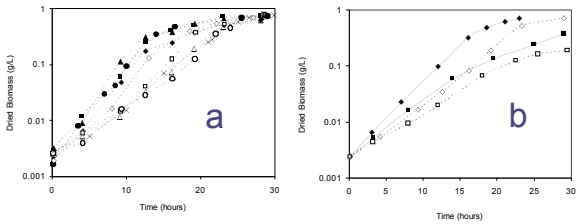

Figure 3

Growth kinetics of *Shewanella oneidensis* MR-1 and other species. (a) *Shewanella* and *E. coli* growth kinetics ×: MR-4, ▲: MR-7, •: ANA-3, ◊: PV-4, ◆: SB2B, □: W3-18-1, ○: CN-32, Δ: MR-1, ×: *E.coli* W3110. (b) *Shewanella oneidensis* MR- 1 growth in different media ◆: MR-1 medium with amino acids supplement; ◊: MR-1 medium with low salt concentration (0.26 M); •: MR-1 medium with high salt concentration (0.33 M) and amino acids supplement (17 amino acids and 25 μM each), □: MR-1 medium with high salt concentration (0.33 M).

Results and Discussion

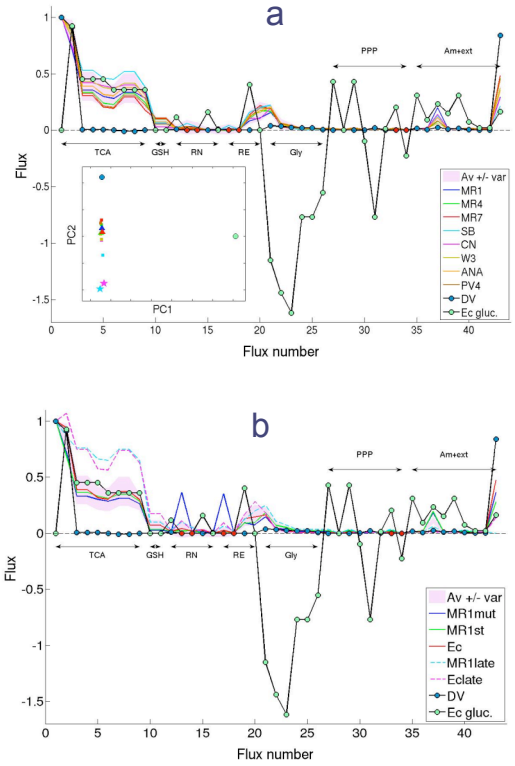

Figure 4

Panel a) Flux profiles calculated for each of the considered lactate fed *Shewanella* species (MR1, MR4, MR7, SB, CN, W3, ANA, PV4) and *E. coli* (Ec) along with profiles for different metabotypes (lactate-fed *Desulfovibrio vulgaris* (DV) and glucose-fed *E. coli* (Ec gluc)) obtained from the literature. Fluxes are normalized to the input lactate flux (flux v_1=1) except for the case of glucose-fed *E. coli.* Red dots indicate fluxes that were not calculated in the flux profiles obtained from the literature (DV and Ec gluc.) and were set to zero. Panel a) shows profiles for the phylogenetically diverse *Shewanella* species and the average of these profiles (av) with the average confidence intervals (var), which defines the metabotype. The metabotype is dependent on both the genome and the culture conditions (e.g., carbon source). A Principal Component Analysis shows the relative location of flux vectors corresponding to the 15 flux profiles. The same symbols used in the main plot identify each species. Points corresponding to profiles shown in panel b are shown as either stars (Eclate and MR1late) or triangles (the rest). It is clear that profiles corresponding to the same metabotype cluster in the same flux space. Panel b): profiles for mutated (MR1mut) and stressed MR1 (MR1st), *E. coli* (Ec), and late profiles of both *E. coli* (Eclate) and MR1 (MR1late). The metabotype (av± var) from panel a, as well as the reference metabotypes for *D. vulgaris* (DV) and glucose-fed *E. coli* (Ec gluc.), are also plotted for comparison. Although late profiles (Eclate and MR1ate) differ from the metabotype, mutated (MR1mut) or stressed MR1 (MR1st) profiles do not.

Abbreviations: TCA cycle + lactate uptake (TCA); Gly shunt (GSH); reversible and C1 metabolism (RN); reversible exchange (RE); glycolysis (Gly); pentose phosphate + ED pathway (PPP); amino acids and external (AM+ext); species abbreviations follow Supplementary Table 1.

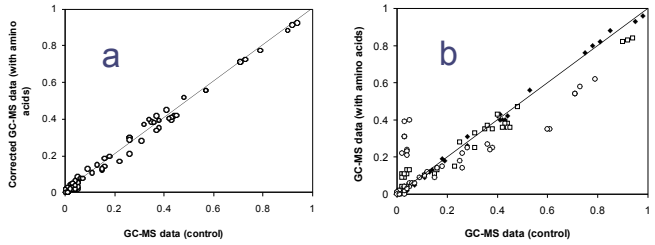

Figure 5

Effect of addition of unlabeled amino acids on central metabolism in MR-1 (illustrated by change of GC-MS data in key amino acids).
a) GC-MS data from the rich medium culture were corrected for unlabeled amino acid effect.
b) Comparison of GC-MS data for proteinogenic amino acids between minimal medium culture (control) and rich medium culture.
Addition of amino acid mixtures, 17 amino acids, 25 mM each. ♦: Ala, Asp, Glu, Gly, Ser; □: Val, Leu, Iso, Pro, Thr, Lys; ○: Met, Phe, His, Tyr.

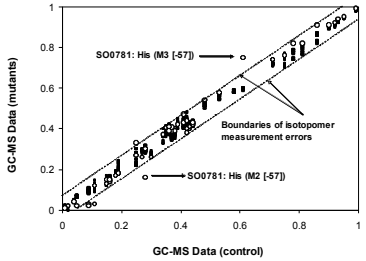

Figure 6

Effect of transposon mutation on central metabolism. The GC-MS data were from 10 different mutants. Nine key amino acids were included (Ala, Asp, Glu, Gly, Ser, Val, Leu, Phe, His). The open dots were GC-MS data from SO0781 (lack of glycine cleavage system P protein). The outliers are histidine labeling data, the M3[-57] fragment is related to labeled histidine and the M2[-57] is related to unlabeled histidine. This increased ^{13}C abundance in histidine is due to the knockout of the glycine dehydrogenase. The measurement noise for isotopic data from independent tracer experiments should be below 5%.

Conclusion

Shewanella and *E. coli* show suboptimal performance under the studied conditions, which provides further evidence beyond genetic perturbations that microbial metabolism is not geared towards growth rate maximization when carbon sources are sufficient. Finally, in addition to phylogenetic, environmental, and genetic robustness, *Shewanella spp.* display a flexible relative metabolic flux distribution aimed towards the progressive utilization (lactate → pyruvate → acetate) of diverse carbon sources.

The relative flux distribution for *Shewanella oneidensis* MR1 is robust with respect to amino acid addition, salt stress, genetic perturbation, gene content and phylogenetic distance. The latter suggests the introduction of the concept of metabotype, or metabolic type, which provides a more natural classification of organisms than phylotypes regarding the characterization of the metabolic activity in a microbial community. Metabotypes depend on growth conditions (e.g., carbon source) and are related to phylotypes, since organisms sufficiently different in phylogenetic terms (e.g., *Shewanella* spp. vs *D. vulgaris*) correspond to different metabotypes. The concept of metabotype has several possible applications: first, it allows us to predict the central metabolism of close species (whose genome may not even be sequenced yet) by only studying one representative species. Second, it paves the way to model the metabolism of whole microbial ecosystems as the sum of a limited number of metabotypes instead of a myriad of phylotypes. Third, it provides a baseline for rational metabolic engineering of microorganism. Since a metabotype encompasses the set of fluxes that define organisms given a growth condition, one can imagine a scenario where a microbial chassis is selected on the basis of optimizing the flux leading to necessary precursor components. Furthermore, the metabotype concept may lead to quick and efficient transfer of constructs from an engineered strain to another in the same metabotype that has a more suitable growth condition.

ACKNOWLEDGEMENT

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