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

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Abstract

Metabolic 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 phylogenetically robustness suggests understanding microbial fluxomics in terms of metabolic types (or metabolotypes), as opposed to phylogeny. In addition to phylogenetic, environmental, and genetic robustness our data shows flexibility in the relative flux profiles when adapting to different carbon sources.

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 isotope level in proteogenic amino acids were measured by GC-MS. For each species and strain, the 13C based flux analysis was performed through genetic algorithms.

Figure 1

Phylogenetic relatedness of sequenced Shewanella genomes. E. coli and Vibrio cholerae were used as the outgroups. Eight Shewanella species were used for this analysis. The tree includes the species, number of genes, and percent of unique genes not found in the other Shewanella genomes.

Results and Discussion

Panel a: Flux profiles calculated for each of the considered lactate fed Shewanella species (MR1, MR4, MR7, SB, CN, ANA, PV4) and E. coli (Ec) along with profiles for different metabolotypes (lactate-fed Desulfovibrio vulgaris (DV) and glucose-fed E. coli (Ec)) obtained from the literature. Fluxes are normalized to the input lactate flux (flux u+1) except for the case of glucose-fed E. coli. Red data 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 most closely related species and the average of these (Central). Panel b) shows profiles for the phylogenetically most different species and the average of these (Divergent). 2. A Principal Component Analysis shows the relative location of flux vectors corresponding to the 10 flux profiles. The same symbols used in the main text identify each species. Points corresponding to profiles shown in panel a are shown as either stars (Central and MR4) or triangles (the rest). It is clear that profiles corresponding to the same metabolotypes are in the same flux space. Panel b) profiles for mutated (MR1str) and stressed MR1 (MR1st) E. coli (Ec), and late profiles of both E. coli (Ec) and MR1 (MR1late) the metabolotypes (arch strain) from panel a, as well as the reference metabolotypes for D. vulgaris (DV) and glucose-fed E. coli (Ec gluc.), are also plotted for comparison. Although late profiles (Ec late and MR1late) differ from the metabolotype, mutated (MR1str) or stressed MR1 (MR1st) profiles do not.

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 skewed towards the pentose phosphate pathway (lactate → glyceraldehyde 3-phosphate) of diverse carbon sources.

The relative flux distribution for Shewanella oneidensis MR1 is robust with respect to amino acid addition, salt stress, growth perturbation, gene content and phylogenetic distance. This latter suggests the introduction of the concept of metabolotype, or metabolic type, which provides a more natural classification of organisms than phylogenies regarding the characterization of the metabolic activity in a microbial community. Metabolotypes depend on growth conditions (e.g., carbon source) and are related to physiologies, since organisms sufficiently different in phylogenetic terms (e.g., Shewanella spp. vs. D. vulgaris) correspond to different metabolotypes. The concept of metabolotype has several possible applications: first, it allows us to predict the central metabolism of close species (where 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 metabolotypes instead of a myriad of separate species. Finally, it provides a framework to encompass the set of fluxes that define organisms given a growth condition, one can imagine a scenario where a metabolotype encompasses the set of fluxes that define organisms given a growth condition, and the metabolotype is related to this condition. Thus, abundance in metabolotypes is due to the knockdown of the glyceraldehyde 3-phosphate dehydrogenase. The measurement noise for isotopic data from independent tracer experiments should be below 5%.

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