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Trait-based approach to bacterial growth efficiency

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Summary

Bacterial growth efficiency (BGE) is the proportion of assimilated carbon that is converted into biomass and reflects the balance between growth and energetic demands. Often measured as an aggregate property of the community, BGE is highly variable within and across ecosystems. To understand this variation, we first identified how species identity and resource type affect BGE using 20 bacterial isolates belonging to the phylum Proteobacteria that were enriched from north temperate lakes. Using a trait-based approach that incorporated genomic and phenotypic information, we characterized the metabolism of each isolate and tested for predicted trade-offs between growth rate and efficiency. A substantial amount of variation in BGE could be explained at broad (i.e., order, 20%) and fine (i.e., strain, 58%) taxonomic levels. While resource type was a relatively weak predictor across species, it explained >60% of the variation in BGE within a given species. A metabolic trade-off (between maximum growth rate and efficiency) and genomic features revealed that BGE may be a species-specific metabolic property. Our study suggests that genomic and phylogenetic information may help predict aggregate microbial community functions like BGE and the fate of carbon in ecosystems.

Introduction

In most ecosystems, heterotrophic bacteria play a pivotal role in determining whether organic carbon is respired as carbon dioxide (CO_2) or converted into biomass and retained in food webs (Pomeroy *et al*., 1998; Bardgett *et al*., 2008; Ducklow, 2000). Many factors control how bacteria process carbon, but one of the most important is bacterial growth efficiency (BGE). BGE is the proportion of assimilated organic carbon that is converted into bacterial biomass (del Giorgio and Cole, 1998). When BGE is high, more carbon is turned into bacterial biomass where it can be retained for longer periods of time while also serving as a resource for other members of the food web. In contrast, when BGE is low, microbially assimilated carbon has a shorter residence time and is released into the environment as CO_2. Typically measured as an aggregate property of the microbial community, BGE is notoriously variable among habitats and has proven difficult to predict (del Giorgio and Cole, 1998; Sinsabaugh *et al*., 2013). While a range of chemical and physical variables influence BGE at the community-level (Apple and del Giorgio, 2007; Hall and Cotner, 2007; del Giorgio and Newell, 2012; Geyer *et al*., 2016), fewer studies have investigated how the traits of species contribute to BGE (Pold *et al*., 2020).

A trait-based approach provides an opportunity for a deeper understanding of how microbial composition and physiology contribute to BGE. By focusing on physiological, morphological, or behavioural characteristics that affect performance, a trait-based approach can be used to predict fitness under a set of environmental conditions (Lennon *et al*., 2012). The distribution of traits among organisms may reflect adaptations, phylogenetic relatedness and metabolic constraints (Martiny *et al*., 2015). In the context of BGE, insight may be gained by identifying taxon-specific differences in microbial metabolism that result from the physiological balance between cellular growth and energetic demands. For example, the bacterial growth strategy is predicted to constrain BGE via physiological trade-offs (Litchman *et al*., 2015). As a result, it has been hypothesized that oligotrophs have higher maximum growth efficiency than copiotrophs (Roller and Schmidt, 2015), and rapidly growing bacteria have been shown to 'spill' up to 20% of their energetic budget due to overflow respiration (Russell, 1991, 2007). Likewise, BGE may be influenced by an organism's capacity to use different types of resources. For example, species that specialize on only a few resources are predicted to be more efficient at using those resources than generalist species that require more cellular machinery to use a larger array of substrates (Dykhuizen and

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Davies, 1980; Glasser, 1984). Therefore, traits such as maximum growth rate and the number of resources used (i.e., niche breadth) could underlie species-specific differences in BGE.

A trait-based approach to BGE also requires that metabolism be examined with respect to the resources that are being consumed. Different resources can affect cellular ATP yield depending on the metabolic pathways used (Fuhrer *et al*., 2005; Flamholz *et al*., 2013), which in turn can influence cellular growth yield (Neijssel and de Mattos, 1994; Russell and Cook, 1995). For example, glucose is metabolized via glycolysis, but growth on more complex, aromatic compounds, such as protocatechuate, requires the β-ketoadipate pathway, which yields less ATP (Gottschalk, 1986). Furthermore, energy-producing catabolic processes and biomass-producing anabolic processes are not independent (Russell and Cook, 1995; Kempes *et al*., 2012). For example, cells have the potential to produce >30 ATP from a single glucose molecule if it is completely oxidized, but there would be no remaining carbon to yield new biomass. Instead, cells must use the intermediate products of glycolysis and the TCA cycle to form proteins and other cellular material, which diminishes the maximum potential ATP yield (Gottschalk, 1986; Flamholz *et al*., 2013). In addition, biomass production requires materials and energy. For example, the synthesis of proteins, which can constitute ~55% of cellular dry mass (Milo, 2013), requires amino acid building blocks and four ATP per peptide bond (Tempest and Neijssel, 1984; Gottschalk, 1986; Lynch and Marinov, 2015). Therefore, because resources differ in their potential energy yield and bacteria differ in their ability to extract energy and form biomass from a given resource, BGE should vary based on the resources available to bacteria.

In this study, we used a trait-based approach to understand how species identity and resource type control BGE. We measured BGE in a diverse set of bacterial isolates supplied with one of three different carbon resources that varied in chemical structure and metabolic pathway (Fig. S1). The trait-based approach provides a framework to understand how and why the composition of microbial communities should affect ecosystem functioning (Wallenstein and Hall, 2012; Krause *et al*., 2014). We used the taxonomic and phylogenetic relatedness of the bacterial isolates to explore the variation in BGE when supplied with different carbon resources. In addition to partitioning variation in BGE based on species identity and resource type, we tested for hypothesized trade-offs with growth rate and niche breadth while taking phylogeny into account. Furthermore, using the genomes of each isolate, we evaluated whether metabolic pathways could explain differences in BGE among diverse representatives of aquatic bacteria from north temperate lakes. Last, to test if resource type affects the metabolic traits that underlie BGE (i.e., production and respiration), we tested for resource-specific relationships between respiration and production rates for each resource. Our trait-based approach provides a framework for understanding linkages between community structure and function due to the physiological constraints on BGE and suggests that large changes in community composition or available resources may alter BGE and therefore carbon cycling in predictive ways.

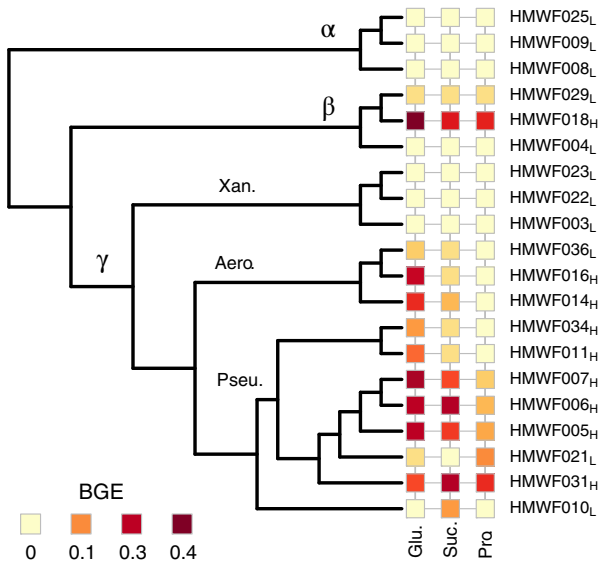

Fig. 1. Bacterial growth efficiency (BGE) of each isolate for each resource. BGE was calculated based on measured production (BP) and respiration (BR) rates using the following equation: BGE = BP/(BP + BR). The cladogram is based on the RAxML consensus tree but is shown without branch lengths for visualization (see Fig. S2 for the complete phylogenetic tree). Taxonomic class and order are included based on the Ribosomal Database Project taxonomy: α = Alphaproteobacteria, β = Betaproteobacteria, γ = Gammaproteobacteria, *Xan*. = Xanthomonadales, *Aero*. = Aeromondales, *Pseu*. = Pseudomondales. The BGE group is indicated for each isolate (H = high; L = low).

Results

Bacterial growth efficiency

Using measures of bacterial productivity (BP) and respiration (BR), we calculated BGE for 20 aquatic bacterial isolates growing on three different resources: glucose, succinate and protocatechuate (Fig. S1). Isolates were enriched from north temperate lakes, and all belonged to the Proteobacteria phylum with representatives from the Alpha-, Beta-, and Gamma-proteobacteria subphyla (Fig. 1, Fig. S2). Across isolates, BGE ranged from <0.01 to 0.32 (Fig. 1). Based on linear mixed-effects models, species identity and resource type explained a

substantial amount of variation in BGE. Across resources, species identity explained 58% of the variation in BGE and 67% of the variation within a resource type. The taxonomic order of each species explained 20% of the variation in BGE across all resources and 28% of the variation within each resource type. Resource type only explained 8% of the variation in BGE across all species, but 63% of the variation within species (see Table S1 for additional information model output).

Next, we tested for phylogenetic signal in BGE. Using Blomberg's K, no phylogenetic signal was detected for BGE when isolates were supplied with succinate ($K = 0.002$, $p = 0.24$) or protocatechuate ($K = 0.001$, $p = 0.146$), but there was a significant phylogenetic signal when isolates used glucose ($K = 0.002$, $p = 0.04$). However, the low K value suggests that BGE is overdispersed (i.e., less phylogenetic signal than expected under Brownian motion). Similarly, when using Page's λ, we found no evidence that BGE had a phylogenetic signal when the isolates were supplied with any of the resources (Glucose: $\lambda = 0.10$, $p = 0.76$; Succinate: $\lambda = 0.13$, $p = 0.66$; Protocatechuate: $\lambda < 0.01$, $p = 0.99$).

Last, we determined if the values of BGE observed across isolates and resources were unimodally distributed. Based on Hartigan's dip test, there was a bimodal distribution of BGE among our isolates when supplied with glucose or succinate ($D_{glu} = 0.07$, $p = 0.58$; $D_{suc} = 0.08$, $p = 0.30$; Fig. S3). Using this distribution, isolates were split into two groups (based on the glucose BGE), which we define as the 'high-BGE' (mean = 19.6% ± 2.8) and 'low-BGE' (mean = 0.5% ± 0.2) groups.

Phenotypic comparisons

Using linear models, we identified phenotypic differences between isolates that were related to BGE (Fig. 2). While there was no relationship between BGE and maximum growth rate in the low-BGE group of bacteria (μ_{max}; $F_{1,7} = 0.035$, $r^2 < 0.01$, $p = 0.86$), there was a significant inverse relationship between BGE and μ_{max} for the high-BGE group ($F_{1,7} = 7.79$, $r^2 = 0.53$, $p = 0.027$). This model predicts a 2.6% decrease in BGE for each per minute increase in μ_{max} in the high-BGE group. In contrast to our predictions, there was no relationship between niche breadth (Levins Index) and BGE for the low-BGE group ($F_{1,7} = 1.42$, $r^2 = 0.17$, $p = 0.27$) or the high-BGE group ($F_{1,7} = 0.92$, $r^2 = 0.11$, $p = 0.37$).

Genomic comparisons

We detected genomic differences related to BGE. First, isolates in the high-BGE group had 26% more annotated metabolic pathways (based on an 80% module completion ratio cut-off) than isolates in the low-BGE group (high = 66 ± 3, low = 52 ± 4, t-test: $t_{18} = -2.64$, $p = 0.02$). Second, we found that the number of metabolic pathways corresponded with BGE when supplied with glucose, but the direction of the relationship depended on the BGE group. For the high-BGE group there was a negative relationship between BGE and the number of pathways ($\beta = -0.006 \pm 0.002$, $r^2 = 0.48$, $p = 0.04$), but for the low-BGE group there was a positive relationship ($\beta = 0.0003 \pm 0.0001$, $r^2 = 0.37$, $p = 0.05$). Next, we found that differences in the metabolic pathway composition could help explain which BGE group an isolate belongs, and the pathway composition of an isolate was related to its BGE. Specifically, three pathways were indicators of an isolate being in the high-BGE group (Table 1). Likewise, within the high-BGE group 24% of the variation in BGE could be explained based on the composition of metabolic pathways (dbRDA: $F_{1,7} = 2.17$, $R^2 = 0.24$, $p = 0.05$). Eight pathways had significant correlations ($|\rho| > 0.7$) with BGE (Table 2). However, there was no

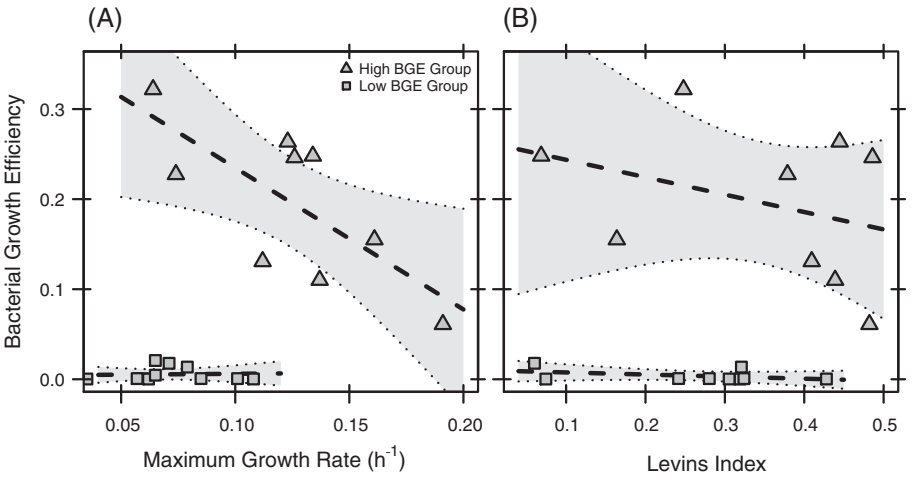

Fig. 2. Phenotypic traits associated with BGE. A. Maximum growth rate, a measure of growth strategy, demonstrates a trade-off (negative relationship) with BGE in the high-BGE group ($F_{1,7} = 9.52$, $r^2 = 0.54$, $p = 0.015$) but not the low-BGE group ($F_{1,7} = 0.51$, $r^2 = 0.06$, $p = 0.50$). B. Levins Index, a measure of niche breadth, does not demonstrate a trade-off with BGE in either the high- or low-BGE groups (high: $F_{1,7} = 0.92$, $r^2 = 0.11$, $p = 0.37$; low: $F_{1,7} = 1.47$, $r^2 = 0.17$, $p = 0.27$). High- and low-BGE groups were determined based on the bimodal distribution of BGE (see Fig. S3).

Table 1. Genetic pathways unique to the high-BGE isolates.

Pathway	Reference function	Prob.
M00045	Histidine degradation (histidine → N-formiminoglutamate → glutamate)	0.02
M00060	Lipopolysaccharide biosynthesis(Kdo2-lipid A biosynthesis)	0.02
M00565	Trehalose biosynthesis (D-glucose-1P → trehalose)	0.03

Functional metabolic pathways were identified from genome sequencing and predicted using Maple. Prob. = probability statistic from indicator species analysis: the probability that the 'species' (i.e., pathway) is not unique to the group.

relationship between pathway composition and BGE for the low-BGE group ($p = 0.45$), nor was the BGE group a strong predictor of pathway composition (PERMANOVA: $p = 0.23$).

Resource effects

Indicator variable linear regression revealed a positive relationship between the per cell respiration and production rates (Fig. 3, $F_{9,42} = 8.07$, $R^2 = 0.63$, $p < 0.001$) with there being a higher *y*-intercept for the high-BGE group of isolates ($\beta_{Group} = 2.7$, $p < 0.001$). Resource type, however, had no effect on the BR–BP relationship or the effect of the BGE group (i.e., no interactions; all $p > 0.25$). Last, the slope of the BR–BP relationship was not different from one ($t_{42} = 0.76$, $p = 0.45$; Fig. 3) indicating that the two measures of bacterial metabolism scale proportionately (i.e., isometrically) with one another.

Table 2. Genetic pathways correlated with BGE in the high-BGE group.

Relationship	ρ	Pathway	Reference function
Positive	0.82	M00025	Tyrosine biosynthesis (chorismate → tyrosine)
	0.72	M00034	Methionine salvage pathway
Negative	−0.73	M00117	Ubiquinone biosynthesis (chorismate → ubiquinone)
	−0.82	M00044	Tyrosine degradation (tyrosine → homogentisate)
	−0.82	M00053	Pyrimidine deoxyribonucleotide biosynthesis(CDP/CTP → dCDP/dCTP, dTDP/dTTP)
	−0.82	M00549	Nucleotide sugar biosynthesis (glucose → UDP-glucose)
	−0.82	M00568	Catechol ortho-cleavage (catechol → 3-oxoadipate)
	−0.82	M00637	Anthranilate degradation (anthranilate → catechol)

Correlations are Spearman's rank correlations between BGE and the pathway presence. Pathways with correlation coefficients (ρ) ≥ |0.70| were considered significant.

Discussion

We measured BGE in 20 aquatic bacterial isolates supplied with one of three resources that varied in their bioavailability, structure and pathways required for metabolism (Fig. S1). While BGE varied across isolates, there was mixed evidence for a phylogenetic signal based on efficiency. However, a substantial amount (20%) of the variation in BGE could be explained by an isolate's taxonomic order while a much smaller amount of the variation (8%) could be attributed to the particular resource used (Fig. 2). We found evidence for a predicted trade-off between maximum growth rate and efficiency, but only on the most labile resource (glucose) (Fig. 2). Even though it explained 63% of the variation in BGE within an isolate, resource type did not alter the relationship between respiration and production rate observed across isolates (Fig. 3). Together, our results support the view that BGE is a complex physiological trait, but resource characteristics may modify species-specific physiological performances. Taxonomic groups of bacteria may have fundamentally different growth efficiencies such that changes in community composition may alter the fate of carbon resources (i.e., biomass versus CO_2) within the ecosystem (Treseder *et al*., 2011; Wallenstein and Hall, 2012).

BGE as a trait

Our results indicate that there are species-specific properties regulating BGE, which may be conserved at higher taxonomic levels. This conclusion is consistent with the view that BGE represents a complex bacterial trait (i.e., an aggregate property of numerous cellular functions) with ecological significance, and that different groups of bacteria have different strategies for carbon allocation. Our phylogenetic analyses suggest that BGE may be an over-dispersed trait (at least with glucose) such that the efficiencies of closely related bacteria are less similar than expected. Though some traits such as phosphorus acquisition, photosynthesis and methanogenesis are phylogenetically conserved deep in the microbial tree of life (Martiny *et al*., 2006, 2013), others such as complex carbon metabolism are not (Zimmerman *et al*., 2013). Therefore, BGE may be similar to traits such as complex carbon metabolism that are not deeply conserved. An alternative explanation for this pattern is that our culture collection lacked phylogenetic resolution within some of our taxonomic groups (e.g., Betaproteobacteria) or that the variation in BGE within a taxonomic group (e.g., order) may not be the same across taxonomic groups. Because our analysis focused on Proteobacteria with a large representation from the Gammaproteobacteria, it is possible that we

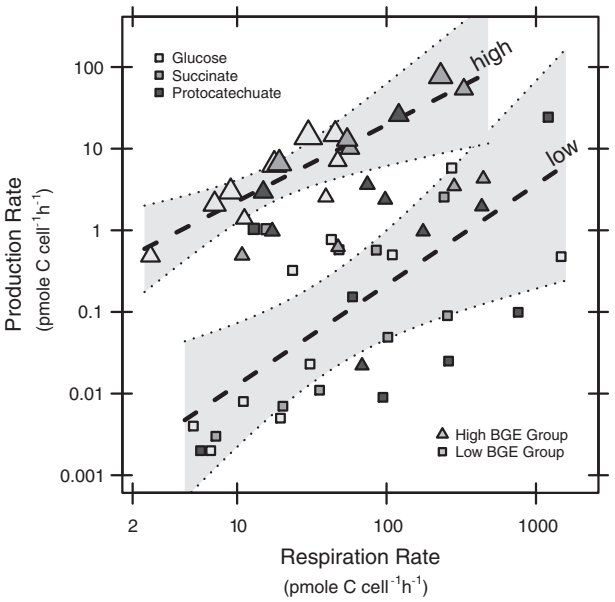

Fig. 3. Relationship between respiration and production rates. Respiration and production rates were compared using an indicator variable linear regression ($F_{9,42} = 4.92$, $R^2 = 0.51$, $p < 0.001$). According to the regression model, production rate increases with the respiration rate proportionally (i.e., isometric scaling: slope not significantly different from one, $t_{42} = -0.26$, $p = 0.79$). In addition, group (high- vs. low-BGE) was a significant factor and isolates in the high-BGE group had a greater *y*-intercept ($p = 0.02$). The two regression lines represent the linear fits for the high- and low-BGE groups. Symbols indicate isolate group (high- and low-BGE) and colour indicates the resource being used. Symbol size is scaled by growth efficiency.

missed important phylogenetic patterns found in other important lineages (e.g., Bacteroidetes). Likewise, the phylogenetic methods used assume that trait variation is proportional to branch length variation and this may not be the case for BGE. Regardless, our data reveal that BGE is a complex bacterial trait that is influenced by taxonomic affiliation. As such, it may be possible to make predictions about BGE and other ecosystem functions given information about the composition of resident microbiomes (Goberna and Verdú, 2016).

BGE on different resources

Differences in resource complexity and the metabolic pathways required for degradation may explain species-specific differences in BGE due to the resource type used. Within an isolate, resource type accounted for 63% of the variation in BGE. Given that different resources are processed via different metabolic pathways, it is perhaps not surprising that we observed resource-based variation in BGE within species. For example, BGE was higher when isolates were supplied with glucose compared with when they were supplied with protocatechuate. Glucose is a simple sugar that is able to be metabolized by numerous pathways and converted to acetyl-CoA (Neidhardt, 2007). Protocatechuate, on the other hand, is a complex aromatic compound that requires a specific metabolic pathway to be converted to acetyl-CoA. Furthermore, because protocatechuate is chemically more complex, it requires more energy (i.e., ATP) to be degraded than more labile resources such as glucose (Harwood and Parales, 1996). Therefore, resource complexity and the metabolic pathways required may explain the within-species variation in BGE. Across species, there were no resource-specific differences in the relationship between respiration and production rate. However, our results may be limited by the number and types of resources used in this study. Regardless, our findings suggest that the energetic demands required to use different resources may be a species-specific trait. That is, the energetic demands for individual species may be constrained and therefore not change much when growing on different resources. These findings suggest that the effect of resources on the efficiency of entire microbiomes may depend on the composition of bacteria consuming those resources.

Low- vs. high-efficiency taxa

Although, the range of BGE measured across isolates is similar to the range observed in many ecosystems (del Giorgio and Cole, 1998), our results suggest that some species of bacteria grow relatively inefficiently, irrespective of resource quality. Across all isolates, we found a bimodal distribution of BGE suggesting that there were two distinct groups with contrasting efficiencies. One group had low BGE (<5%) across all resource types while the other group ranged in BGE from 7% to 30% (Figs 1 and 2). One explanation is that the minimum cellular energetic demand (i.e., cellular maintenance costs) is higher in some bacteria than others (Russell and Cook, 1995); however, this would likely only have an impact when growth rates are low. Furthermore, energetic demand may be higher when bacteria are grown in minimal media where they must produce all cellular components from a single carbon resource (Tao *et al*., 1999). Alternatively, nutrient concentrations (e.g., phosphorus) and other physical properties (e.g., temperature) may regulate efficiency (Smith and Prairie, 2004; Frey *et al*., 2013) and the effects of these properties may be species-specific. As such, it is possible that maintenance costs, resource imbalances and the physical growth conditions affected the BGE of our isolates. Furthermore, differences in low-BGE and high-BGE isolates were also reflected in genomic content, including the number and presence–absence of metabolic pathways. However, these genomic features seem to best explain large-scale

rather than fine-scale differences in BGE. These findings suggest that there are fundamental differences between bacterial species that determine BGE.

Physiological trade-offs

We found evidence to support a trade-off between maximum growth rate and BGE (Fig. 2), which is predicted in microbial and non-microbial systems (Glasser, 1984; Roller and Schmidt, 2015). For example, theoretical models of microbial communities predict a rate-efficiency trade-off (Allison, 2014), which has been observed across microbial taxa (Lipson, 2015). Physiologically, the trade-off is based on allocation constraints imposed by the balance between energy requirements and biomass yield: organisms with higher maximum growth rates may have greater energetic requirements and thus lower BGE than organisms with lower maximum growth rates (Russell and Baldwin, 1979; Russell and Cook, 1995). Furthermore, processes that limit respiration, such as oxygen availability, have been shown to suppress bacterial growth rate (Meyenburg and Andersen, 1980). Therefore, the respiration rate is likely a major control on biomass production and BGE. Consistent with this, we observed an isometric scaling relationship between respiration and production rates (Fig. 3). The non-zero intercept of this relationship suggests that there is a minimum respiration rate required before any biomass can be produced, which is commonly interpreted as the cellular maintenance requirement. Therefore, it is possible that the maintenance energy demand of a bacterial species explains the physiological trade-off between maximum growth rate and growth efficiency.

The theory also predicts a trade-off between resource niche breadth and growth efficiency (Glasser, 1984). This trade-off is based on the assumption that there is an energetic cost to maintaining numerous metabolic pathways (Johnson *et al*., 2012). As such, species with more metabolic pathways should have more energetic requirements and thus lower BGE; although, the effects of genome reduction has been debated (Giovannoni *et al*., 2005; Livermore *et al*., 2014). In this study, physiological profiling using Biolog EcoPlates was conducted to quantify resource niche breadth based on 31 unique carbon sources, but we did not find evidence of a trade-off between resource niche breadth and BGE (Fig. 2). Likewise, there was no evidence that the number of genes or genome size directly influenced BGE (Tables S2–S3), but we did find an inverse relationship between the number of pathways and BGE for the high-BGE group. One possible explanation for the lack of a niche breadth-efficiency trade-off is that the resources used in our phenotypic assay (i.e., Biolog EcoPlates) did not reflect the full metabolic potential of our isolates. Alternatively, there may not be a strong trade-off between niche breadth and efficiency, but further experiments with additional isolates and resources are required to test this prediction more rigorously.

Genomic signatures

In addition to the physiological differences documented among our isolates, we found genomic evidence of metabolic pathways that are associated with BGE. Specifically, there were genomic differences between isolates belonging to the low-BGE and high-BGE groups. Isolates from the high-BGE group had 26% more annotated metabolic pathways than isolates in the low-BGE group. Furthermore, three pathways were unique to the high-BGE group (Table 1) and a number of pathways were correlated with the observed BGE (Table 2; Table S2). Our findings suggest that there are genomic features that may contribute to or regulate BGE.

In general, the genomic composition of BGE groups appears to reflect differences in cellular biosynthesis. It is possible that species with particular biosynthesis pathways may generate essential cellular components with less energetic demand. For example, the low-BGE isolates lacked some metabolic pathways, including pyridoxal biosynthesis and histidine degradation, which were present in the high-BGE group. The pyridoxal biosynthesis pathway produces vitamin B_6 from erythrose-4-phosphate (Mukherjee *et al*., 2011). Because vitamin B_6 is essential for growth, the isolates lacking the pyridoxal pathway must use alternatives such as uptake from the environment if they are auxotrophic (i.e., unable to synthesize) or other synthesis pathways such as the deoxyxylulose-5-phosphate synthase (DXS) pathway (found in all but three of the genomes in this study; Table S4; Mukherjee *et al*., 2011). However, the DXS pathway requires pyruvate (a precursor for Krebs cycle) and thus may limit central metabolism and possibly lead to lower BGE. Likewise, the histidine degradation pathway is used to breakdown histidine into ammonium and glutamate (Bender, 2012). Alternatively, glutamate can by synthesized from α-ketoglutarate; however, because α-ketoglutarate is an intermediate component of the Krebs cycle this may limit central metabolism and possibly lead to reduced BGE if the energetic requirements are maintained but the ability to recycle biomass is reduced.

Conclusion

A trait-based approach can provide a mechanistic link between the structure and function of bacterial communities. At the cellular level, BGE reflects the balancing of energetic and cellular growth demands. We found

evidence of this based on physiological trade-offs (i.e., maximum growth rate) as well as metabolic pathways. As such, changes in community composition and resource availability have the potential to alter the food web and ecosystem function due to changes in BGE. For example, communities dominated by species with low BGE should yield a higher net release of CO_2 from the ecosystem. Alternatively, communities comprised of individuals with high BGE could yield increased carbon and energy flow within aquatic food webs, depending on trophic transfer efficiency. However, variation in BGE can arise within a species due to the ways in which it processes different resources. Therefore, changes in the resource supply will alter the performance of individual taxa, but we predict that these changes will not be as strong as changes in BGE that arise owing to differences in community composition. Our results highlight how bottom-up, trait-based approaches may be useful for understanding complex microbial communities in nature.

Methods

Bacterial isolates

Using a novel cultivation approach, we isolated 20 bacterial strains from lakes in the Huron Mountain Research Preserve (Powell, MI, USA) by incubating inert carbon beads (Bio-Sep Beads) in the water column for 1 week. Prior to the incubations, the beads were saturated with a sterile, complex-carbon substrate, i.e. Super Hume (CropMaster, United Agricultural Services of America, Lake Panasoffkee, FL, USA). Super Hume is a lignin-rich resource comprising 17% humic and 13% fulvic acids and has been shown to be an analog of terrestrial DOC in aquatic ecosystems that can be used by diverse bacteria (Lennon *et al*., 2013). We used this enrichment technique to select for bacteria with a range of metabolic potentials (Ghosh *et al*., 2009). After the incubation, beads were rolled on R2 agar plates (BD Difco, Sparks, MD, USA) and incubated at 25 °C. Random colonies from plates were picked and serially transferred until axenic. All isolates were preserved in 25% glycerol at −80 °C.

Each bacterial strain was identified by direct sequencing of the 16S rRNA gene using genomic DNA extracted from log-phase cultures using the FastPrep DNA extraction kit (MP Biomedical). After amplifying the 16S rRNA gene using the 27F and 1492R bacterial primers (See for primer sequences and PCR conditions), we sequenced the PCR products at the Indiana Molecular Biology Institute (IMBI) at Indiana University (Bloomington, IN, USA). Raw sequence reads were quality-trimmed based on a Phred quality score of 25. Forward and reverse reads were manually merged after aligning sequences to the Silva 16S SSU rRNA reference alignment (release 132) using SINA v. 1.2.11 and the Bacteria variability profile. After merging into full-length 16S rRNA sequences, we used mothur (Schloss *et al*., 2009) to check the quality of sequences, and alignments were checked using the ARB software package (Ludwig *et al*., 2004). Finally, sequences were compared with the Silva All-Species Living Tree Project database (Yilmaz *et al*., 2014) for taxonomic identification (Fig. S2).

Bacterial growth efficiency

We measured BGE for each isolate when supplied with one of three different carbon substrates: glucose, succinate, or protocatechuate (Fig. S1). These carbon sources (i.e., resources) were chosen based on differences in their bioavailability and structure but also the required pathways for metabolism (see Fig. S1). We measured bacterial respiration and production rates and then calculated BGE as BP/(BP + BR), where BP is bacterial productivity and BR is bacterial respiration (del Giorgio and Cole, 1998). BP and BR were measured using triplicate cultures of each isolate. Cultures of each isolate were grown in R2 broth (BD Difco) until mid-log phase. Hundred microliters of culture was then transferred into 10 ml of M9 broth (Green and Sambrook, 2012) with the appropriate carbon source (25 mM C) and allowed 24 h to acclimate. We then transferred 100-μl of culture into 10 ml of fresh carbon-amended M9 broth and incubated 1–3 h to replenish nutrients. Using these transfers, we were able to establish populations of each isolate at target cell densities between 10^4 and 10^5 cells ml^{-1}. We used the populations to measure BP and BR, which were normalized to cell density using plate counts of colony-forming units. BP was measured using the ^{3}H-Leucine assay (Smith and Azam, 1992) with 1.5 ml of cultures in triplicate. We added ^{3}H-Leucine to a final concentration of 50 nM and incubated for 1 h. Following incubation, production was terminated with trichloroacetic acid (final concentration 3 mM), and we measured leucine incorporation using a liquid scintillation counter. BR was measured using an automated O_2 measurement system (PreSens Sensor Dish System, PreSens, Regensburg, Germany) with 5 ml of cultures in triplicate. We estimated BR based on the slope of O_2 concentration (i.e., O_2 consumption rate) during a 3-h incubation using linear regression. A respiratory quotient conversion factor was used to convert O_2 depletion into C respiration assuming aerobic growth (del Giorgio and Cole, 1998).

Taxonomic and phylogenetic relationships

We compared differences in BGE across isolates and resources using linear models. First, a taxonomic

framework was used to compare BGE between isolates (Lennon *et al*., 2012). Isolates were classified into taxonomic groups based on the species tree constructed in ARB. Mixed linear models were used to compare BGE across taxonomic groups and resources. To test the hypothesis that taxonomy (i.e., at the order level) affects BGE, we nested resource type within isolate in the linear model. To test the hypothesis that the specific resource used affects BGE, we nested isolate within resource type. The best statistical models were identified based on the variation explained (R^2) and AIC values. Second, we tested if phylogenetic relationships between isolates could explain the differences in BGE across isolates. We created a phylogenetic tree based on the full-length 16S rRNA gene sequences. Sequences were aligned using the SINA aligner (Pruesse *et al*., 2012) and checked alignments using ARB. A phylogenetic tree was generated using RAxML (v.8.2.12; Stamatakis, 2006) using the CIPRES science gateway (Miller *et al*., 2010). We used the GTRGAMMA DNA substitution model and the rapid hill-climbing algorithm to build our maximum likelihood trees and used the extended majority rule to find the consensus tree. Blomberg's K and Pagel's Lambda were used to compare trait variation (as a continuous variable) across the tree and test if phylogenetic relationships between isolates could explain differences in traits (Pagel, 1999; Blomberg *et al*., 2003). Blomberg's K is a test for the phylogenetic signal that determines if trait variation is better explained by phylogenetic relationships or Brownian motion. Pagel's Lambda is a test of phylogenetic signal that determines if trait variation differs from Brownian motion. Last, to determine if the distribution of BGE across isolates was unimodal, we used Hartigan's dip test for unimodality (Hartigan and Hartigan, 1985). Hartigan's dip test is used to determine if a distribution is unimodal by testing the null hypothesis that there is a dip in the distribution. A significant Hartigan's dip test would suggest that the distribution is unimodal. Alternatively, the distribution has an internal 'dip' (reported as D). All statistical tests were conducted in the R statistical environment (R Core Development Team, 2013). We used the *nlme* package (Pinheiro and Bates, 2011) for the mixed-effects linear models, the *picante* package (Kembel *et al*., 2015) for the phylogenetic methods and the *diptest* package (Maechler, 2015) for Hartigan's dip test.

Phenotypic comparisons and trade-offs

To test the hypothesis that phenotypic differences and physiological trade-offs underlie BGE variation, we compared the maximum growth rate (μmax) and niche breadth of each isolate. First, to test whether BGE was affected by growth strategy (i.e., copiotrophs vs. oligotrophs), we measured the maximum growth rate of each isolate. Bacterial growth rates were measured based on changes in optical density during 18-h incubations. Bacterial isolates were grown in R2 broth in 48-well plates. Plates were incubated with continuous shaking and optical density was measured every 15 min using a plate reader (Synergy MX, BioTek, Winooski, VT, USA). Growth curves were analysed by fitting a modified Gompertz growth model (Zwietering *et al*., 1990; Lennon, 2007) to the observed growth curves using maximum likelihood fitting. We used the model fit as our estimate of μmax.

Second, to test whether BGE was affected by niche breadth, we generated carbon usage profiles using BioLog EcoPlates (Garland and Mills, 1991). The EcoPlate is a phenotypic profiling tool consisting of 31 unique carbon sources. In addition to the carbon source, each well contains a tetrazolium dye, which in the presence of NADH is reduced resulting in a colour change. This colorimetric assay was used to generate carbon usage profiles for each strain. We standardized profiles for each strain by subtracting water blanks (average water blank +1 SD), and relativizing across substrates. Using these data, resource niche breadth was calculated using Levins Index (Colwell and Futuyma, 1971).

We used an indicator variable linear regression to test for changes in the BGE rate due to the maximum growth rate and niche breadth. The BGE group (high- versus low-BGE) was included as the categorical predictor and BGE as the continuous predictor (Lennon and Pfaff, 2005). In addition, we included the interactions term. Where the interaction term was significant, the main effects are reported for each categorical predictor (i.e., BGE group). All statistical tests were conducted in the R statistical environment.

Genomic comparisons

To test the hypothesis that variation in metabolic pathways could explain differences in BGE, we compared the genomes of each isolate. First, whole-genome sequencing was used to characterize each isolate and determine the metabolic pathways present in the genome. Genomic DNA libraries for each isolate were prepared using the Illumina TruSeq DNA sample prep kit using an insert size of 250 bp. Libraries were sequenced on an Illimina HiSeq 2500 (Illumina, San Diego, GA) using 100-bp paired-end reads at the Michigan State University Research Technology Support Facility. Raw sequence reads (FASTQ) were processed by removing the Illumina TruSeq adaptors using Cutadapt (Martin, 2011), interleaving reads using Khmer (McDonald and Brown, 2013) and quality filtering based on an average Phred score of 30 using the

FASTX-toolkit (Hannon Lab, 2010). Finally, we normalized coverage to 25 based on a k-mer size of 25 using Khmer and assembled the genomes using Velvet (Zerbino and Birney, 2008) after optimizing assembly parameters for each isolate with Velvet Optimizer (Gladman and Seemann, 2012). We annotated contigs larger than 200 bp using Prokka (Seemann, 2014) and predicted metabolic and physiological functions using MAPLE with bidirectional best-hit matches (Takami *et al*., 2012). Functional pathways (modules) were identified based on the presence of intermediate genes within a pathway and scored pathways as present if more than 80% of the intermediate genes were recovered based on the module completion ratios.

To test the hypothesis that metabolic pathways affect BGE, we used multivariate methods to compare the pathways of each isolate. First, PERMANOVA was used to determine if there were differences in pathways associated with the different levels of BGE, and indicator species analysis (Dufrene and Legendre, 1997) was used to determine which metabolic pathways contributed to group-level differences in BGE. Next, to determine if metabolic pathways could explain differences in BGE within a group, we used distance-based redundancy analysis (dbRDA), which is a multivariate technique that tests if a quantitative predictor can explain differences in multivariate datasets (Legendre and Legendre, 2012). Because pathways were scored as present or absent, metabolic distances between isolates were calculated using the Jaccard Index. Significance was tested using a permutation test. If the dbRDA model was significant, Spearman's rank-order correlation was used to test for correlations between BGE and individual metabolic pathways. We used the *vegan* R package (Oksanen *et al*., 2012) for multivariate analyses.

Resource effects

To test the hypothesis that resources have different effects on components of metabolism that affect BGE, we used a linear model to test for a relationship between BR and BP. Because BP requires energy through respiration, the production rate was used as the dependent variable and respiration rate as the independent variable. An indicator variable linear regression was used to test for changes in the BP rate due to BR. We included the specific resource used and group (high- versus low-BGE) as the categorical predictors and BR as the continuous predictor (Lennon and Pfaff, 2005). In addition, we included all interaction terms. Respiration and production rates were $\log_{10}$-transformed to meet model assumptions. Last, to determine if the relationship between BR and BP rates was isometric (proportional scaling, slope = one) or allometric (disproportional scaling, slope = one), a one-sample *t*-test was used to determine if the slope was different from one. All statistical tests were conducted in the R statistical environment.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supporting information
Appendix S2: Supporting information
Appendix S3: Supporting information