Prokaryotes Become Larger at High Temperatures but They Do Not Grow Faster

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Abstract

Metabolic theory posits that metabolism governs the rate at which organisms transform energy into biological work and growth. Thus, it constitutes the main mechanism driving the evolution of organismal growth and size across almost all domains of life. One general prediction of metabolic theory suggests that populations of larger organisms grow more slowly than populations of smaller organisms. However, increasing evidence show that prokaryotes seem to be the exception for such a trend. Larger prokaryotes appear to grow faster, challenging the standard theory and stimulating a further reevaluation of the current evidence. Here, I report a broad comparative analysis of the evolution of growth rate and cell size in prokaryotes. As opposed to previous investigations, my analysis relies on the concept of the thermal performance curve and the effects of its parameters on growth rate and cell size. Based on such approach, I found that prokaryotes evolved larger sizes at relatively high grow temperatures but their growth rates do not scale with size. At the optimum temperature for growth, the relationship between growth rate and cell size becomes unclear. These results call for a careful interpretation of the current evidence and highlight the importance of understanding the thermal sensitivity of the biochemical reactions that take place in the cells. Specifically, the metabolic reactions that regulate the protein synthesis in the cell, which are controlled by the translation machinery. In this regard, this study describes how different components of this machinery, such as the rRNA genes and the tRNA genes, interact to shape the evolution of growth rate and cell size across prokaryotes. Overall, I present more accurate results compared to previous evidence and suggest new hypotheses that can be applied to a wide range of taxa, paving the way for others to validate them at the intraspecific level.

Keywords: Archaea, Bacteria, life history, RNA, thermal adaptation.

Introduction

A long-standing prediction of metabolic theory suggests that populations of larger organisms grow more slowly than populations of smaller organisms (Savage et al., 2004). A general thought is that metabolism is responsible for this trend because it determines the rate at which organisms transform energy into biological work and growth (Hatton et al., 2019; Damuth, 1981; Savage et al., 2004). Because larger organisms have lower metabolic rates relative to their size, one may expect them to grow slower as described by the following mathematical model:

$$r = M^{B-1}$$

Where r (min^{-1}) corresponds to the intrinsic growth rate, M constitutes the mass of the organisms (gr), and B an exponent linking their mass to metabolic rate. In theory, absolute metabolism scales hypoallometrically with body size with an exponent of B, whereas mass-specific metabolism scales at $B - 1$. This theory holds for many organisms, ranging from multicellular metazoans and plants, to unicellular eukaryotes. For instance, in a comprehensive analysis of the scaling laws across multicellular eukaryotes, Hatton et al. (2019) showed that B is typically ≈ 0.75. Thus, r should scale around -0.25. Similarly, Lynch et al. (2022) showed that for five unicellular eukaryotic groups of heterotrophs, B is -0.208. Accordingly, the data for this broad set of heterotrophs are consistent with the view that growth rate declines approximately as the -0.208 power of adult mass. Contrary to most organisms, however, some evidence suggest that unicellular prokaryotes show a hyperallometric scaling between growth rate and adult mass with an exponent of 0.286 (Lynch et al., 2022), chal-

lenging the prevailing views of metabolic theory and encouraging us to look for an alternative theory that can be applied to all domains of life.

Although metabolic theory successfully predicts variation in growth and size across many taxonomic groups, these predictions are often based on general measures of metabolism, such as oxygen consumption (in $joules \times cell^{-1} \times min^{-1}$; Marshall et al., 2022) or activation energy estimates (in $Watts$; Hatton et al., 2019). But little do we know about the metabolism of protein synthesis, which provides a more intuitive link to the growth of unicellular organisms, because much of the cell content corresponds to proteins Bremer and Dennis (2008). Because ribosomes are required for protein synthesis, their number and their rate of function determine the rate of protein synthesis and cytoplasmic mass accumulation. Mathematically, the relationship between growth rate (u) and the translation machinery of an organism can be described as follows:

$$u = (60/ln2) \times (N_r/P) \times e_r$$

Where the growth rate of an organism u (min^{-1}) equals the product of the number of ribosomes per amount of total protein present (N_r/P), times the rate of protein synthesis per average ribosome, known as "ribosome efficiency" (e_r; Bremer and Dennis, 2008). Consistent with this idea, Schaechter et al. (1958) showed that the amounts of RNA and proteins per cell are tightly linked to the growth rate of a bacterium. More recently, the relationship between the translation machinery and growth rate has received increasing attention. For instance, Lynch et al. (2022) provided a hypothetical upper bound on growth rates across prokaryotes and unicellular eukaryotes dictated by the translational constraints imposed by the properties of ribosomes. Considering the time required for ribosomal-protein replacement, the authors suggest an upper limit to the exponential growth rate of $50/day$ for prokaryotes, and an upper

limit to growth rate of ≈ 2.25x lower in eukaryotes than that for prokaryotes (i.e., $20/day$).

As described earlier, inherent to the link between growth and size of an organism is the role of metabolism, which depends on temperature (Angilletta, 2009). Temperature determines the proportion of reactants that possess the free energy required for reaction. Thus, as the temperature increases, more reactants exceed the energy of activation. Because enzymes lower the energy required for activation and thus speed the reaction at any given temperature (Hochachka and Somero, 2002), the metabolic rate and hence the growth rate of an organism should scale proportionally with the growth temperature. In fact, comparative analyses of the evolution of growth rate across many taxa may only be accurate when growth rate is normalized to a specific temperature for growth (e.g., Lynch et al., 2022; Hatton et al., 2019). However, this temperature correction seems to be done arbitrarily in some cases, leading to potential misleading results because no two organisms have exactly the same thermal restrictions (Angilletta, 2009). A better approach may be to consider the thermal performance curves of the organisms involved in the investigation. One can capture the general characteristics of the thermal performance curves with specific parameters: (1) the thermal optimum (T_{opt}); (2) the thermal limits, referred to as the critical thermal minimum (CT_{min}) and the critical thermal maximum (CT_{max}). Ideally, comparisons across many taxa should be done at the optimum temperature for growth, assuming that organisms would likely perform best at such temperature.

Here, I examine the evolution of growth rate and cell size across prokaryotes based on a large comparative analysis. In doing so, I test some of the principal predictions of metabolic theory and present results that challenge previous findings. Particularly, this analysis links the evolution of growth and cell size to important foundational features of biology that have

not been properly considered in previous investigations, such as the translation machinery and the thermal sensitivity of organisms. Accordingly, this study provides novel insights that enable us to better understand the evolution of the life history in prokaryotes.

Materials and Methods

Data source and description of variables

Trait datasets are increasingly being used in studies investigating evolutionary theories and global conservation initiatives (e.g., `https://opentraits.org/datasets.html`). These datasets allow for integrating a diverse range of genomic, physiological, ecological, morphological, and life-history data to explore organismal evolution. This study used a unified microbial trait dataset suitable for investigating evolutionary correlations between traits across many species. The dataset spans the full range of bacterial and archaeal habitats, including fresh and marine waters, soils and sediments, animal and plant hosts, and thermal environments (Madin et al., 2020). Data sources include well-established repositories, such as GenBank, Bergey's Manual of Systematics of Archaea and Bacteria, and a number of compilations published in the primary literature (e.g., Lynch et al., 2023; Gonzalez-de Salceda and Garcia-Pichel, 2021).

To study the evolution of growth rate and cell size across prokaryotes, I collected data of minimum doubling time ($log_{10}\ h$), cell volume ($log_{10}\ um^3$), and cell diameter ($log_{10}\ um$) for the species under investigation. The minimum doubling time is best interpreted as a key life-history trait associated with the r/K selection theory (Boyce, 1984), having relevant connection to the metabolic rate of organisms. With data of doubling time (T_d), one can easily compute the intrinsic growth rate (r) as follows: $r = \frac{ln(2)}{T_d}$. In prokaryotes, cell volume

might be the only obvious difference between some species. Unfortunately, data of cell volume across the domains of life are rather scarce. By contrast, cell diameter seems to be the most common proxy for cell size across prokaryotes. Interestingly, cell diameter is a good predictor of cell volume in some species (Figure S1), enabling us to use it as a good indicator of cell size in this study.

To investigate the scaling relationship between growth rate and metabolism, I examined the translation machinery of an organism; here referred to as the number of ribosomal RNA genes (rRNA) and transfer RNA genes (tRNA). The rRNA genes are often assumed to be a robust measure or proxy for ribosome content in prokaryotes (Bremer and Dennis, 2008). Indeed, ribosomal gene copy number per genome seems to correlate with a microbe's life-history traits, where fast growth is associated with higher copy number (Klappenbach et al., 2000; Roller et al., 2016). By contrast, the effects of tRNA gene copy number on growth has received less attention in prokaryotes. This might be associated with the fact that 14% of the total stable RNA in a cell is tRNA, and 86% is rRNA Bremer and Dennis (2008).

Lastly, I examined the effect of temperature on the evolution of growth rate and cell size across prokaryotes. To do so, I used specific parameters of the thermal performance curves of each species. Performance can be defined as any measure of an organism's capacity to function, usually expressed as a rate or probability (Angilletta, 2009). Common measures of performance include growth and cell size. To accurately compare the evolution of these traits among species, I analyzed the intrinsic growth rate of the species (r) and cell diameter (mu) at the optimum temperature for growth ($log_{10}\ C^{\circ}$). The same procedure was done at temperatures below and above the optimum; here referred to as lower temperature and upper temperature, respectively.

Comparative analysis

Because species are part of a hierarchically structured phylogeny, and thus cannot be regarded for statistical purposes as if drawn independently from the same distribution (Felsenstein, 1985), I used a subset of recently published phylogenies available in the primary and secondary literature (see `https://timetree.org/`), enabling me to account for similarity by descendant. Most of the sources I compiled consisted of dated posterior tree distributions derived to infer evolutionary relationships for Archaea and Bacteria. I produced trees with subsets of taxa based on the species for which I had available data. These trees were rescaled to absolute time and then joined to form a full species-level tree of prokaryotes (e.g., Figure 1). To do so, I used the Build a Timetree function available at The TimeTree of Life database (see url link above), which outputs a timetree of the taxa of interest extracted from the global timetree connecting species and publication-specific timetrees in the database. For instance, one can input a species list or simply enter a taxon name to see the clade-specific portion of the global timetree. Also, one can restrict the timetree produced to contain tips at a desired taxonomic level, including species, genus, or family.

Figure 1: Phylogenetic relationship between some of the species involved in this study.

Statistical Analysis

I used phylogenetic generalized least squares (PGLS) to model the evolution of growth rate and cell size across prokaryotes. As mentioned earlier, PGLS models enable one to account for non-independence of the data (Felsenstein, 1985). To do this, I used the *gls* function from the "nlme" package of R, v. 4.2.2 (Team, 2000; Pinheiro et al., 2017). I fitted all models assuming the species trait values evolved via a Brownian motion model. Alternatively, I used

the standard ordinary least squares regression method (OLS; type I regression) to calculate regression statistics, which is the standard approach in bivariate power law regression (Sibly et al., 2012). But this method assumes that all error is in the Y-axis variable. This tends to underestimate the slope as error in the X-axis variable increases. To evaluate the models' goodness of fit, I used information-theoretic criteria such as AIC_c and selected the most likely one for inferences.

Results

A phylogenetic-informed model describing the relationship between cell volume and intrinsic growth rate was strongly supported, as opposed to a standard OLS regression model. Specifically, the model indicates that growth rate increased as cell size increased (Figure 2A). However, this result may be misleading because the data of cell volume consisted of species that grew at different temperatures, preventing one from making accurate comparisons. For species that grew at their optimum temperature, the analyses revealed no significant relationship between growth and cell diameter (Table 1; Figure 2B).

Table 1: Scaling coefficients estimated by a model describing the evolution of cell size and growth rate in prokaryotes.

Figure 2: Scaling relationship between cell size and growth rate across prokaryotes. (A) Scaling of cell volume with growth rate across species grown at different temperatures. (B) Relationship between cell diameter and doubling time across species grown at their optimum temperature ($n = 40$). Regression lines were displayed only when significant effects were observed.

The evolutionary relationship between growth rate and the translation machinery shows

that both the rRNA genes and the tRNA genes increased with growth rate. Though, the rate of increase in rRNA genes was faster than that of tRNA genes in species that grow faster (Figure 3). This evidence supports the existence of an interaction between rRNA genes and tRNA genes across the range of growth rates examined (Table 2). In other words, fast-growing prokaryotes tend to have a high number of rRNA genes but a low number of tRNA genes. By contrast, slow-growing prokaryotes tend to have a low number of rRNA genes but a high number of tRNA genes (Figure 3).

Table 2: Contrast of parameters estimated by an OLS regression model describing the evolution of the translation machinery as a function of growth in prokaryotes.

Figure 3: Relationship between growth rate and the translation machinery in prokaryotes ($n = 413$).

Strikingly, I found that cell size increased with temperature, but only when organisms grew at temperatures higher than the optimum temperature for growth (Table 3; Figure 4A). Importantly, this model only accounted for an effect of cell size alone. A model that examined the interaction between cell size and growth temperature was not supported by the data. When grown at any of the temperatures suggested by the thermal performance curves, organisms seemed to grow generally faster regardless of their cell sizes (Figure 4B).

Table 3: Scaling coefficients estimated by an OLS regression model of the evolution of cell size as a function of temperature in prokaryotes.

Figure 4: Evolutionary relationships between cell size, growth, and temperature across prokaryotes. (A) From left to right, prokaryotic cell size as a function of the lower, optimum, and upper temperatures of the species. (B) Similarly, the bottom panel shows the relationship between growth and the aforementioned temperatures. Regression lines were displayed only when significant relationships were observed.

Discussion

Although previous evidence from interspecific and intraspecific studies suggest that growth rate increases with cell size in prokaryotes, this study shows no evidence of such trend. Instead, I found that larger cells only evolved at relatively high temperatures, whereas faster growth generally evolved at any of the temperature ranges examined regardless of the size of organisms. A hypermetric correlation between cell size and cell growth contradicts the expectation based on standard theory, but plausible hypotheses in favor of this observation have been proposed by some researchers. First, Marshall et al. (2022) suggested that larger cells of *Escherichia coli* are cheaper to maintain and build per unit volume, such that the scaling of the total cost of production is far less than proportional to cell size. Second, DeLong et al. (2010) hypothesized that the relatively large genomes of prokaryotes enable them to produce a large number of enzymes involved in more complex biochemical networks. These networks would confer them an increased metabolic power because enzymes could bind to substrates more completely, thereby producing more ATP molecules per unit substrate and per unit time. Although the current literature provides compelling arguments about the unexpected hypermetric correlation between cell size and and cell growth in prokaryotes, none of them consider the greater catalytic capacity of biochemical systems operating at high temperatures; a capacity which in itself may have been an important factor in the evolution of fast growth and large cells in prokaryotes. Furthermore, the idea that an increasing genome size with cell size might explain the hypermetric scaling of growth with cell size in prokaryotes sounds appealing, but a detailed analysis on the mechanics of metabolism-related genes that

affect growth and cell size is still needed. Below, I not only describe the ways in which protein-synthesis genes interact to shape the evolution of growth and cell size in prokaryotes, but also, I provide a comprehensive discussion about the effects of temperature on their life history.

Among all of the metabolism-related genes present in the genome of an organism, the rRNA genes and the tRNA genes are perhaps some of the most important ones because they regulate the production of macromolecules required for growth and cell size. Consistent with this idea, a wealth of evidence seem to indicate that the amounts of RNA genes and protein scales with growth and cell size (Klappenbach et al., 2000; Bremer and Dennis, 2008; Lynch and Marinov, 2015). Furthermore, early studies suggest a growth limitation imposed by DNA concentration in the cell, such that DNA limits mRNA synthesis and mRNA limits protein synthesis (Maaløe, 1979). But that claim is no longer supported by the current literature. By contrast, the ribosome concentration and the protein synthesis rate per average ribosome, both of which are regulated by the RNA genes, seem to be growth limiting (Bremer and Dennis, 2008). Given the high demand for rRNA transcription and the central role of rRNAs in the regulation of ribosome synthesis, an increasing rRNA gene copy number should scale with growth, because it dictates how quick microbes can synthesize ribosomes (Klappenbach et al., 2000). Indeed, the results of this study show that both the rRNA genes and the tRNA genes increase with growth among prokaryotes. Interestingly, the tRNA genes increase with growth at a lower rate than that of the rRNA genes, supporting the idea that in slow-growing species there appears to be a slight excess in the synthesis rate of stable RNA, such that the excess rRNA is rapidly degraded, whereas the tRNA accumulates (Jinks-Robertson et al., 1983; Norris and Koch, 1972). In sum, the translation machinery of the cell is rather complex and a better understanding of its regulatory effects on growth and cell size may only be

attained if we examine not only the variation in RNA genes, but also the thermal conditions in which the protein synthesis occurs.

Temperature constrains the rates of biochemical reactions in the cell, leading to different thermal sensitivities of growth and cell size across prokaryotes. While prokaryotic cells function within a range of $-5°C$ to $110°C$ (Jaenicke, 1991, 1993), eukaryotic cells are relatively restricted, tolerating temperatures between $-2°C$ and $60°C$ (Tansey and Brock, 1972). The wide range of variation in the thermal tolerance of prokaryotes may then reflect important differences in growth and cell size across species. This study shows that both growth and cell size span three orders of magnitude across species. Such variation provides good conditions for natural selection to operate. In general, natural selection favors mutations that alter the conformational stability of enzymes (Hochachka and Somero, 2002; Marx et al., 2007). A more flexible structure helps enzymes to change shape faster during catalysis. If enzymes with greater conformational stability function better at high temperatures (Fields, 2001; Somero, 1995), species should evolve larger sizes at a relatively high temperature for growth, as indicated by the results of this study. Such hypothesis could be validated if growth and cell size are compared between Archaea and Bacteria. Because there is evidence of adaptation to high temperatures across the evolutionary history of archaeal species (Groussin and Gouy, 2011), one should expect those species to exhibit larger sizes than do species of the bacterial domain. My analysis supported this expectation; on average, archaeal species are larger than bacterial species, but bacteria seem to grow faster (Figure 5). On one hand, this result is consistent with the prediction that populations of large organisms grow more slowly than populations of small organisms. On the other hand, this findings align with the idea that "the hotter is better", which proposes that genotypes or species with relatively high optimal temperatures also have relatively high maximal performance or fitness (Savage et al., 2004;

Angilletta, 2009; Kingsolver and Huey, 2008). As discussed earlier, this argument is based on empirical evidence suggesting that metabolic reactions inevitably increase with absolute temperature. Consequently, maximum biochemical reaction rates of species adapted to warm temperatures are higher than those of species adapted to cold temperatures, when each is measured at its optimal temperature (Kingsolver and Huey, 2008).

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Figure 5: Violin plots displaying the distribution of growth and cell size in prokaryotes. (A) Mean difference in growth between Archaea and Bacteria. (B) Mean difference in cell size between Archaea and Bacteria. The dashed lines represent mean values (μ).

Based on a broad comparative analysis, this study evaluates the intriguing superlinear scaling of growth rate with cell size in prokaryotes. This pattern challenges the predictions of metabolic theory, motivating me to conduct a further examination of the data currently available in the literature. Contrary to most studies, I found that larger cells evolved at a relatively high temperature for growth. However, there seems to be no relationship between growth and cell size across species at an optimum temperature for growth. As opposed to most studies, my analyses account for important characteristics of the thermal biology of organisms, enabling me to provide more reliable evidence. In addition, this study describes novel ways in which the translation machinery may affect the evolution of life-history traits in prokaryotes, paving the way for others to validate my results at the intraspecific level. For instance, the idea that "hotter is better" seems plausible, but little evidence in favor of its predictions is available in microbes. Similarly, the thermal sensitivity of the metabolic reactions that control protein synthesis in the cell, and hence growth and organismal size, seems poorly understood. Because each species comprises a unique set of biochemical reactions, studying the thermal biology of many species would enable us to determine the severe limits that

temperature imposes on life.

Acknowledgements

N/A

Data Accessibility Statement

A fully reproducible workflow of the data analyses, including R scripts and additional supporting material, can be downloaded in the following repository: `https://dylan-padilla.github.io/cell-growth-size-paper/`, a Dryad link will be available upon acceptance .

Conflict of interest

The author declares no conflict of interest.

Author Contributions

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Tables with captions

Table 1: Scaling coefficients estimated by a model describing the evolution of cell size and growth rate in prokaryotes.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-1.305	0.169	-7.724	0.000
log_{10}(diameter)	0.169	0.139	1.214	0.232

Table 2: Contrast of parameters estimated by an OLS regression model describing the evolution of the translation machinery as a function of growth in prokaryotes.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	1.569	0.010	158.646	< 0.001
log_{10}(r)	-0.038	0.010	-3.734	< 0.001
log_{10}(rRNA)	0.359	0.015	24.426	< 0.001
log_{10}(r):log_{10}(rRNA)	0.117	0.019	6.281	< 0.001

A colon punctuation mark (:) denotes an interaction term.

Table 3: Scaling coefficients estimated by an OLS regression model of the evolution of cell size as a function of temperature in prokaryotes.

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.043	0.293	0.148	0.883
$log_{10}(T_{upper})$	0.615	0.199	3.087	0.002

Figures with captions

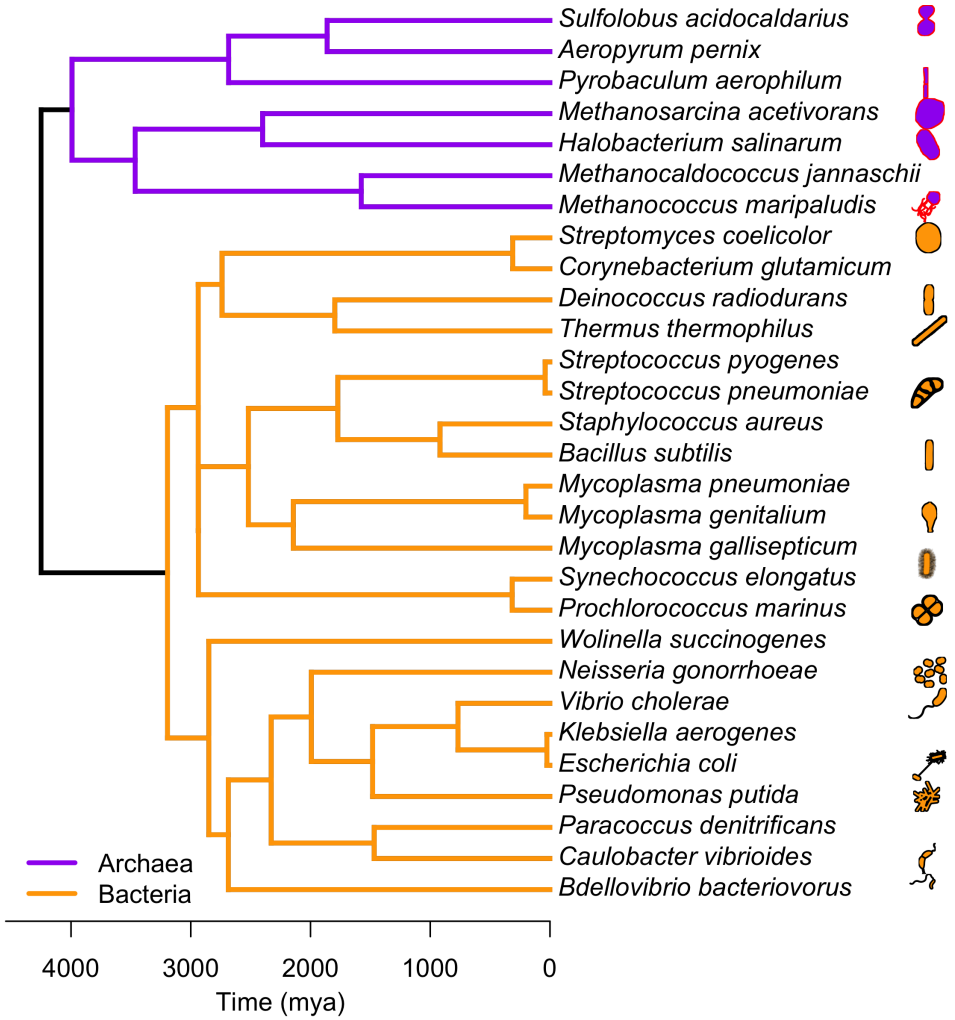

Figure 1: Phylogenetic relationship between some of the species involved in this study.

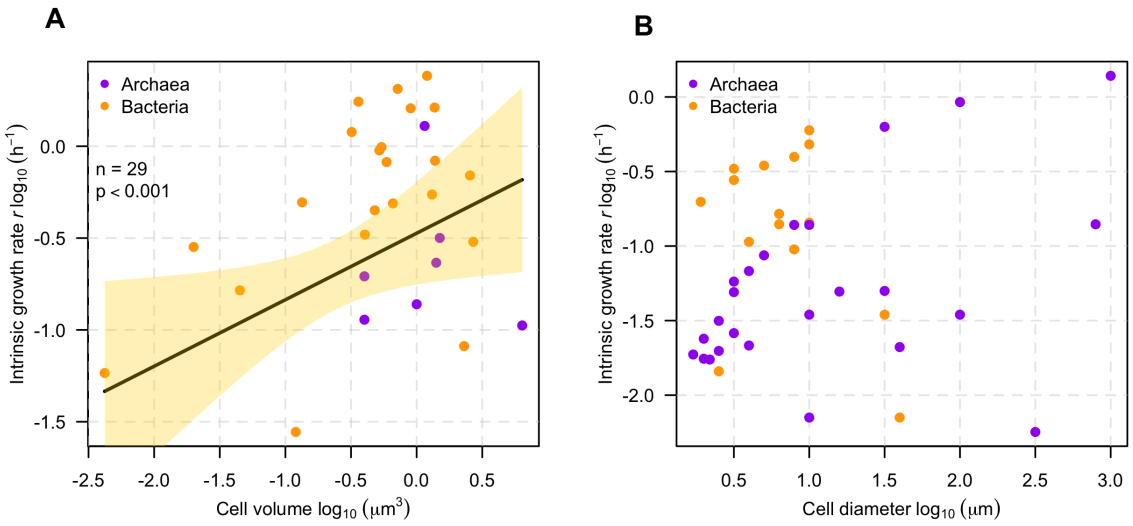

Figure 2: Scaling relationship between cell size and growth rate across prokaryotes. (A) Scaling of cell volume with growth rate across species grown at different temperatures. (B) Relationship between cell diameter and doubling time across species grown at their optimum temperature ($n = 40$). Regression lines were displayed only when significant effects were observed.

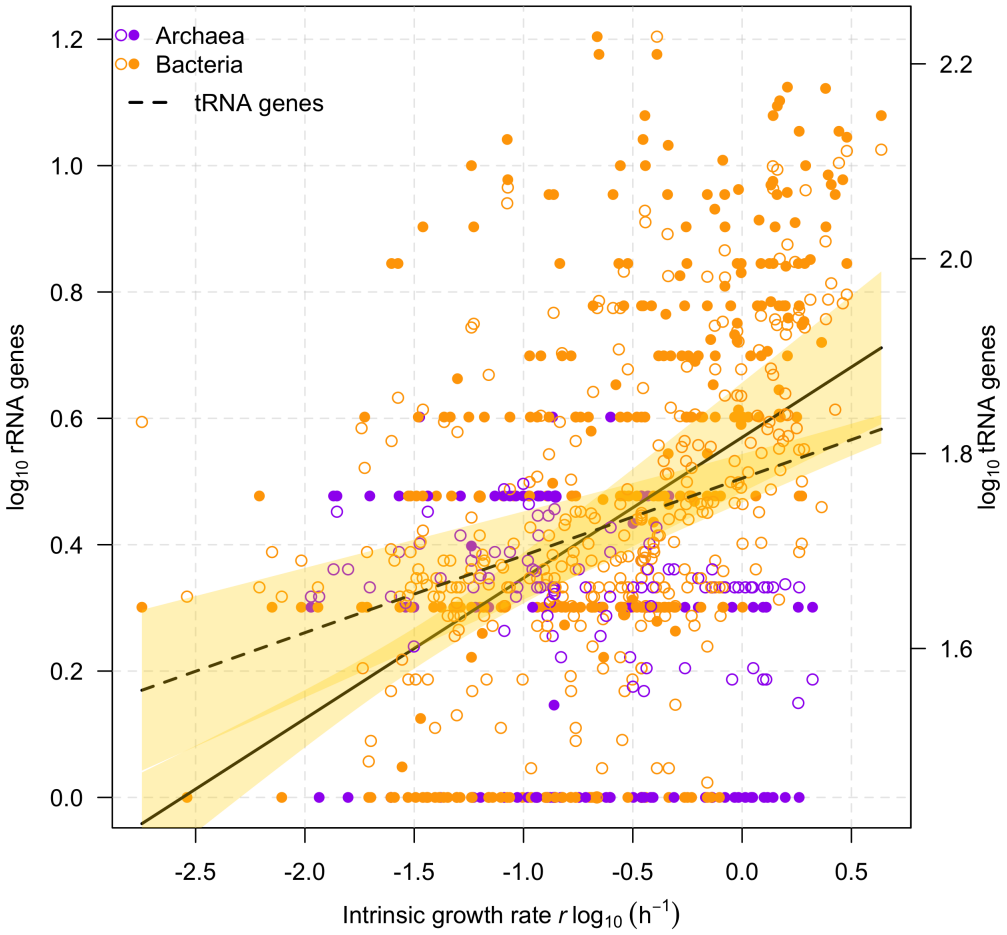

Figure 3: Relationship between growth rate and the translation machinery in prokaryotes ($n = 413$).

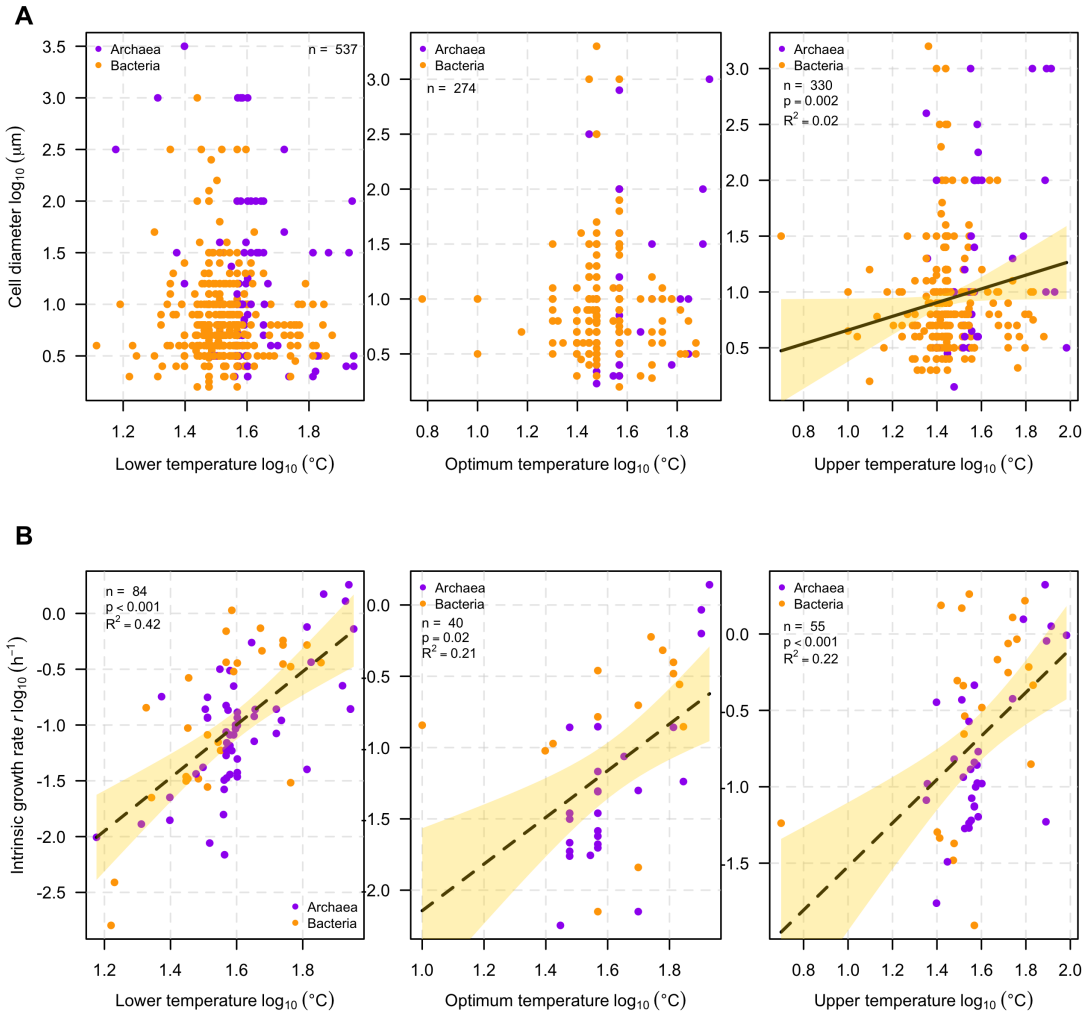

Figure 4: Evolutionary relationships between cell size, growth, and temperature across prokaryotes. (A) From left to right, prokaryotic cell size as a function of the lower, optimum, and upper temperatures of the species. (B) Similarly, the bottom panel shows the relationship between growth and the aforementioned temperatures. Regression lines were displayed only when significant relationships were observed.

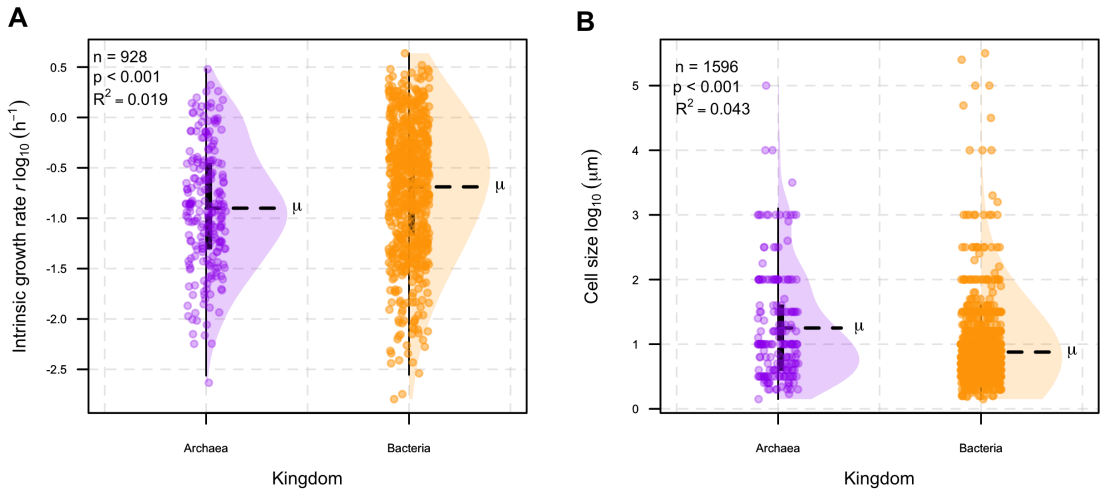

Figure 5: Violin plots displaying the distribution of growth and cell size in prokaryotes. (A) Mean difference in growth between Archaea and Bacteria. (B) Mean difference in cell size between Archaea and Bacteria. The dashed lines represent mean values (μ).

Supplementary material

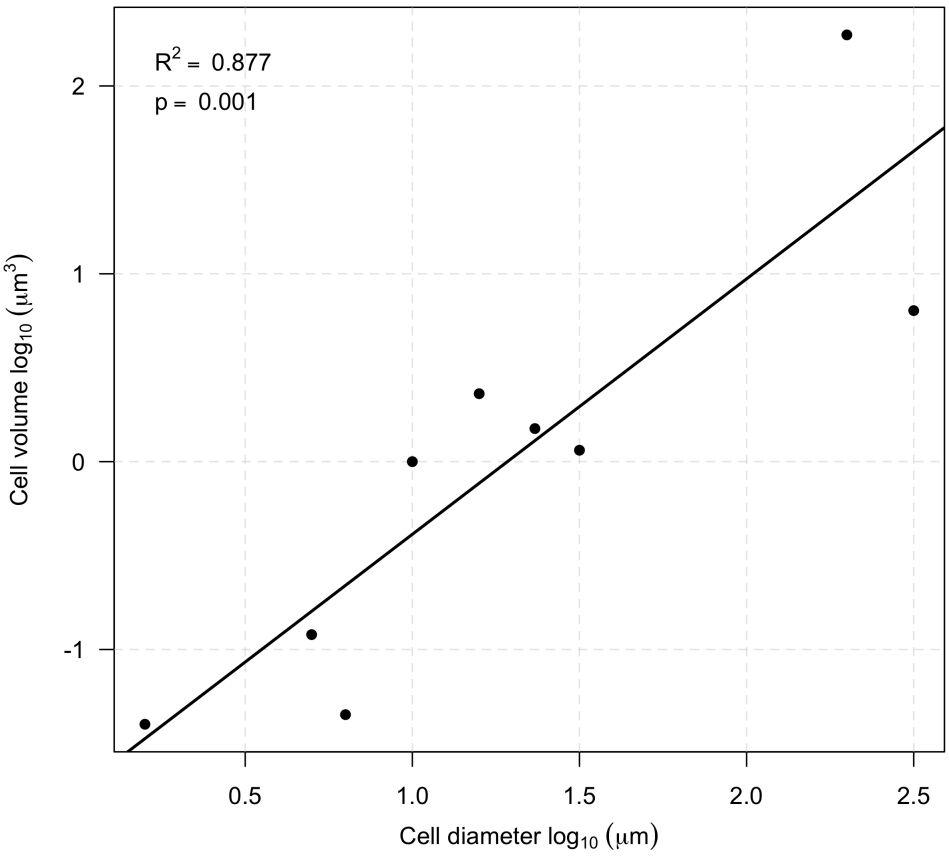

Figure S1: Correlation between cell diameter and cell volume in prokaryotes ($n = 9$).