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General Patterns in the Thermal Responses of Microbial Lag and Exponential Growth Phases

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IMPERIAL COLLEGE LONDON THESIS DECLARATION

I declare that this thesis represents my own work for the degree of Master at Imperial College London. It not substantially the same as any that I have submitted, or, is being concurrently submitted for other institution for a degree, diploma or other qualifications.

The data in this dissertation is provided to you by Dr. Samraat Pawar's laboratory, Department of Life Science, Imperial College London. All code, figures and analysis were done by me.

Abstract

Microorganisms play a vital role in ecosystems globally. Microbial population dynamics is the fundamental process of microbiology, understanding it helps us learn more about the underlying metabolic strategies. While throughout the whole life cycle temperature takes a critical role in constraining the biological rates, recent studies do not take this constraint into consideration. With a deeper understanding of the thermodynamic constraints on metabolic traits, we could improve those models to a level with higher practical application value. In this study, I aim to find general patterns of temperature-related metabolic traits. By comparing the thermal responses within and across species, I find that the intensity of adaptation is stronger observed in the lag phase, which means the ability to surpass the thermodynamic constraint is higher in the lag phase. Under the same conditions, microorganisms spontaneously putting more effort into overcoming the thermodynamic constraint in the lag phase. This adaptive phenomenon leads to interesting questions about how the underlying mechanisms allocate limited resources in coping with changing environments to make the organisms more competitive.

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1 Introduction

Microbes, as one of the earliest life forms, are ubiquitous on earth. They support all higher trophic organisms and maintain the health of the ecosystems (Whitman et al. 1998). Their immense diversity, varied responses to environmental change and fast-changing generation features make it a valuable research model organism. Conversely, it is because of its complexity and diversity, studying every specific microbial system may be arduous and unrealistic. Therefore, exploring the common physiological mechanisms under microorganisms may give us more insights.

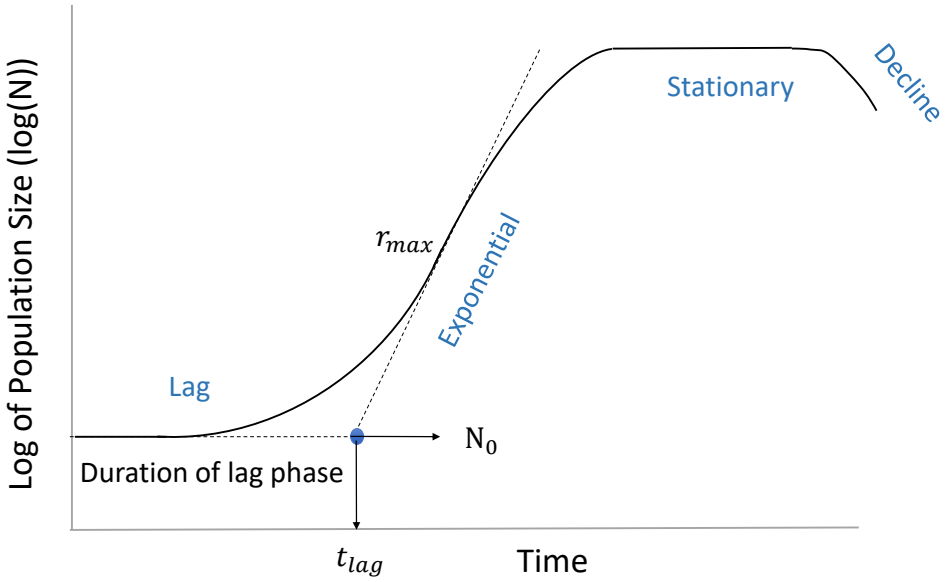

Figure 1: Typical Microbial Growth Pattern

Typical microbial growth pattern which can be divided into 4 phases: lag, exponential, stationary and decline phase. The sloped dashed line represents the maximum growth rate (r_{max}) which is the growth curve across the inflection point. This line intercept with the dashed horizontal line which shows the initial population size (N_0), gives back the blue dot has the x axis value defined as the duration of the lag phase (t_{lag}).

Microbial growth is a fundamental process in microbiology. Monitoring, interpreting and predicting the population growth of microbes inspire us to learn more about microbes. The growth curve of the microbial population can be divided into 4 phases (Fig. 1): lag, exponential, stationary and decline. When encountering a new environment, microbes do not proliferate immediately, they adapt themselves to it. After this period, the population comes into its cell doubling phase. In this phase, the number of new individuals in the population appears to be proportional to the last generation. This proportion measured in the infinitesimal generation time interval, in the mathematical form $\frac{dN}{dt}$, is defined as the growth rate. The biggest growth rate at inflection point in this phase is defined as the maximum growth rate (r_{max}). The population cannot grow infinitely, and becomes relatively constant in the subsequent phase called the stationary phase. It may come about because of some growth-limiting factors, such as resources depletion. Due to the same or any other reasons like cell damage as stationary phase, when the fertility lies below the mortality, the population passes into the decline

phase.

In those growth phases, the intriguing phenomenon is that after inoculation, the microbial cells do not duplicate instantly. Although this was observed by Müller in 1895, after more than a century, there is still too little knowledge about them has been gained. Understanding the lag phase can deepen our understanding of microorganisms and have an application value (Bertrand 2019). For instance, the lag phase is commonly taken as the preparation period to respond to the changing environment and some suggest that microorganisms will repair some damage in the lag phase (Rolfe et al. 2012). Understanding the strategies microbes take to cope with those damage or old cells may give back heuristic insight about ageing and longevity (Pin et al. 2009). The longer lag phase may help pathogens dodge the host immune responses, which confer increased antibiotic tolerance (Li et al. 2016). Also, the seemingly maladaptive strategy of iron accumulation in the lag phase when the pathogen is exposed to antibiotics can reveal some underlying microbial regulation mechanisms and that instruct the use of antibiotics (Rolfe et al. 2012).

Precisely modelling and predicting the growth pattern in the lag phase allows the modern refrigeratory food industry to preserve food from any organism contamination by prolonging the lag phase (Swinnen et al. 2004, Pérez-Rodríguez 2013, Ghidelli & Pérez-Gago 2018, Adams & Moss 2020). Therefore we could boost the development of food safety and amplify the profitable fields of the food industry. In the food industry, the most commonly used and the main tool to suppress microbial growth in perishable foods is controlling temperature. In consideration of that, temperature with its critical influence on microbial metabolism (Brown et al. 2004), provide a fundamental basis for understanding the latent mechanistic kinetic. Beyond that, the response of the soil microbes, because of the temperature perturbation, significantly influences the biogeochemical cycles (Davidson & Janssens 2006), which no creatures on earth could escape.

Swinnen et al. 2004 provided a framework explaining the mechanistic concept underlying the lag phase, which they define as a function of environmental change. The speed that microbes finish their work to adapt to the new environment can be expressed as $\frac{Workload}{\Delta time}$, in which $Workload$ is proportional to $\Delta Environment$, similar to Bertrand 2019. Recent work has shown that the duration of the lag phase is correlated to the rate of the environmental conditions change (Chu & Barnes 2016). Facing the rapidly changing environment, the lag phase tends to be shorter and vice versa. From research by Chu & Barnes 2016, we also get an insight into the microbial ability to adapt faster or grow faster. The authors believe that there is a trade-off between these two abilities. In which 'grow faster' means microbe perform better in the subsequent exponential growth phase. However, as the research Chu & Barnes 2016 takes carbon source in the substrate as the environmental change, it is not clear that the same would be observed with the other environmental changes. Actually, the opposite result can be found in the study (De Silvestri et al. 2018) that there is not any trade-off between the ability to grow faster and adapt faster, rather they are positively correlated. Those microbes are studied under optimal growth conditions, except temperature and inoculation size as independent variables, monitored in the laboratory. However, in a recent study Hamill et al. 2020 tried to explain the lag phase as the indicator of the stress, they claims there is no stable correlation between the length of the lag phase and the rate of growth or germination.

Based on the growth curve of microorganisms, is there a constant growth rate pattern? As growth rate is influenced by too many factors, such as temperature, body size, medium, inoculation size etc. It could be complicated and unrealistic to test each specific condition with an experiment. Rather than comparing the growth rate in specific conditions, there is a need to test it more generally using a mechanistic model to scale it into a generalized biological pattern. The Metabolic Theory of Ecology (MTE) (Brown et al. 2004) presents an approach. The MTE considers the dynamics of ecological systems as consequences of biological metabolism and deems the phenotypic variations within constraints on metabolic rate mainly influenced by body size, temperature and stoichiometry. In other words, most of the biological rates are governed by metabolism, so growth rate as a form of biological rate, could also be explained using MTE.

So far, there has been no research into the relationship between the lag phase and the exponential phase of microbes in terms of their short-term and long-term thermal responses taken into metabolism. It is therefore valuable to study the influence temperature has on the microbial growth dynamic of the lag and exponential phase focusing on the underlying metabolic relationship. In this study, I collected the microbial population growth data from Samraat Pawar's laboratory, the Department of Life Science, Imperial College. Those data are from 21 previous studies on microbial population growth that incorporated 94 species. By fitting the Boltzmann–Arrhenius model according to MTE, using data points within the Operational Temperature Range (OTR), we can get metabolic activation energy which represents the thermal responses of microbes in lag and exponential phase. Smith et al. 2019 studied the adaptation by comparing the short-term and long-term thermal response of microbe using the maximum growth rate (r_{max}). I used the same method to compare the degree of adaptation between lag and exponential growth phases. I aim to infer some properties, for example, adaptability and potential metabolic mechanism, of microbes in the lag and exponential phases.

2 Methods

I used the parameters r_{max}, t_{lag} (Fig. 1) as the real and reciprocal of the metabolic rate to estimate the thermal response (E) of microbes in exponential and lag phases. These metabolic parameters were obtained by fitting the modified Gompertz model. And the activation energy E is by fitting the Boltzmann–Arrhenius model according to MTE. All the computation, analyses and plotting were done in R 4.1.1.

2.1 Data Collection

The data were collected from 21 papers on microbial population growth under different abiotic conditions. The whole data set comprises around 94 species and across temperature range from -4°C to 37°C (for the detail refer to supplementary information A.1 on page 17).

2.2 Estimating Growth Rate Parameters

$$log(N_t) = N_0 + (N_{max} - N_0)e^{-e^{r_{max}exp(1)\frac{t_{lag}-t}{(N_{max}-N_0)log(10)}+1}} \quad (1)$$

The modified Gompertz Model (Zwietering et al. 1990) is wildly validated as an appropriate model, which is frequently used to describe the growth of population and each parameter in the equation has biological meaning. Therefore, I use it to estimate the two metabolic rate parameters: r_{max} and t_{lag}, which are taken as fitness measurements in this study too.

Before fitting the Gompertz model, I deleted data with incorrect species names and with negative population sizes, which do not have biological meanings. Then, as the model has used the logarithm of population size, I also deleted the population size that has the value of 0 and takes the logarithm of it ($log(N)$). And for fitting the model, it also requires a sufficient degree of freedom in each fitting. There are 4 parameters in the Gompertz model (equation 1), so the data set that has less than 5 points were also deleted. Moreover, each fitting means fitting the model on each data set under the same experimental conditions. So the ID value contains information of species, medium, temperature and citation is given to each data point. The data points with the same ID value are taken as a data set. After the above steps, 809 valid data sets could be used in model fitting.

The model was fitted using Non-linear Least-squares, `nlsLM()` function in R based on the Levenberg-Marquardt algorithm. The estimated parameters in fitting the Gompertz model (equation 1) were sampled 1000 times and retained the parameters in the fitting with the minimum AIC value. The AIC value is a criterion for evaluating the goodness of fit.

2.3 Determining the Operational Temperature Range

The organisms have their own Operational Temperature Range, studying their metabolic rate out of this range does not provide too much practical significance. And also the Arrhenius model would not be applicable out of the bound, for it requires the reaction rates to increase monotonically with temperature (Peleg et al. 2012). The normal temperature range for most organisms falls into 0 to 40°C (Thompson & Thompson 1942) and I scatter plotted the growth rates changing V.S. temperature. By roughly examining the general trend of it, I take 30°C as the upper limit of the operational temperature range.

2.4 Calculating the Activation Energy

The MTE equation (equation 4) builds on allometric equations (equation 2) (Kleiber et al. 1932) and the Boltzmann–Arrhenius model (equation 3) that explains the mass- and temperature-correlated rate respectively. However, studies have already proven that only mass-specific metabolic rates change in response to temperature (Schramski et al. 2015). So the mass-specific rate of metabolism B scales as the equation 5 by dividing $M^{\frac{3}{4}}$ on both sides of the equation 4.

$$Y = Y_0 M^{\frac{3}{4}} \tag{2}$$

$$B = B_0 e^{-\frac{E_a}{kT}} \tag{3}$$

$$I = I_0 M^{\frac{3}{4}} e^{-\frac{E}{kT}} \tag{4}$$

$$B = B_0 M^{-\frac{1}{4}} e^{-\frac{E}{kT}} \tag{5}$$

This dissertation takes temperature as the critical confounding factor, so incorporate the $M^{-\frac{1}{4}}$ into constant B_0 in equation 5 comes to the same pattern as Boltzmann–Arrhenius equation 3, the actual model being fitted. So that B_0 is the normalization constant independent of body size, temperature and other abiotic factors. Y_0 in equation 2 and I_0 in equation 4 are also constants. B in equation 3 is explained as the frequency that the collisions result in a reaction. Under this specific dissertation, it is taken as the mass-specific biological growth rate under consideration of MTE. Y in equation 2 and I in equation 4 represent whole-organism metabolic rate. E_a is the apparent activation energy, correspondingly E is the metabolic activation energy. K is the Boltzmann constant, T is the absolute temperature in Kelvin.

In the same circumstance as the Gompertz model, the log transformation process and sufficient degree of freedom requirements need to be handle. Those two metabolic trait parameters, r_{max} and $1/t_{lag}$, were log-transformed. Before that, non-positive estimated parameters were deleted. Each within species fitting used the data sets with the same species, more than one temperature and more than 2 data points data sets. The across species fitting used the highest metabolic rate parameters in the fitting within species. Both fitting used `lm()` function in R 4.1.1.

$$ln(B) = ln(B_0) - \frac{E}{kT} \tag{6}$$

2.5 Comparison of Thermal Response Patterns

The estimated parameters $log(1/t_{lag})$ and $log(r_{max})$ are scatter plotted to get an overview of the general relation between them. The regression line was gotten by using the 'loess' method in the ggplot2 package with 95% prediction bounds. Then, a more precise quantitative comparison is exerted to get the activation energy by fitting the Boltzmann-Arrhenius model.

As shown in Fig. 2, the short-term ($\bar{E}_S$) and long-term (E_L) activation energy in both lag and exponential phases had been computed and compared to infer the thermal sensitivity and the degree of adaptation in each growth phases. Each short-term Arrhenius fitting used the data set with the same species and got the short-term activation energy E_S. Intra-specific (short-term) thermal response ($\bar{E}_S$) of microbes were calculated by taking the average of intra-species activation energy E_S. The long-term fitting used the data points across species, each at the optimal temperature in the short-term fitting. The optimal temperature at which these data points are located has the largest average growth rate in each short-term fitting.

3 Results

3.1 Correlation Between Growth Rate Parameters

By plotting metabolic rate parameters of exponential phase ($log(r_{max})$) and lag phase ($log(1/t_{lag})$) (Fig. 3), it shows a positive correlation between those two parameters.

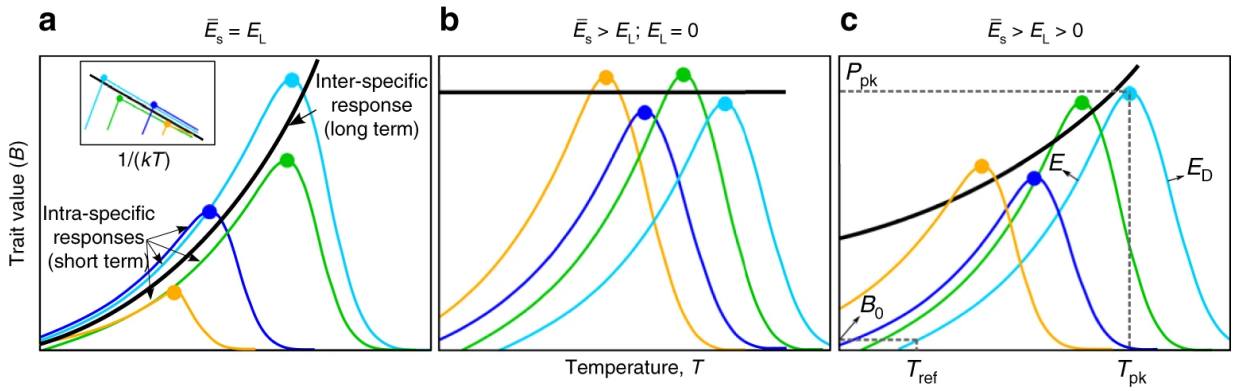

Figure 2: Thermal Response Patterns

copy from (Smith et al. 2019). The coloured lines are intra-specific Thermal Performance Curve (TPC) used to calculate intra-specific activation energy, which represents the short-term thermal response. The black line, using only the trait value under the optimum condition (P_{opt}) to generate inter-specific activation energy, corresponds to long-term thermal response. Inset panel in a shows how the plot would be like in the Boltzmann-Arrhenius model. $\bar{E}_S$ is the mean intra-specific activation values (the absolute values of the slope of the colored lines in panel plot), correspondingly E_L is the black line. Larger activation energy value represents larger thermal constrains (larger thermal sensitivity), short-term. So by comparing the difference of the short-term and long-term activation energy, we can infer the degree of the adaptation. In this plot, the three circumstances are: **a** there is no adaptation, the short-term activation energy ($\bar{E}_S$) is statistically indistinguishable from the long-term (E_L). The metabolism rate is completely constrained by the thermodynamic **b** Natual selection defeats thermoconstrains completely. **c** Intermedium senarial that the microbes is partialy adapted.

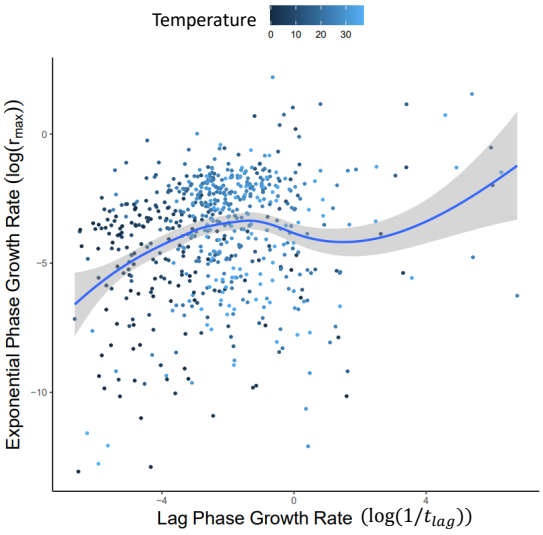

Figure 3: Rough correlation between metabolic traits

The correlation between metabolic rate parameters of exponential and lag phases under different temperature. The regression line is fitted using 'loess' method in ggplot2 package with 95% prediction bounds.

3.2 Operational Temperature Range

Visually, the data with the temperature above 30°C would largely not follow the Arrhenius model that the reaction rate increase monotonically with temperature (Fig. 4, 6) (Peleg et al. 2012). So I take 30°C as Operational Temperature Range upper bound.

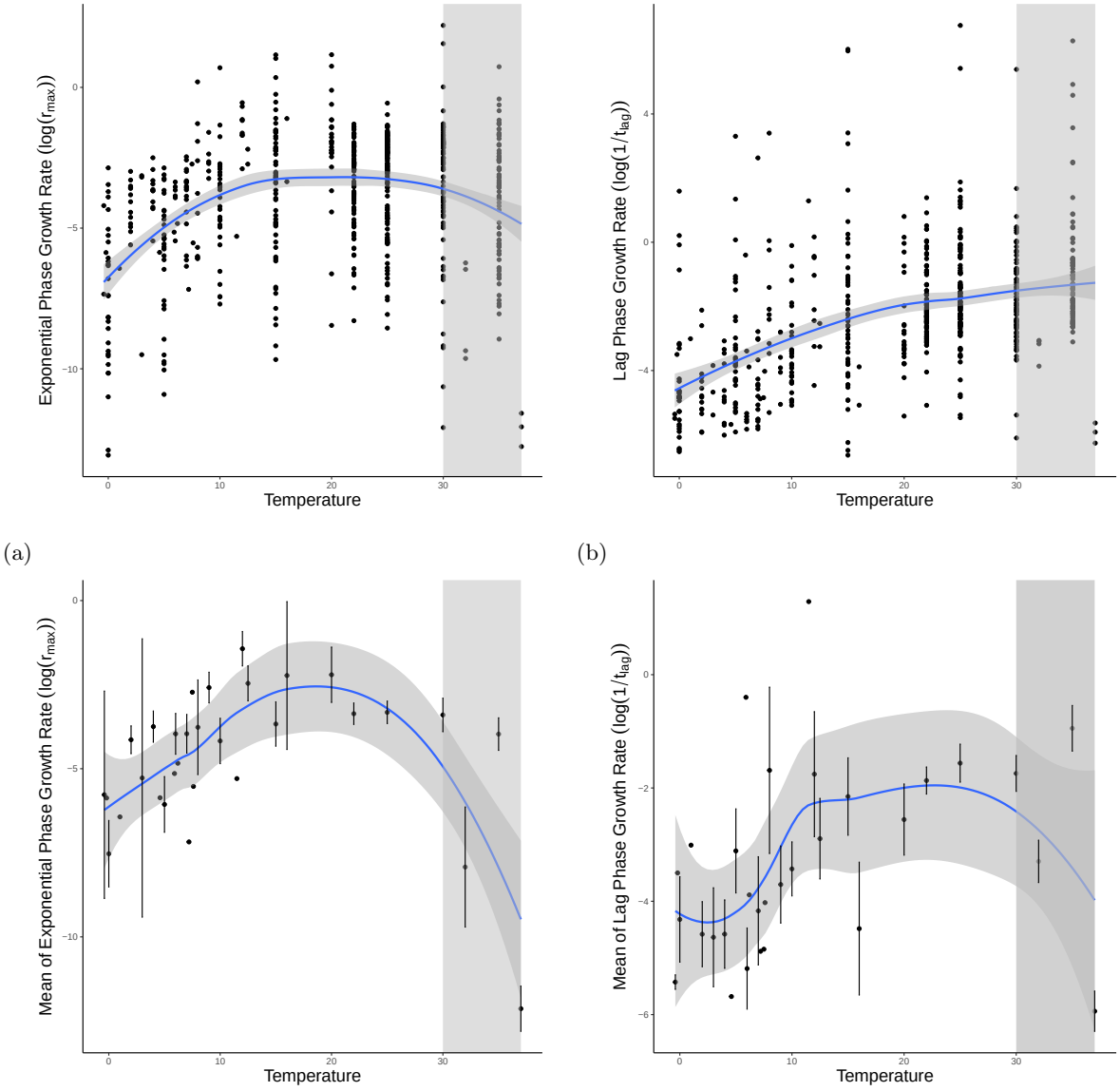

Figure 4: Operational Temperature Range

General plot the trending of the temperature-dependent metabolic rate parameters. **a, c** are calculated from r_{max}, **b, d** are from t_{lag}. The curve regression line is fitted using 'loess' method in ggplot2 package with 95% confidence interval. The plot **c, d** show the mean metabolic rate values. Error bars represent 95% confidence interval calculated from normal distribution. By roughly scanning the trend, 30°C is taken as Operational Temperature Range upper bound.

3.3 Thermal Response Comparison

For both growth phases, the $\bar{E}_S > E_L > 0$ (Fig. 5, table 1), follows the pattern in **c** of Fig. 2. As explained in Fig. 2 about inferring the strength of the thermodynamic constraints on ectotherms. My result shows the thermal sensitivities are partially constrained by thermodynamic. In other words, selection does not completely override thermodynamic constraints, but there is some biochemical adaptation. Further detail about the activation energy of each species can be found in the supplementary information (Fig. 8, table 3 and table 4).

Time Scale	$E_{r_{max}}(eV)$	N_{rmax}	$E_{t_{lag}}(eV)$	N_{tlag}
short term	1.02 ± 0.24	42	1.20 ± 0.27	37
long term	0.68 ± 0.30	93	0.44 ± 0.33	90

Table 1: Two Phases short-/long-trem Activation Energy Comparison

Comparison of short-term and long-term activation energy values calculated from lag phases and exponential growth phase ± 95% confidence interval. Data size is denoted by N

Moreover, if we take the difference between short-term and long-term activation energy ($\bar{E}_S - E_L$) estimated from metabolic traits as the degree of adaptation. In my result, estimating this measurement from parameter $1/t_{lag}$ is higher than from r_{max} means the intensity of adaptation is stronger observed in the lag phase.

4 Discussion

This study examined the thermal response of two key metabolic traits (duration of lag phase and exponential growth rate) of microbes within and across species. I find stronger evidence for adaptation of the lag phase compared to the exponential phase. As under same constant experimental conditions, the stronger ability to suppress the thermodynamic constrain in lag phase could be achieved by the underlying metabolic mechanism of microbes. It got me to think that if this adaptation is autonomous, it is likely a response to selection for the ability of microorganisms to allocate limiting resources adjusting to the better metabolic rate in coping with constantly changing environmental conditions, such as different temperatures.

The result that the short-term thermal sensitivity is higher in the lag phase than in exponential phase is contradicts to the results of De Silvestri et al. 2018, who found that r_{max} has the higher short-term thermal sensitivity (table 2). The predicted activation energy values from two metabolic traits in this study were both relatively higher than those found by previous works De Silvestri et al. 2018 and Smith et al. 2019. This could be because I used `lm()` function in R to fit the Boltzmann-Arrhenius model, it may probably happen to use the steepest curve of the Temperature Performance Curve so got the higher value. Despite this potential limitation my result comparing the activation energy from both exponential and lag phases would not be affected, because the comparison is under the same temperature range. Further work could use the Sharpe–Schoolfield model (Shi & Ge 2010) as an alternative to Boltzmann–Arrhenius model. It would obtain a more precise Operational

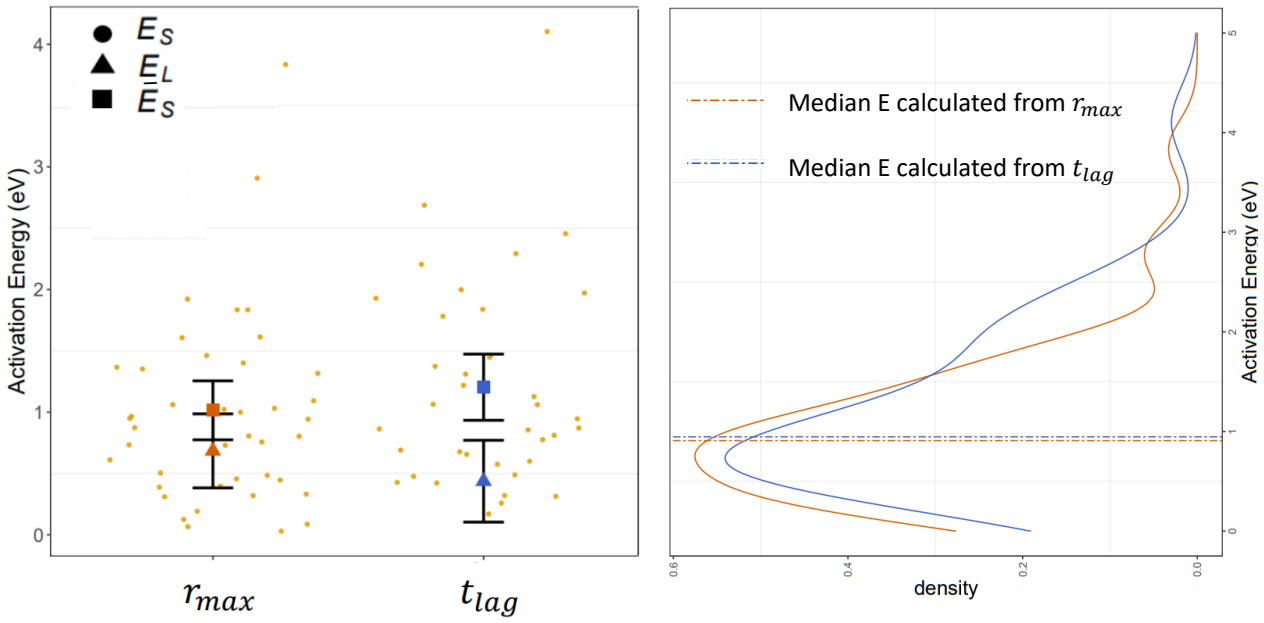

Figure 5: Activation Energy Comparison

a The thermal responses of each species (E_S, yellow dots). The mean value ($\bar{E}_S$, squares) of them is taken as the short-term thermal responses of exponential and lag phases. The long-term thermal responses (E_L, triangles) were calculated using the best performance metabolic rate parameters in each species. Both short-term and long-term thermal responses have error bars with 95% confidence intervals. Orange symbols denote values calculated from exponential phase, blue ones denote lag phase. **b** Distribution of activation energy of species estimated from exponential (orange) and lag (blue) phases. The two "twodash" lines represent median activation energy values.

Temperature Range below the highest metabolic traits. So that the activation energy values may not as higher as in my study, it may lead to different result in further research.

Multi carbon substrate supply is another widely concerning environmental factor that could cause the lag phase in the microbial growth curve. Facing several carbon resources, the microbes switch hierarchically between them from the most favourite to the least, leading to biphasic growth curves also called diauxic growth (Monod 1949). During these diauxic phases, the microbes could be vulnerable to harsh perturbations such as heat, as they are undergoing processes adjusting to the new environment. Could the duration of the lag phase be a stress indicator (Hamill et al. 2020)? Understanding the underlying mechanism of microbes' special sensitivity to temperature change could make them the eco-friendly guide organisms used to monitor environmental change.

My result that the difference of activation energy calculated from the lag phase is higher than from the exponential phase suggests that there is more adaptation in the lag phase. In other words, it is less restricted by thermodynamics. Following the approach of Hamill et al. 2020, further research could also investigate whether

Reference	$E_{r_{max}}(eV)$	$E_{t_{lag}}(eV)$
current study	1.02 ± 0.24	1.20 ± 0.27
(De Silvestri et al. 2018)	0.95	0.83
(Smith et al. 2019)	0.87 ± 0.05	None

Table 2: Activation Energy Comparison with Previous Works

Comparison of short-term activation energy values calculated from the lag phase and the exponential growth phase in the current study and in previous work (De Silvestri et al. 2018, Smith et al. 2019)

the reduced thermal restriction or also higher degree of adaptability of the lag phase, could be an indication of stronger competitive ability. Furthermore, as the lag phase is the initial growth phase that microbes enter, the metabolic strategies they employ could have a profound influence on their competitiveness in later life cycles.

If we want to analyse the competitive ability of species from the perspective of thermal response patterns of metabolic traits, the trait values estimated in my study alone are not enough. Because in my research, the fitness of the population is assessed independently of other perturbations or interactions in the systems, which is a less perfect reflection of what really happens in the real world. In the real world, microbial populations mostly exists as a component of communities interacting with other species and influenced by environmental conditions. If we want to understand the broader microbial communities, we need to understand those interactions incorporating microbial community structures, dynamics, functions and networks etc.

For obtaining more reliable predictions of competitiveness, a more precise determination of the fitness of a genotype is required. The in-silico simulation could be an ideal way to achieve this. For example, a recent study has constructed an approach to predict relative density-dependent fitness accounting for the effects of each growth cycle on fitness (Ram et al. 2019). This research took culture medium as a variable, a similar future work could be constructed to predict relative fitness based on thermal response.

Models incorporating species interactions and environmental perturbations can also be found in recent studies (Rodrigues et al. 2021, Pacciani-Mori et al. 2020, Li et al. 2020). Rodrigues et al. 2021 considered resource competition. They incorporated demographic composition and found that cooperative behaviour in the early and late life span are both not favoured. They also found that niche expansion can promote mutualism. Pacciani-Mori et al. 2020 studied dynamic metabolic adaptation as a mechanism to maintain biodiversity. Incorporating thermal constraints in their model could make it more useful in practical applications. While Pacciani-Mori et al. 2020 just assign the metabolic rate value in the model, (Li et al. 2020) built a model that makes it dynamic, mapping from metabolic strategies to fitness consequences. Their model is based on resource-competition models using the adaptive dynamic technique, but in contrast to the adaptive dynamic technique, it takes the metabolic rate as dynamic. In their study, Li et al. 2020 found the dynamic fitness landscape is changing with species-environment feedback. On that basis, Li et al. 2020 studied how could the microbes allocate the resource to maintain the most fitness metabolic behaviour. Further research similar to Li et al. 2020 could incorporate temperature as a critical factor influencing metabolic strategies.

My study has some limitations that could be addressed with future work. The calculation of short-term thermal responses $\bar{E}_{S_{rmax}}$ and $\bar{E}_{S_{tlag}}$ are simply the average of activation energy of each species. An alternative approach would be to cancel the bias from poor fitting data sets by taking the weighted average instead. The data sets in my study were collected for analysing the growth rate of the microbes, it is not guaranteed that each species strain contains a series of temperatures so that it can be used to examining the microbes' response to temperature. For this reason, in my study, many data sets were invalid. A larger data size (table 1) may lead to a different result and could be more persuasive. And collecting experiment data use temperature as the independent variable is more suitable for this study.

In this study, by comparing the metabolic traits I found different degrees of adaptation in life cycles, which could lead to stronger competitiveness of microbes. While throughout the whole life cycle temperature takes a critical role in constraining biological rates, recent studies do not take this constrain into consideration. The results of this study deepen our understanding of thermodynamic constraints on metabolic traits, thus laying the foundation for understanding mechanisms underlying competition between microbial taxa across environmental gradients.

Data and Code Availability

All the code for replicate this dissertation can be found in the github repository: https://github.com/zongyi2020/CMEEProject

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A Supplementary Information

A.1 Data

(Bae et al. 2014, Blagodatskaya et al. 2007, Galarz et al. 2016, Gill & DeLacy 1991, Heo et al. 2009, INOUE 1977, Kapetanakou et al. 2019, Kirchman & Rich 1997, Koutsoumanis et al. 2006, Lee et al. 2007, Phillips & Griffiths 1987, Roth & Wheaton 1962, Silva et al. 2018, Sivonen 1990, Stannard et al. 1985, Vankerschaver et al. 1996, Willocx et al. 1993, Zwietering et al. 1994)

A.2 Operational Temperature Range

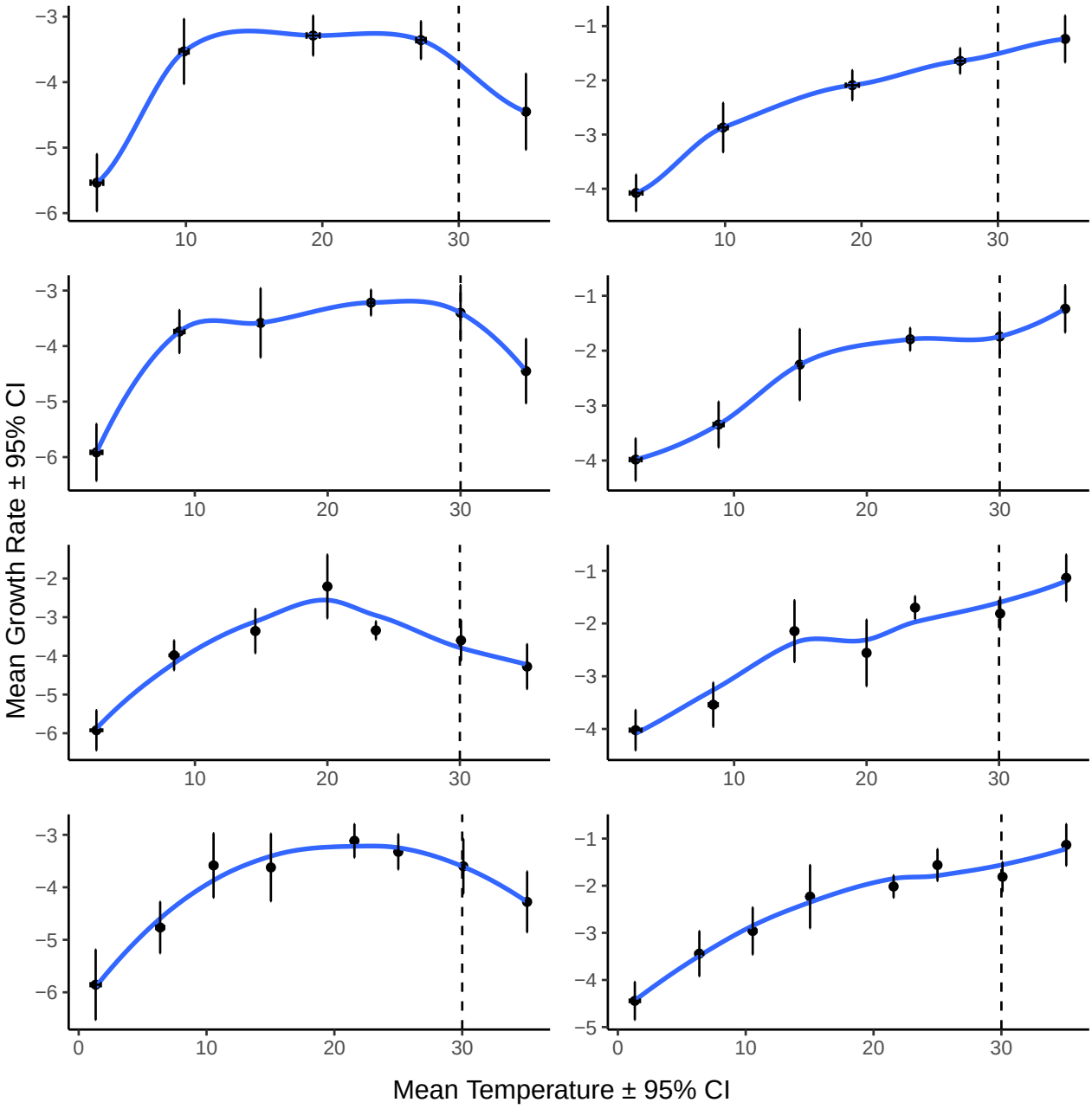
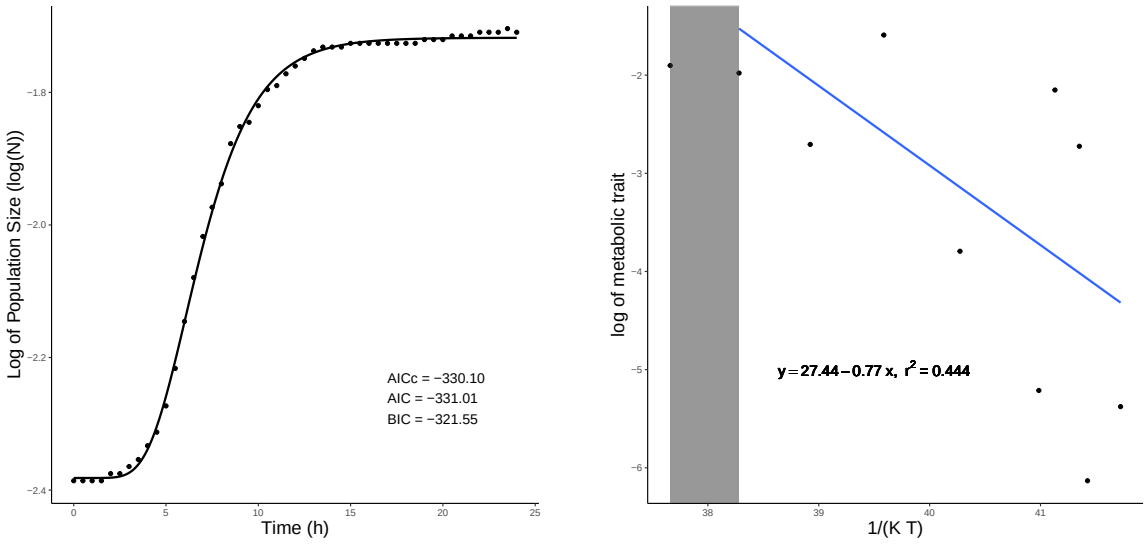


Figure 6: Operational Temperature Range Grouped by Temperature

Across species mean maximum growth rate ($log(r_{max})$, left) and lag phase growth rate ($log(1/t_{lag})$, right) with 95% confidence interval in different groups of temperature, from the first to the forth row represents 5-8 groups respectively.

A.3 Typical Model Fitting Plot



(a) Typical Gompertz Model Fitting Plot

(b) Typical Boltzmann-Arrhenius Model Fitting Plot

Figure 7: Typical Model Fitting Plot

The typical model fitting plots. **a** A typical Gompertz Model fitting plot with *AICc, AIC, BIC* as goodness of fit criteria. **b** A typical Boltzmann-Arrhenius model fitting plot with r^2

A.4 Activation Energy

Table 3: E_S E value calculated from exponential phase

No.	Species	Activation Energy	Confidence.Interval
1	Pantoea.agglomerans	0.61	1.25
2	Clavibacter.michiganensis	1.00	2.18
3	Stenotrophomonas.maltophilia	0.50	1.21
4	Klebsiella.pneumonia	2.66	4.56
5	Dickeya.zeae	2.91	2.83
6	Acinetobacter.clacoaceticus	0.40	6.84
7	Bacillus.pumilus	0.48	2.63
8	Pseudomonas.fluorescens	1.02	4.70
9	Staphylococcus	0.95	0.48
10	Pseudomonas	0.33	0.26
11	Aerobic.Psychotropic	0.31	0.36
12	Aerobic.Mesophilic	0.97	0.47
13	Spoilage	0.81	0.71
14	Escherichia.coli	1.06	1.50
15	Salmonella.Typhimurium	1.92	0.67
16	Curtobacterium.psychrophilum	0.76	0.87

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No.	Species	Activation Energy	Confidence.Interval
17	Cytophaga.antarctica	1.83	1.32
18	Cytophaga.xantha	0.07	0.99
19	Spirillum.pleomorphum	0.73	0.82
20	Pseudomonas.flourescens	1.83	2.69
21	pseudomonas	0.94	1.35
22	yeasts.moulds	1.32	0.69
23	enterobacteriaceae	1.46	1.00
24	lactic.acid.bacteria	1.61	0.74
25	pseudonomads	0.73	2.23
26	brochothrix.thermosphacta	3.83	0.95
27	aerobic.bacteria	0.03	0.89
28	Serratia.marcescens	1.40	0.78
29	Arthrobacter	0.32	0.75
30	Arthrobacter.aurescens	0.46	0.18
31	Arthrobacter.globiformis	1.09	2.72
32	Lactobacillus.plantarum	1.03	0.87
33	Lactobacillus.sakei	0.19	1.24
34	Lactobaciulus.plantarum	0.87	0.97
35	Rahnella	0.39	0.75
36	Raoultella	0.13	0.99
37	Citrobacter	0.45	0.59
38	Curtobacterium	1.37	1.94
39	Microbacterium	0.80	0.85
40	IsoMix	0.09	0.71
41	Novosphingobium	1.35	1.68
42	Serratia	1.61	1.80
43	Mean Value	1.02	0.24

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Table 4: E_S E value calculated from duration of lag phase

No.	Species	Activation Energy	Confidence.Interval
1	Pantoea.agglomerans	0.32	2.03
2	Clavibacter.michiganensis	1.37	2.74
3	Stenotrophomonas.maltophilia	1.22	0.57
4	Klebsiella.pneumonia	1.97	2.31
5	Dickeya.zeae	2.46	1.39
6	Acinetobacter.clacoaceticus	2.29	1.64
7	Bacillus.pumilus	4.10	2.79

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No.	Species	Activation Energy	Confidence.Interval
8	Pseudomonas.fluorescens	1.20	4.59
9	Staphylococcus	1.45	0.46
10	Pseudomonas	0.81	0.28
11	Aerobic.Psychotropic	0.87	0.46
12	Aerobic.Mesophilic	0.78	0.57
13	Spoilage	1.31	1.60
14	Escherichia.coli	1.93	3.95
15	Curtobacterium.psychrophilum	0.58	0.83
16	Cytophaga.antarctica	0.86	0.56
17	Cytophaga.xantha	0.68	0.82
18	Spirillum.pleomorphum	1.78	0.87
19	Micrococcus.cryophilus	0.26	1.07
20	Pseudomonas.flourescens	2.69	2.78
21	pseudomonas	0.42	1.28
22	yeasts.moulds	0.86	1.61
23	lactic.acid.bacteria	0.43	2.58
24	pseudonomads	1.46	1.44
25	Serratia.marcescens	2.21	1.24
26	Arthrobacter	0.31	0.58
27	Arthrobacter.aurescens	1.84	0.17
28	Arthrobacter.globiformis	0.48	1.41
29	Lactobacillus.sakei	0.95	1.01
30	Lactobaciulus.plantarum	1.06	0.63
31	Rahnella	0.17	0.80
32	Sphingobacterium	0.69	1.79
33	Rhizobium	1.06	0.96
34	Curtobacterium	1.13	1.39
35	IsoMix	0.66	0.60
36	Paenibacillus	0.60	1.18
37	Bacillus	0.49	1.57
38	Mean Value	1.20	0.27

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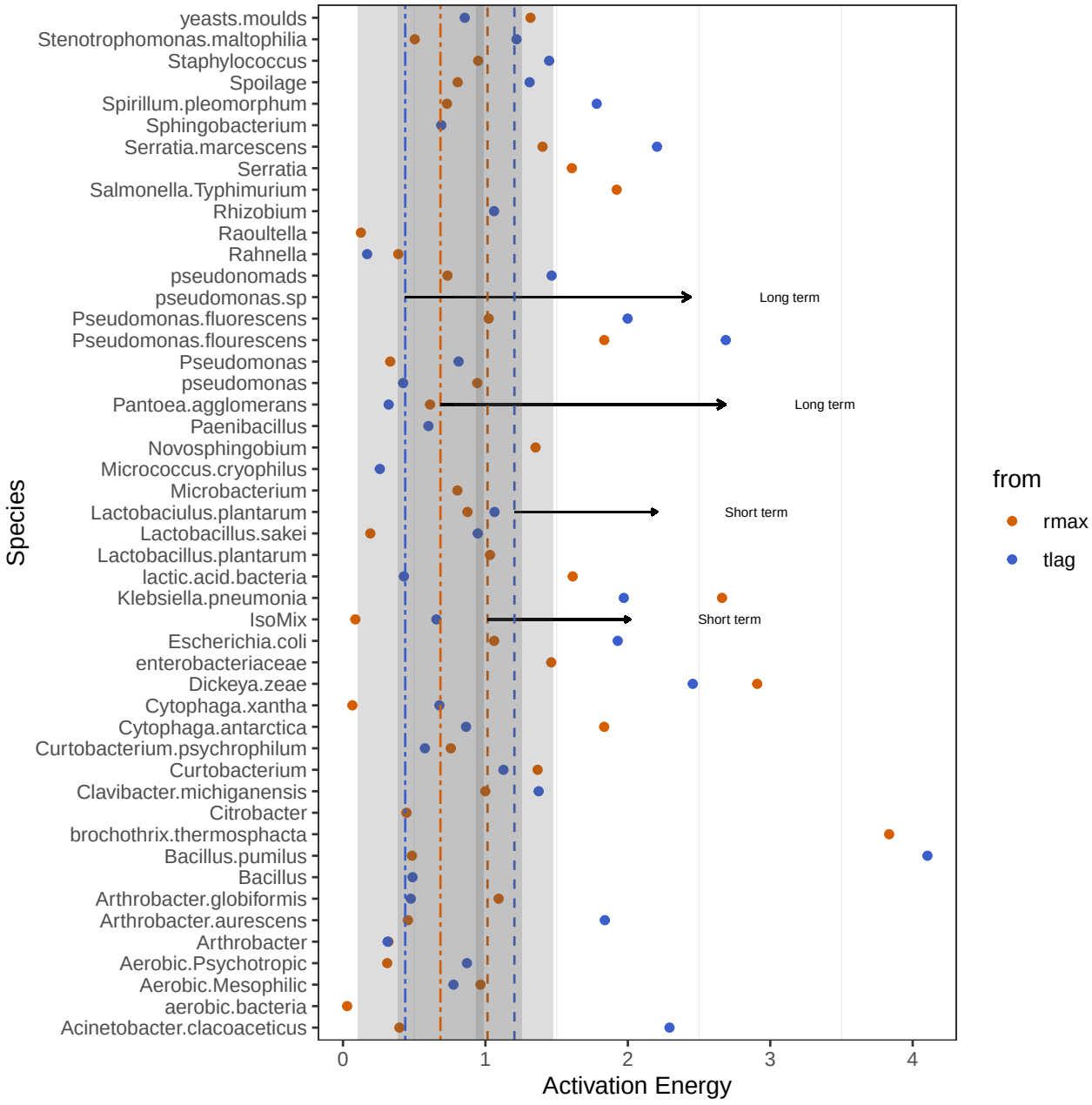

Figure 8: Activation Energy of Each Species

Variation in short-term thermal sensitivity(E_S) amongst bacteria groups, with vertical dash lines annotating the mean variations($\bar{E}_S$, short-term) and the long-term(E_L) sensitivity both with 95% confidence interval