Machine learning for optimal growth temperature prediction of prokaryotes using amino acid descriptors

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Abstract

The optimal growth temperature of organisms is valuable in bioprospecting enzymes that work under extremophilic temperature conditions. Existing prediction models achieve high accuracy, but they mainly capture the trends of the mesophiles (OGT = 15-45°C) and taxonomies that are abundantly present in the training set. In this study, we investigated the use of a weighted root mean square deviation (RMSE) and phylogenetic splits to improve the generalizability of the prediction models trained on amino acid descriptors. To do this, we first built a new OGT database of more than 10,000 species distributed over 51 phyla of Bacteria, Archaea and Eukaryota with special attention to include extreme temperature data. Then, we trained machine learning models on 6,401 observations with available genomes. The best performance was from the multi-layer perceptron with an RMSE of 5.05°C and an R^2 of 0.81. The most important model descriptors were related to charged residues, as well as bulky, hydrophobic residues.

1 Introduction

Bioprospecting identifies novel molecules, enzymes and microbes relevant to biological and biochemical research and industrial applications. Of particular interest are process parameters such as temperature for bioprospecting of enzymes and microbes. Each organism is adapted to a specific temperature range that influences its growth rate (Corkrey et al., 2016). At the optimal growth temperature (OGT), an organism achieves its maximal growth rate. Organisms are broadly classified based on their OGT into psychrophiles (OGT < 15°C),

mesophiles (15°C < OGT < 45°C), thermophiles (45°C < OGT < 80°C), and hyperthermophiles (OGT > 80°C), although these temperature boundaries are not fixed (Jensen et al., 2012). Temperature affects the internal energy of the cell,

and changes in temperature directly impact every cellular component (Ramón et al., 2023; Wang and others, 2015). For instance, temperature has a large influence on protein stability, with higher temperatures leading to denaturation, protein aggregation, and eventual loss of function, while low temperatures lead to reduced enzyme kinetics and eventual inactivation (Plaza Del Pino et al., 2000; D'Amico et al., 2002). OGT data is valuable to understand general trends

in thermal adaptation, or specific ones through comparative analysis. Correlations have been found between OGT and properties of the genome (Kawashima et al., 2000; Hu et al., 2022; Zeldovich et al., 2007; Sabath et al., 2013), rRNA and tRNA (Galtier and Lobry, 1997; Hurst and Merchant, 2001; Khachane and others, 2005), codon usage (Lobry and Chessel, 2003; Sauer and Wang, 2019), proteome (Zeldovich et al., 2007; Kurokawa et al., 2023; Sauer and Wang, 2019; Tekaia and Yeramian, 2006; Villain et al., 2022), and metabolic network (Weber Zendrera et al., 2019; Farrell et al., 2025). Furthermore, OGT correlates with enzyme optimal catalytic temperatures (r = 0.76; Wang et al. 2024), and current models for predicting enzyme optimal catalytic temperature rely heavily on OGT (Li et al., 2019; Gado et al., 2020; Li et al., 2022). Thus, OGT can indirectly estimate optimal enzyme catalytic temperatures and inform species selection for bioprospecting enzymes. Experimentally determining OGT is a

laborious and time-consuming process, as it requires incubation and growth analysis at various temperatures (Sauer and Wang, 2019; Yang et al., 2024). This method is limited to culturable organisms, which are biased towards certain phyla and only account for a small fraction (roughly 3%) of all prokaryotes (Lewis et al., 2021). Culturability depends on the range of conditions tested, and a lack of knowledge about an organism's environmental requirements, including temperature preference, can impede its culturability (Lewis et al., 2021). In addition, environmental data from metagenomics can be inaccurate due to local temperature gradients (Khachane and others, 2005), or influx of microorganisms (Kurokawa et al., 2023). Finally, organisms can tolerate temperatures far from their OGT, such as facultative thermophiles whose OGT is larger than 45°C, but can also grow at moderate temperatures (Bergey, 1919). Similarly, psychrotrophs are cold-tolerant but have an OGT above 15 °C (Morita, 1975). OGT predictions can thus (1) enhance the culturability of organisms and (2) help identify their optimal growth conditions. Given the challenges of

experimental determination and the importance of OGT in thermal adaptation research, enzyme prospecting, and increasing culturability, predicting OGT has become a popular research topic. Several OGT prediction models have been developed based on genomic (Wang et al., 2022), tRNA (Cimen et al., 2020), proteomic features (Li et al., 2019; Kurokawa et al., 2023; Barnum et al., 2024),

or a combination thereof (Jensen et al., 2012; Sauer and Wang, 2019). Furthermore, the continuous release of dedicated databases, such as TEMPURA (Sato et al., 2020) and ThermoBase (DiGiacomo et al., 2022), further underscores the ongoing interest in understanding optimal growth conditions, especially OGT. Despite the progress, current OGT prediction models face several limitations.

One key issue involves the phylogenetic signal in OGT data Hu et al. (2022). Random train-test splits may cause models to overfit on taxonomic patterns, resulting in overly optimistic test performances, and limiting their ability to generalize to distantly related organisms and gain biological insights Cimen et al. (2020). Moreover, mesophiles are often overrepresented in the training data, reducing model performance at the extremes, especially at low temperatures Sauer and Wang (2019); Li et al. (2019). While some studies have attempted to address this bias through undersampling of mesophiles Wang et al. (2022); Cimen et al. (2020), others did not Li et al. (2019); Sauer and Wang (2019). Finally, these models have often provided limited insights into the underlying biological mechanisms. Here, we developed an OGT prediction model based on physicochemical amino acid properties Osorio et al. (2015) from predicted proteome information. Our approach includes phylogenetically informed data splits, weighted RMSE for better predictions in extremophiles, and the SHAP framework for model interpretation Lundberg and Lee (2017).

2 Materials and Methods

2.1 Optimal growth temperature database construction

2.1.1 Data retrieval

Optimal growth temperature (OGT) data was downloaded from four databases (ThermoBase (DiGiacomo et al., 2022), TEMPURA (Sato et al., 2020), aciDB (Neira et al., 2020)), MediaDB (Richards et al., 2014)) and the supplementary material of Lyubetsky et al. (2020). In addition, growth temperature data was obtained from three culture collection centers (CCUG [1], CECT [2], NIES [3]) through Python web scraping from February to April 2023. Finally, temperature records were extracted from the bacterial metadatabase BacDive using the API (Reimer et al., 2022).

2.1.2 Data preprocessing

All records were mapped to a unique taxonomic identifier, the NCBI TaxId, based on organism name(s) and with the help of the NCBI datasets Python

[1] https://www.ccug.se/

[2] https://www.uv.es/uvweb/spanish-type-culture-collection/en/spanish-type-culture-collection-1285872233521.html

[3] https://mcc.nies.go.jp/

API and the Taxonomy browser. If no matching TaxId could be found, manual annotation was attempted by looking up the cross-referencing culture collection numbers, or through other metadata. The preferred TaxId rank was "strain". Records that could only be identified down to the genus level were removed. Any out-of-use TaxIds were replaced.

Temperature data cleaning In the case of BacDive, records were of type minimal, maximal, optimum or growth. Minimal and maximal temperatures were removed as these correspond to conditions of no growth. For the remaining databases, all records were uniformly annotated as "optimum" or, if no indication of optimality was present, "growth" for cultivation growth temperature. Temperature ranges were averaged. Two records of *Levilactobacillus acidifarinae* and *Levilactobacillus zymae*, both with an OGT of 7,5°C in BacDive, were excluded because the annotations could not be verified against the original paper.

2.1.3 Genome extraction and annotation

All prokaryotic TaxIds were mapped to their reference genome or, if unavailable, all their genomes in NCBI using the NCBI datasets command line tool. The species level was used if no genomes were available at the lowest taxonomic level. High-quality genomes were selected based on 1) the average nucleotide identity (ANI) report, and 2) the assembly level. The ANI report for prokaryotes contains the result of an ANI-based analysis that evaluates whether the assigned taxonomy of an assembly is correct. All genome assemblies with a 'taxonomy-check-status' of "inconclusive" or "failed" were removed, leaving only unassessed genomes and genomes with a correct taxonomic identity. Next, if there were still multiple genome assemblies for a given TaxId, only assemblies with the highest assembly level were kept following the order 'Complete Genome > Chromosome > Scaffold > Contig'. All 10,652 assemblies left after filtering were downloaded

using the NCBI Datasets command line tool. Genomes were annotated with Prokka (version 1.14.6, Seemann 2014) using the default installation. The taxonomic domain and genus names were provided.

2.2 Machine learning

2.2.1 Features

Previous OGT prediction models used amino acid (AA) composition to describe the proteome (Sauer and Wang 2019; Li et al. 2019; Barnum et al. 2024). Here, we extracted 58 amino acid descriptors from the descriptor sets z-Scales (Sandberg et al., 1998), T-scale (Tian et al., 2007), ST-scale (Yang et al., 2010), MS-WHIM (Zaliani and Gancia, 1999), VHSE (Mei et al., 2005) Kidera (Kidera et al., 1985), Cruciani (Cruciani et al., 2004), Blosum (Georgiev, 2009) and Fasgai (Liang and Li, 2007) with the R package "peptides" (version 2.4.4, Osorio et al. 2015). We summarized them on the proteome level by averaging

them across all proteins. The descriptors capture key amino acid characteristics derived from dimensionality reduction on physicochemical and/or structural properties. The hypothesis is that the additional information in the descriptors, compared to the composition, further improves the model. In addition, taxonomic domain information was coded into a dummy variable.

2.2.2 Dataset composition

While in the past, OGT prediction models were trained on cultivation temperatures (Li et al., 2019; Sauer and Wang, 2019), most recent models have been trained on OGT data (Wang et al., 2022; Barnum et al., 2024). Here, we only used OGT data for modelling. Still, the cultivation temperature records have two purposes: (1) to compare with OGT and (2) to provide temperature growth conditions for more microorganisms, including extremophiles, for further studies. Our machine learning dataset contains one observation per TaxId with the

proteome descriptors averaged over its genomes, the domain, and the median OGT. We performed a phylogenetically informed train-test split (90.0%-10.0%), keeping observations of the same family together (Barnum et al., 2024). Hyperparameter tuning and performance evaluation were carried out using 5-fold cross-validation on the training set, while the test set was reserved for the final model comparison.

2.3 Machine learning algorithms

We trained 9 linear models (lasso, ridge, Bayesian ridge, elastic net, LARS, LARS-lasso, Huber, PLS, and stochastic gradient descent), 5 non-linear models (kernel ridge, Gaussian process, SVR, multi-layer perceptron and k-nearest neighbor), and the ensemble model histogram gradient boosting using the packages scikit-learn (version 1.5.1, Pedregosa et al. 2011). Hyperparameter tuning was performed in Optuna (version 3.6.1, Akiba et al. 2019) using 5-fold cross-validation over 200 trials with a weighted root-mean-square (RMSE) loss function to address the temperature imbalance in the dataset. The weights were calculated in two steps. For observation i with OGT value x:

1. Calculate a weight proportional to the inverse of the probability density function:

$$\text{weight}(x) = \frac{1}{\text{KDE}_{\text{Gaussian}}(x) + \delta} \tag{1}$$

   With $\delta = 0.01$, and $\text{KDE}_{\text{Gaussian}}(x)$ estimated on the training data with *scipy.stats.gaussian_kde*.

2. Min-max normalize

2.4 Model interpretation

The most influential descriptors to the model were identified using the SHAP (SHapley Additive exPlanations) framework. SHAP values, expressed in target units (°C), are estimates of each feature's marginal contribution to deviations of the predictions from the expected value. SHAP values were calculated with the Python package shap (version 0.46.0, Lundberg and Lee 2017) using the default KernelExplainer and the background set to 10 K-means samples.

2.5 Amino acid descriptor interpretations

Some amino acid descriptors applied in this work lack a clear biological explanation. Therefore, we investigated the correlation of key descriptors with 586 physico- and biochemical amino acid properties from the AAindex (Kawashima et al., 2008), and two additional sources (Koehler et al., 2009; Lin et al., 2003). The indices and their hierarchical categorization were retrieved from the AA-analysis package (Breimann et al., 2024). Pearson correlations with $|r| \geq 0.9$ were considered.

2.6 Correlation analysis

The Pearson correlation coefficient was used unless stated otherwise. Correlations were classified into strong ($|r| \leq 0.7$), moderate ($0.7 < |r| \leq 0.5$), and weak ($0.5 < |r| \leq 0.3$). To address the temperature imbalance, the dataset was sorted by OGT and divided into 5 equal-sized bins (n = 1,280), with correlations calculated within each temperature bin. The fifth bin, comprising of the top 20% of OGT values contains temperatures of 37°C or higher.

3 Phylogenetic signal

The phylogenetic signal in the amino acid descriptors and OGT was measured with Pagel's lambda (Pagel, 1999) on a time-scaled phylogenetic tree retrieved from TimeTree for each domain (Kumar et al., 2022). The tree contained 298 of the 401 archaea and 3,941 of the 5,958 bacteria on our training set. Pagel's lambda was calculated with the function phylosig from the R package phytools (Revell, 2012).

4 Results and Discussion

4.1 Data

Over 34,000 records were collected and assigned to a taxid, of which 21,881 were cultivation temperatures and 12,866 optimal growth temperatures (Table 1). The dataset contains mainly Bacteria, and almost no eukaryotic OGT data. Some records are expected to be duplicates because of, for example, the requirement for novel species to be deposited in multiple culture collection centers (Rule

Table 1: Distribution of species and genera by domain.

#	Cultivation		Optimal growth	
	TaxIds	Species	TaxIds	Species
Archaea	290	269	756	624
Bacteria	7,026	6,287	9,801	9,405
Eukaryota	1,758	1,734	27	27

30 (3b), Oren et al. 2023) and shared references among optimal growth temperature databases. The OGTs range from 2°C to 114°C. The GC-content is rather balanced with 57% of entries having a GC-content of 50% or higher.

4.2 Cultivation versus optimal growth temperature

4.2.1 OGT data is biased towards common culturing temperatures

In the past, OGT prediction models were trained on cultivation temperature data (Li et al., 2019; Sauer and Wang, 2019) before switching to OGT (Wang et al., 2022; Barnum et al., 2024). While cultivation temperature refers to any temperature at which an organism can grow, OGT is the condition that leads to the highest growth rate. However, in practice, the OGT is derived from a subset of tested conditions, with subsets varying in granularity, leading to differences in OGT data quality (Barnum et al., 2024). In our dataset, based on the kernel density estimations of the median temperature per species TaxId, the cultivation temperatures of Eukaryota and Bacteria show distinct peaks at common culturing temperatures (Supplementary Figure 1). The reduced, although remaining bias toward CCTs in bacterial OGT data is likely due to the selection of evaluation temperatures during the experimental setup. We also compared the median cultivation temperature with the median OGT for the 2,598 species for which both were available. The overall Pearson correlation was 0.90. 14.6% of the species had a deviation larger than 5°C, which is the lower bound of RMSE for current OGT prediction models. In 2.6% of the cases (n = 67), the deviation was larger than 10°C (Figure 1, 2).

Because of the sometimes large deviations of cultivation temperature from OGT, only the latter was considered for model training. In addition, given the negligible amount of eukaryotes with OGT data, we, from this point onwards, focus on the prokaryotic subset.

4.3 PCA on prokaryotic OGT

4.4 Nucleotide bias follows the direction of maximum variance

The first principal component (PC1) from principal component analysis (PCA) explains 65.68% of the total variance in the features. PC1 shows a near-perfect

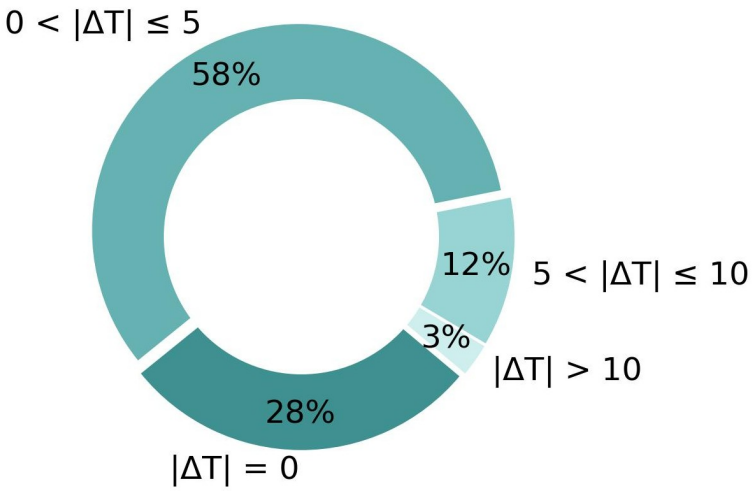

Figure 1: Distribution of the absolute deviations between median cultivation temperature and median OGT for 2,598 species.

inverse correlation (r = -0.97) with GC-content (Figure 3A). GC-content is strongly reflected in the frequencies of amino acids with GC-rich codons: proline (r= 0.95), glycine (r = 0.95), alanine (r = 0.96), and arginine (r = 0.96), as well as AT-rich amino acids lysine (r = -0.96), isoleucine (r = -0.95), glutamine (r = -0.95), tyrosine (r = -0.90) and phenylalanine (r = -0.88). Methionine (r = -0.47) is the exception among AT-rich amino acids.

4.4.1 Differences in environmental conditions define PC2

The second principal component, explaining 14.39% of the variance, separates organisms living in extreme conditions, namely, the archaeal class *Halobacteria* at low values and to a lesser extent, the thermophiles at high values (3B). The distinct grouping of *Halobacteria* is not due to a domain split, as other archaea cluster together with bacteria (Supplementary Figure 2).

PC2 correlates strongly negative with the fraction of aspartate (r = -0.80) and moderately positive with the fraction of leucine (r = 0.66). The aspartate fraction combines two trends in extremophilic adaptation. Thermophiles avoid aspartate due to its chemical instability at high temperatures (Villain et al., 2022). In contrast, halophiles prefer negatively charged amino acids as a negative design mechanism promoting repulsion in misfolded proteins (Amangeldina et al., 2024).

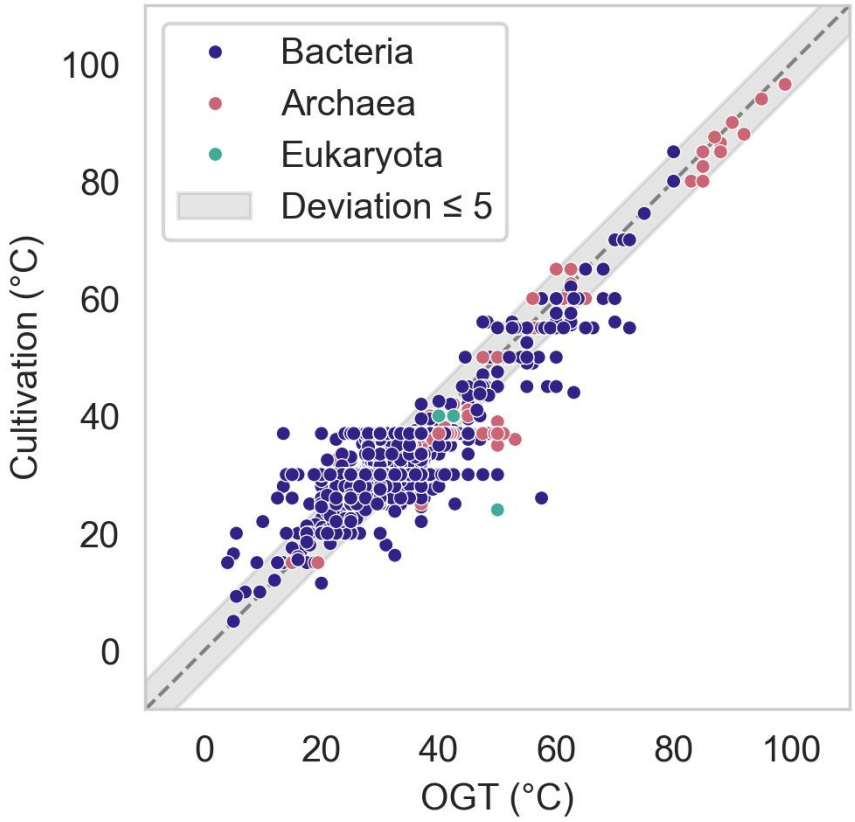

Figure 2: Absolute differences between optimal growth and cultivation temperatures for 2,598 species. Notably, at common culturing temperatures 30°C and 37°C, the range of deviations for cultivation temperatures versus OGT exceed 5°C, indicating significantly higher data quality for OGT measurements.

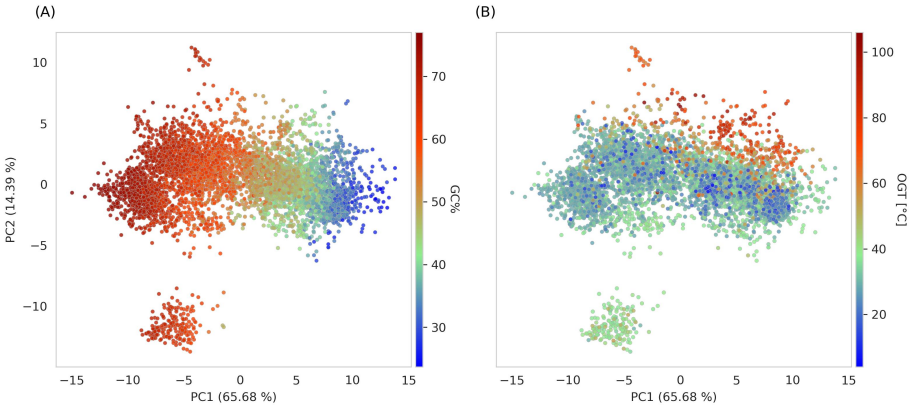

Figure 3: First two principal components (PCs) for the 6,401 taxids in the OGT dataset, colored by (A) the average GC percentage, and (B) the median optimal growth temperature. PC1 follows the direction of decreasing GC percentage. High PC2 values indicate thermophilicity. The outlying observations with low PC2 values belong to the class Halobacteria.

4.4.2 Amino acid usage bias captures both GC-content and adaptation to environment

The main driver of amino acid composition is nucleotide bias, followed by temperature (Hickey and Singer, 2004). In our dataset, the amino acid usage bias, measured by the standard deviation of the 20 common amino acid frequencies, is strongly correlated with GC-content in GC-rich genomes (GC% >= 50%: r = 0.94; GC% < 50%: r = -0.49). This trend remains across temperature bins. AAUB correlates moderately with OGT only for AT-rich genomes and in the top 20% of the dataset (r = 0.57) Supplementary Figure 3).

4.5 Phylogenetic signal in the training set

Both OGT and the descriptors contain significant evolutionary signal under the Brownian motion model of evolution. For archaea, Pagel's lambda was 0.96 ($p < 0.001$), and for Bacteria 0.92 ($p < 0.001$). Therefore, under a random training-test split, data leakage occurs and an alternative phylogeny-based split should be performed. In reality, the best model is a trade-off between generalization and performance (Cimen et al., 2020). Here, we kept observations of the same genus level together.

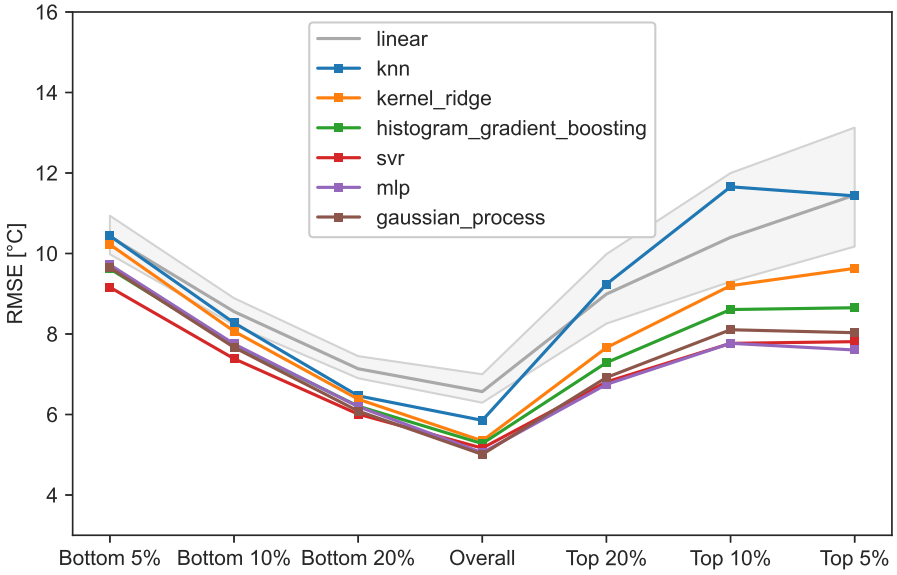

Figure 4: Cross-validation RMSE for the individual non-linear models compared to a linear model background (CI = 95%). The percentages indicate the upper and lower 5%, 10%, and 20% quantiles. Improvement over the linear models is stronger at higher temperatures

4.6 Machine learning results

4.6.1 Non-linear models show the most improvement in thermophiles

Non-linear models have consistently better cross-validation RMSEs compared to the linear models (RMSE = 6.57°C) overall and specifically in the thermophilic region 4. The best model based on overall metrics is the Gaussian process model with RSME = 5.01 and $R^2 = 0.815$, followed by the multi-layer perceptron (mlp) model (RMSE = 5.05°C and $R^2 = 0.812$).

4.6.2 Poor fit for psychrophiles

All models capture thermophilic trends better than the psychrophilic ones. The reason is two-fold. First, the dataset consists for 88.9% (n = 5,689) of mesophiles, followed by 10.2% (n = 656) (hyper)thermophiles and only 0.9% (n = 58) psychrophiles. Given the few psychrophilic observations, we opted for weighted RMSE instead of under- or oversampling of the dataset. Secondly, psychrophiles have weaker signals in their amino acid composition (Yang et al., 2015).

4.7 Model interpretation

According to the SHAP analysis on the mlp, SVR and kernel ridge model, the most important descriptors are consistently K6, followed by T4. Both K6 and T4 have an inverse relation with OGT. The taxonomic domain, included as a dummy variable in the training set, has only a small contribution in comparison.

4.7.1 K6

The descriptor K6 is a mixture of amino acid properties (Kidera et al., 1985), and shows strongest negative correlations with AA indices related to typical amino acid compositions of nuclear, membrane or anchor proteins. K6 thus indicate a general deviating composition. Lowest K6 values are observed for the positively charged lysine and hydrophobic leucine, which also correlated moderately with PC2 capturing environmental factors. An increase in charged residues including lysine, has previously been described as a trend in thermoadaptation (Villain et al., 2022).

In contrast, the fractions of sulfur-containing amino acids cysteine (C) and methionine (M) contribute most positively to K6. Previously, no significant correlations were found between these amino acids and OGT (Villain et al., 2022; Sauer and Wang, 2019). However, in our dataset, the fraction "CM" is negatively correlated to OGT in the top 20% for AT-rich genomes (r = -0.45), but not in GC-rich genomes (r = -0.09), which may have previously obscured the relation.

4.7.2 T4

Finally, the descriptor T4 is the second most important descriptor with low values associated with high OGT. According to the the AA indices T4 is negatively correlated to side chain volume. Lowest T4 values are observed for the amino acids W,Y,F, and H. More bulky, hydrophobic residues have previously been shown to be associated with adaption to high temperatures as the bulkiness leads to denser packing of the protein core (Smole et al., 2011).

4.8 Comparison to other OGT prediction models

Comparing OGT prediction models directly requires an independent test set, which is challenging due to different training datasets and the need for distantly related species because of the strong phylogenetic signal. Furthermore, comparison of RMSE and R^2 is unreliable as many models did not include a phylogenetically informed split, resulting in overoptimistic estimates. Still, our mlp model has an RMSE of 5.05°C, which is similar to that of the best existing prokaryotic OGT prediction model (RMSE = 5.17, Sauer and Wang 2019), while including a phylogenetic split on the genus level.

5 Conclusion

In this work we constructed a new (optimal) growth dataset containing OGT values for 10,584 unique taxids from all three domains of life, although eukaryotes are heavily underrepresented. For the 6,401 taxids with available genomes we built OGT prediction models. The best model was the multi-layer perceptron with an RMSE of 5.05°C and an R^2 equal to 0.81. This performance is similar to that of the state-of-the-art prokaryotic OGT prediction model, even though we included a phylogenetic split on the genus level to reduce data leakage between training and test set. The most important predictors were related to charged residues (K6), and bulky hydrophobic residues (T4). Moreover, fractions of the sulfur-containing amino acids cysteine and methionine were significantly decreased in (hyper)thermophiles compared to mesophiles, but only significantly in AT-rich genomes.

6 Competing interests

No competing interest is declared.

7 Acknowledgments

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