Proposal for new halophile classification system based on Statistical Rarity definition of extremophiles

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Abstract

Although current classification systems for halophilic and halotolerant microorganisms have contributed significantly to the field, there remain opportunities to enhance their theoretical foundation and broader adoption. In this study, we introduce a new classification system grounded in the Statistical Rarity definition of extremophiles. To support this proposal, a comprehensive bibliographic survey of 1,298 species described as 'halophile', 'halophilic', or 'halotolerant' from 1967 to 2021 was conducted. Of these, 1,196 species with complete optimal NaCl growth data were analyzed using the Counting Method to determine species frequency across NaCl concentrations. Quartile metrics were applied to define new subclasses: halotolerant (< 0.6 mol·L^{-1} NaCl), slight halophile (0.6–1.2 mol·L^{-1}), moderate halophile (1.2–2.2 mol·L^{-1}), and extreme halophile (> 2.2 mol·L^{-1}). The proposed system reclassifies a significant portion of species and emphasizes biological relevance by correlating rarity of optimal growth conditions with extremity. This paradigm shift addresses inconsistencies in halophile classification and standardizes the definition of halophilic and halotolerant microorganisms, providing theoretical meaning to those terms.

Introduction

The most general definition of extremophile is that they are organisms capable of surviving or thriving in extreme physical or chemical conditions (Rothschild and Mancinelli 2001). The word “extremophile” comes from the Latin root *extremus* (meaning the end or to the end) added to the Greek root *philiā* (meaning extreme love or affection). At first glance, this may appear to be straightforward and even simple definition. Nevertheless, it presents several biological and conceptual challenges, the most significant being how to define what can be considered a physical or chemical extreme (Mariscal and Brunet 2020).

Not all physical-chemical conditions interact the same way with living organisms. As an example, while ionizing radiation is always harmful to living organisms and the 0 Gy dose is the optimal growth condition regarding this condition (*e.g.* the radiotolerant model *Deinococcus radiodurans* in gamma radiation (Daly et al. 2004)), a numeric 0 on the Celsius temperature scale represents a more extreme condition to most living organisms than a 25°C (Kumar et al. 2023). Considering temperature, some organisms grow better at lower values (*e.g. Planococcus halocryophilus* (Mykytczuk et al. 2012)) or higher values (*e.g. Thermus aquaticus* (Brock and Freeze 1969)) on the Celsius scale, and from their perspective, surviving in an intermediate temperature value is considered extreme. Hence an important concept is highlighted: while some physical-chemical conditions are invariably harmful (*e.g.* ionizing radiation, desiccation, heavy metal), others are necessary in moderation and different organisms have different optimal growth ranges (*e.g.* temperature, pressure, salinity). Therefore, defining the extremes of physical chemical conditions is neither biologically nor quantitatively straightforward.

There are some known definitions of extremophiles that are slightly different from each other: Anthropocentric, Edge of Morphospace, Statistical Rarity, Objective Limits and Near Impossibility

(Table 1) (Mariscal and Brunet 2020). Ideally, there would be one absolute definition of extremophiles. However, for certain concepts this is not possible (*e.g.* concept of life) because they do not constitute natural kinds (Bird and Tobin 2024). Each definition has its strengths and limitations and should be chosen thoughtfully. Usually, scientists do not specify their working definition of extremophiles or rely on general meaningless definitions. This lack of clarity can lead to confusion and misinterpretation of research findings.

Table 1
List of definitions of extremophiles.

Adapted from Mariscal and Brunet 2020

Definition	Description
Anthropocentric	Extremophiles are organisms that thrive in environments that humans or human's cells cannot.
Edge of Morphospace	Extremophiles are known organisms that inhabit the limits of life as we know regarding some physical or chemical continuum.
Statistical Rarity	Extremophiles are organisms that thrive in conditions that most other organisms cannot.
Objective Limits	An extreme is the limit of some physical or chemical phenomena. Extremophiles do well in these environments.
Near Impossibility	Extremophiles (when they exist) are at the limits of what life's mechanisms can possibly handle.

Halophiles constitute a specialized category of extremophiles encompassing representatives from Archaea, Bacteria, and Eukarya domains that have developed adaptive mechanisms to withstand elevated concentrations of sodium chloride (Cavicchioli 2002; Ventosa et al. 2004). Halophilic microbes are widely used in biotechnology and play a significant role in the food industry, since salt is commonly used to preserve food (Rathod et al. 2023; Xu et al. 2024; Thompson and Gilmore 2024). Halophiles, as a specialized category of extremophiles, face the same challenge of definition and categorization. Furthermore, salinity is one of those physical-chemical conditions that can be beneficial, leading to a further subclassification between those organisms that have optimal growth in low NaCl concentrations and can survive in higher salinity, usually known as *halotolerants*, and those organisms that thrive in high NaCl concentrations, strictly known as *halophiles* (Larsen 1986). However, a critical question remains: what defines high or low NaCl concentration? This classification likely depends on the chosen definition of extremophile. While several acknowledged numerical classifications exist for halophiles and halotolerants, none appear to have obvious biological or theoretical foundation. Table 2 presents several widely recognized classification systems for halophiles with their primary references.

Table 2
Three current halophiles classification systems converted to SI units

	DasSarma and DasSarma 2012	Kushner and Kamekura 1988	Larsen, 1986
Slight	0.2–0.85 mol·L^{-1}	0.2–0.5 mol·L^{-1}	0.34–0.86 mol·L^{-1}
Moderate	0.85–3.4 mol·L^{-1}	0.5–2.5 mol·L^{-1}	0.86–3.42 mol·L^{-1}
Extreme	3.4–5.1 mol·L^{-1}	> 2.5 mol·L^{-1}	3.42–5.13 mol·L^{-1}

Biological classification systems have evolved over the decades as our understanding of living organisms deepened, including the classification of halophiles. However, the classification system used to classify halophilic microorganisms in articles describing the species is not always explicit. Even if the classification system used is explicitly written, the criteria across systems are not fully consistent, which is natural with the constant advances of scientific knowledge. As a result, an organism designated as a halophile under one system may not meet the requirements for another, which can cause major confusion and miscommunication in literature. It is necessary to unify the halophile and halotolerant classification systems and standardize a clear definition for each subcategory, as has been discussed for other classes of extremophiles such as psychrophiles (Cavicchioli 2016).

In this context, the present work proposes a new classification system for halophiles based on the Statistical Rarity definition of extremophile. For that, we conducted a large-scale bibliographic survey of microorganisms described as halophiles. Microorganisms were chosen for being the majority of halophilic organisms, which is precisely what is needed for working with Statistical Rarity.

Methods

Bibliography survey

The bibliographic survey of halophilic and halotolerant microorganisms was carried out to obtain information on: (i) NaCl concentration range for optimal growth, (ii) maximum and minimum concentrations at which growth is observed; and (iii) the classification given by the authors (slightly, moderately or extremely halophilic, halotolerant or unclassified). Articles describing new species as “halophile”, “halotolerant” or “halophilic” in the International Journal of Systematic and Evolutionary Microbiology were accessed by searching the website of the Microbiology Society and the data contained therein were tabulated by species.

The dataset used was derived from the original species description, with subsequent updates incorporated only when published in the same journal as a correction of the species description. Species names were corrected when necessary following information on the List of Prokaryotic Names with Standing in Nomenclature (LPSN) (Parte et al. 2020). Multiple strains of the same species were consolidated into a single entry within the dataset, in which only the most extreme values were considered for the species, creating a “chimera” that does not represent any specific strain. All raw data can be found in Online Resource 1 and all references for the data can be found in Online Resource 2.

Data processing

The data from the survey of halophilic microorganisms were processed using authorial scripts in R programming language (version 4.3.3). During processing, only species with complete data and with NaCl concentration were retained. Some papers used a mixture of salts such as marine salts or total salts to measure growth; others simply did not provide this information; in both cases the species were excluded from posterior analysis as it is not possible to classify any microorganism as halophile or halotolerant without information of growth with NaCl. The NaCl concentration data were converted to $mol \cdot L^{-1}$ unit to enable direct comparison across studies. The data was then processed using the Counting Method, which consist of counting how many species can grow or grow optimally in an 0.1 $mol \cdot L^{-1}$ interval (Fig. 1). Each species can appear more than once depending on the NaCl concentration range.

The quartile metrics were then applied to the distribution created by the Counting Method of optimal growth ranges of the species with complete information. With that, the NaCl concentration continuum was divided into four intervals with 25% of occurrence of species each. These intervals define the new classification system of halophilic microorganisms in halotolerant (first quartile), slightly halophilic (second quartile), moderately halophilic (third quartile) and extremely halophilic (fourth quartile).

All data processing and graph producing scripts are available in Online Resource 3.

Results and discussion

The bibliography survey resulted in information from the year 1967 to 2021 for a total of 1298 species described as halophilic or halotolerant. As shown in Fig. 2, the number of species described had increased significantly since 2003 (Fig. 2). This survey encompasses 1298 species from 527 different genera, 164 families, 68 orders, 30 classes, 15 phyla, 7 kingdoms and all 3 domains, demonstrating its comprehensive nature and ensuring statistical power for our analysis. The Eukarya domain was underrepresented with only 6 species described.

A total of 1196 species had complete optimal growth information and therefore were used to determine the frequency of species that could grow optimally in each NaCl concentration interval through the Counting Method. This distribution was used to resolve the statistical rarity of species in the NaCl biospace by quantifying the number of species that would thrive in each NaCl concentration interval. Then the Statistical Rarity definition of extremophile was applied by means of quartile metrics of the distribution to define the new intervals of halophile classification: from 0.6 $mol \cdot L^{-1}$ to 1.2 $mol \cdot L^{-1}$ NaCl for slight halophile, from 1.2 $mol \cdot L^{-1}$ to 2.2 $mol \cdot L^{-1}$ NaCl for moderate halophiles and greater than 2.2

$mol \cdot L^{-1}$ NaCl for extreme halophiles. The microorganism is classified as halotolerant if the optimum growth occurs in NaCl concentration lower than 0.6 $mol \cdot L^{-1}$ but growth is supported above that value. The establishment of 0.6 $mol \cdot L^{-1}$ NaCl as the lower limit for a halophile is biologically meaningful, as this concentration meets the mean salinity of the oceans. It would not make sense for a Statistical Rarity-based classification to characterize one of the Earth's most extensive biomes (marine environments) as extreme. Notably, this conceptual inconsistency persists across every previous classification system. Our proposed system resolves this limitation by approaching a theoretical and biological framework in which the methodology was based (Statistical Rarity).

The procedure for classification using the system proposed here is as follows (illustrated in Fig. 3): (i) The microorganism is classified as "halophile" if the optimal growth occurs in NaCl concentrations higher than 0.6 $mol \cdot L^{-1}$ and the microorganism is classified as "halotolerant" if the optimal growth occurs below 0.6 $mol \cdot L^{-1}$ and growth can occur above this value; (ii) When the minimum and maximum of optimal growth range fall in the same category interval, the microorganism is classified within this category; (iii) When the minimum and maximum of optimal growth range are not in the same category, the microorganism is classified according to the category containing the majority of its growth range; if the range is equally distributed between categories (exactly 50% in one category and 50% in the other), classification follows the category containing the upper range limit (maximum of the optimal growth concentration).

We compared the original classification of species description with the literature classification systems for species with complete optimal growth and growth range information (n = 1154) to assess the degree of alignment among scientists. This comparison is presented in Table 3. The classification system by Kushner and Kamekura (1988) showed the highest concordance with the original species descriptions, though this value remained below 70%. These findings suggest that, at present, the classification of a species as halophile (particularly its subclassification as slight, moderate, or extreme) varies considerably depending on the system used, with no single approach achieving broad consensus. Our proposed classification system coincides with 47.63% of the authors' original classifications. While this represents the lowest overlap among the systems evaluated, it highlights the distinct perspective introduced by our approach and underscores its potential to contribute to a paradigm shift in halophile classification.

Table 3
Coincidence between categorization of halophiles and halotolerants by author describing the species versus three classifications presented in the literature and the proposed classification (Schiavo et al. 2025)

Classification system	Coincidence of category of halophile
DasSarma and DasSarma 2012	60.53%
Kushner and Kamekura 1988	69,02%
Larsen 1986	56.45%
Schiavo et al. 2025	47.63%

The current classification stands with 167 halotolerants, 140 slight halophiles, 435 moderate halophiles, 165 extremes halophiles and 247 halophiles without subclassification. The classification of the same species with our proposed system stands with 387 halotolerants, 295 slight halophiles, 207 moderate halophiles and 247 extremes halophiles; 18 species presently classified as halophiles cease to be considered extremophiles. The enrichment of the “halotolerant” (from 167 to 387) and “slight halophile” (from 140 to 295) categories reflects the theoretical basis of the classification system in Statistical Rarity definition of extremophiles: less extreme conditions should have more species able to grow. This enrichment can be seen graphically in Fig. 4, where only the proposed classification system shows a skew toward the “Tolerant” category. Moreover, the proposed system exhibits categories with similar quantities of species, as is expected by a quartile-based method of categorization.

While the proposed classification system demonstrates considerable robustness, it is important to remark certain limitations inherent to the current dataset. Firstly, not all halophilic microorganisms are included in the data acquisition, only those described in the International Journal of Systematic and Evolutionary Microbiology. For instance, no data from *Eukarya* Domain is used as none had complete information on NaCl concentration of optimal growth. We know that some eukaryotes grow in high NaCl concentration and could skew the intervals to higher values, for example the genus *Dunaliella* (Oren 2014). Additionally, the limit values for the interval of each category of halophilic microorganisms should be updated with every new species description. Despite this dynamic aspect, the substantial number of species currently considered (n = 1196) provides a strong foundation and is expected to ensure the stability of the classification for the foreseeable future.

Conclusion

We propose a new classification system of halophilic and halotolerant microorganisms based on the Statistical Rarity definition of extremophiles, by means of quartile of the distribution of optimal growth ranges of halophiles described in the International Journal of Systematic and Evolutionary Microbiology. The new intervals of halophiles classification are from 0.6 mol·L^{-1} to 1.2 mol·L^{-1} NaCl for slight halophile, from 1.2 mol·L^{-1} to 2.2 mol·L^{-1} NaCl for moderate halophiles and greater than 2.2 mol·L^{-1} NaCl for extreme halophiles; halotolerant microorganisms have optimal growth range bellow 0.6 mol·L^{-1}

NaCl but can grow in higher NaCl concentrations. The proposed system aims to unify and standardize the definition of halophilic and halotolerant microorganisms, providing theoretical meaning to those terms as well as to the subclasses of halophiles (slight, moderate and extreme). We also bring to light the discussion of microorganism's classification as extremophiles, for the inaccuracies and inconsistencies presented for halophiles can appear in other extremophilic classes (e.g. acidophiles, radiotolerantes etc) as previously discussed for psychrophiles in the work of Cavicchioli (2016). We encourage that more theory-based classification be developed to other kinds of extremophiles. Ultimately, we recognize that, as with all scientific knowledge, the Statistical Rarity-based system may eventually require revision or even become obsolete as new discoveries are made.

Declarations

Competing Interests

The authors have no competing interests to declare that are relevant to the content of this article.

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Author Contribution

The study conception and design were performed by A.P.M.S. Funding acquisition was performed by F.R.. Material preparation and data collection were performed by A.P.M.S. Data analysis was performed by A.P.M.S. and R.A.V. The first draft of the manuscript was written by A.P.M.S. and all authors commented on previous versions of the manuscript. All authors read and approved of the final manuscript.

Data Availability

Data is provided within the manuscript or supplementary information files.

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Figures

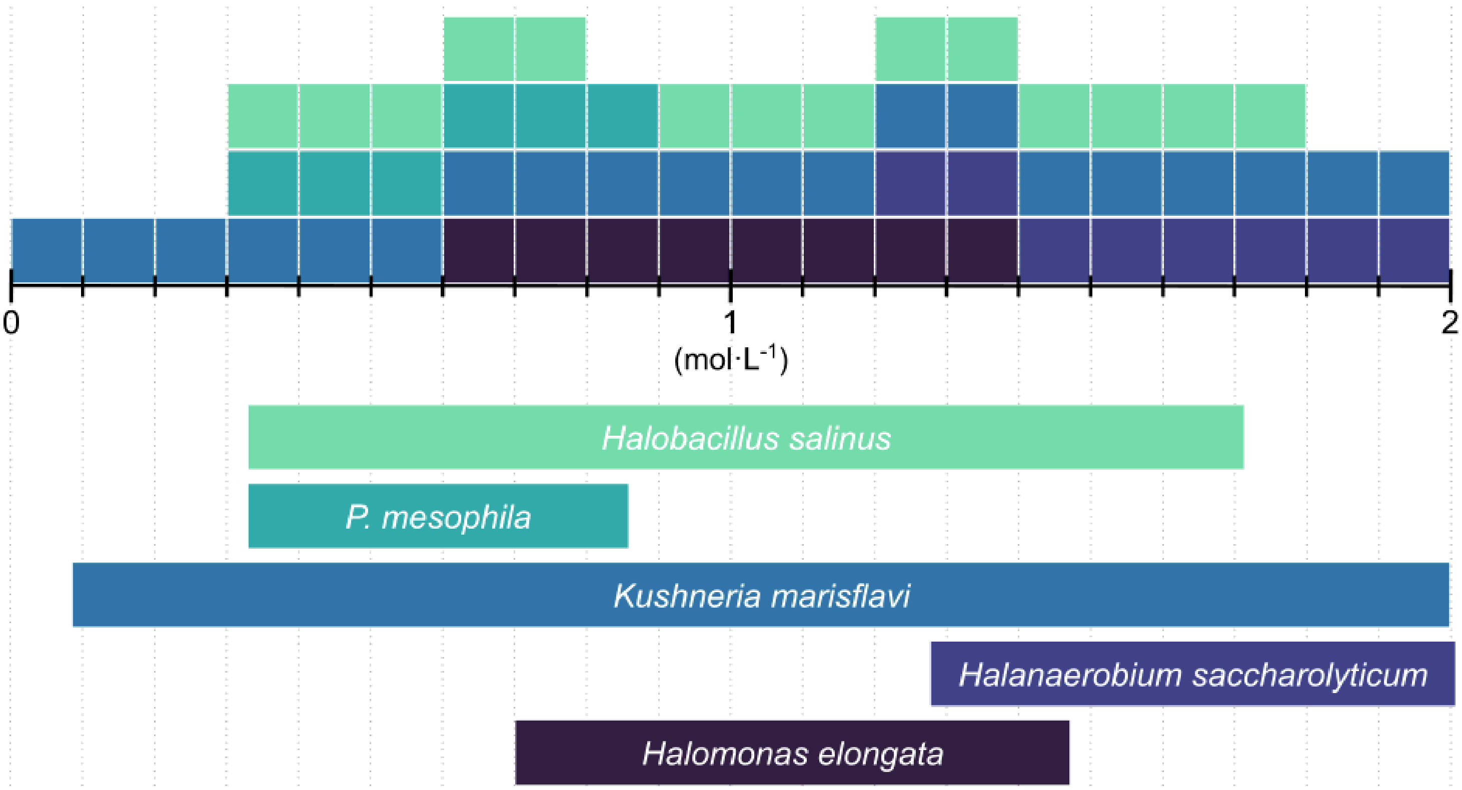

Figure 1

Graphical representation of the Counting Method: the number of species that can grow optimally within the specified 0.1 $mol{\cdot}L^{-1}$ interval is counted

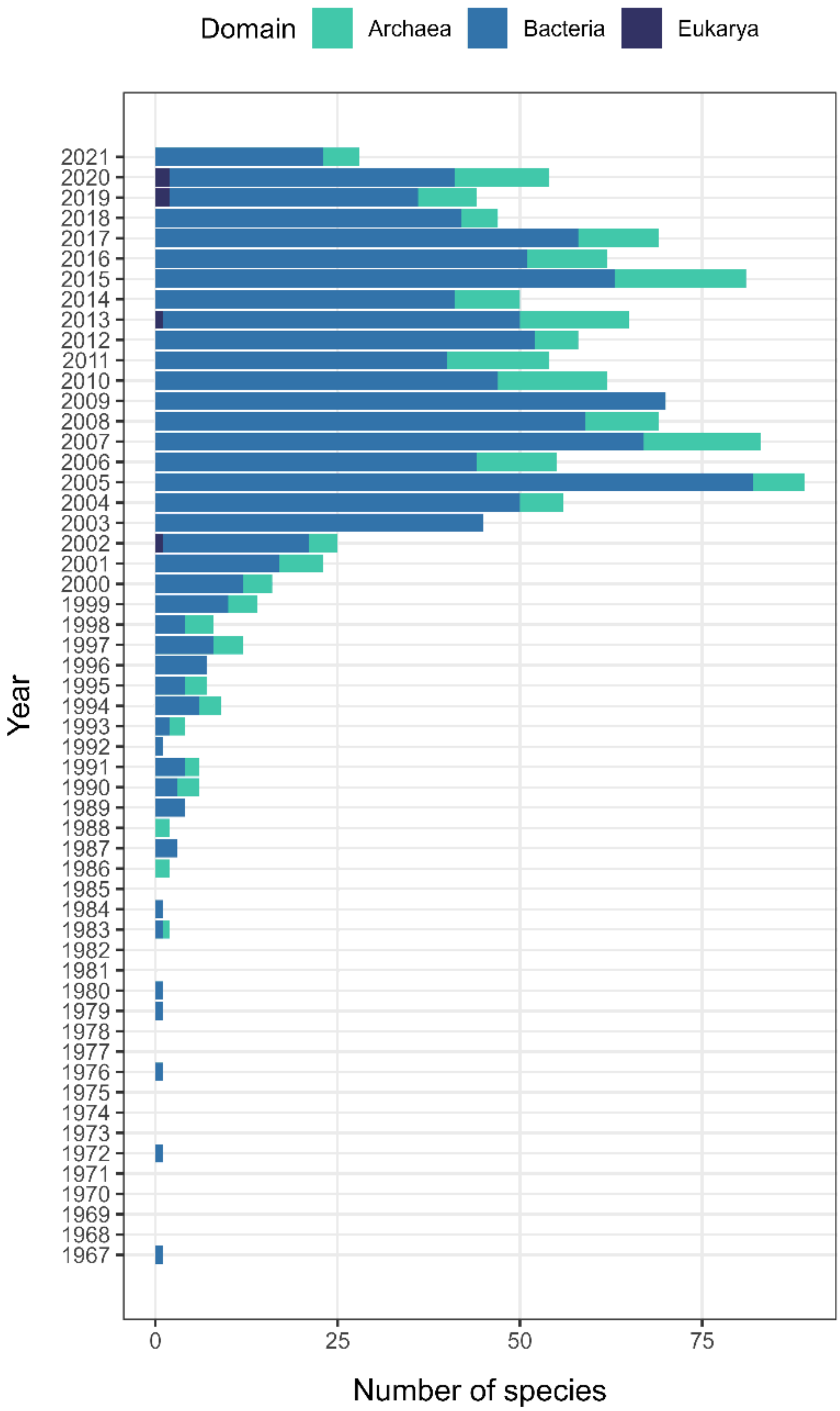

Figure 2

Histogram of number of species described per year per Domain in the International Journal of Systematic and Evolutionary Microbiology classified by the authors as “halotolerant”, “halophile” or “halophilic”

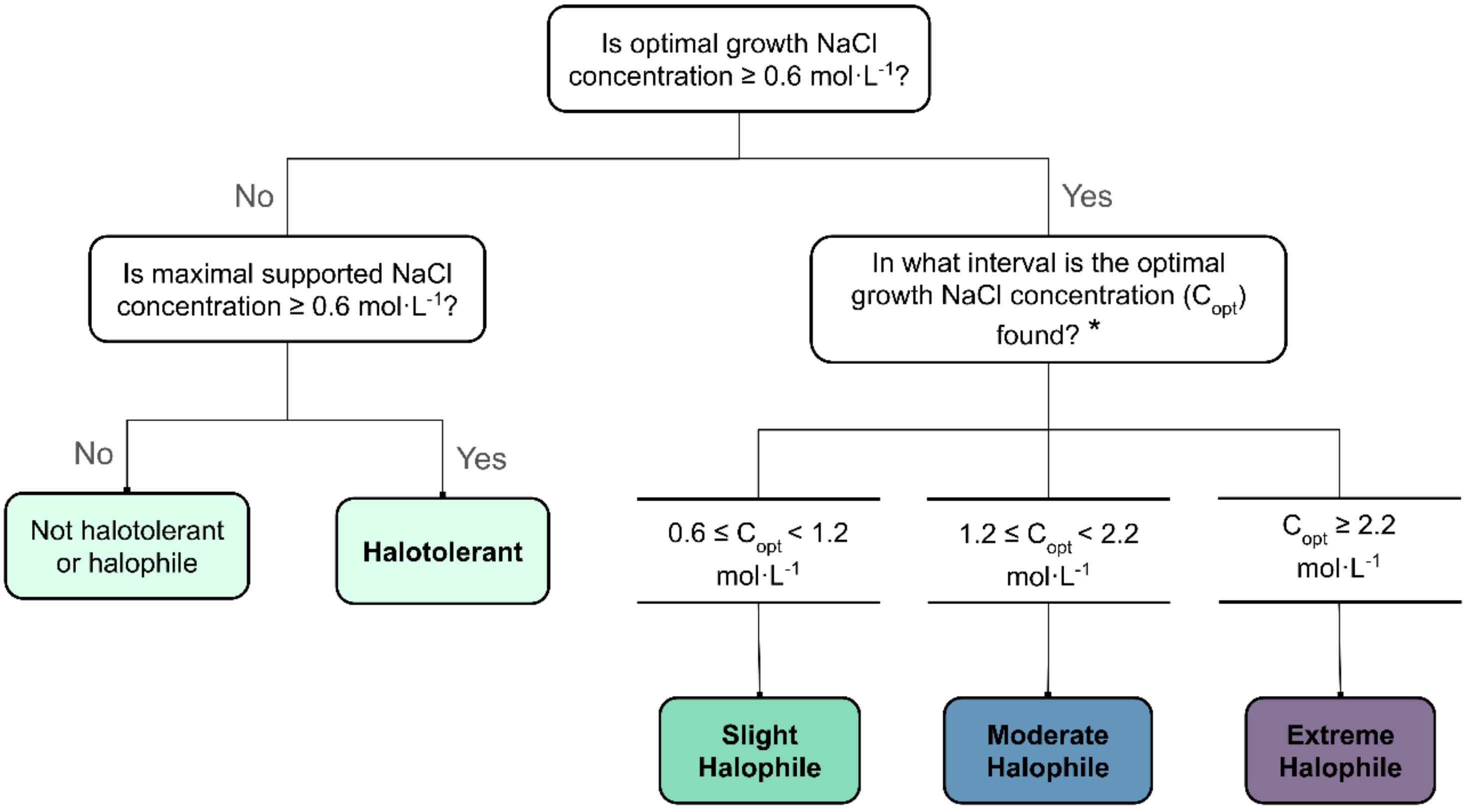

Figure 3

Flowchart for the classification of microorganisms as “not tolerant to NaCl”, “halotolerant”, “slight halophile”, “moderate halophile” or “extreme halophile” in accordance with the proposed classification system. * If the microorganism growths optimally in a range NaCl concentrations, consider the category in which most of the optimal growth range is. If the range is evenly split between categories, use the category that includes the highest value in the range

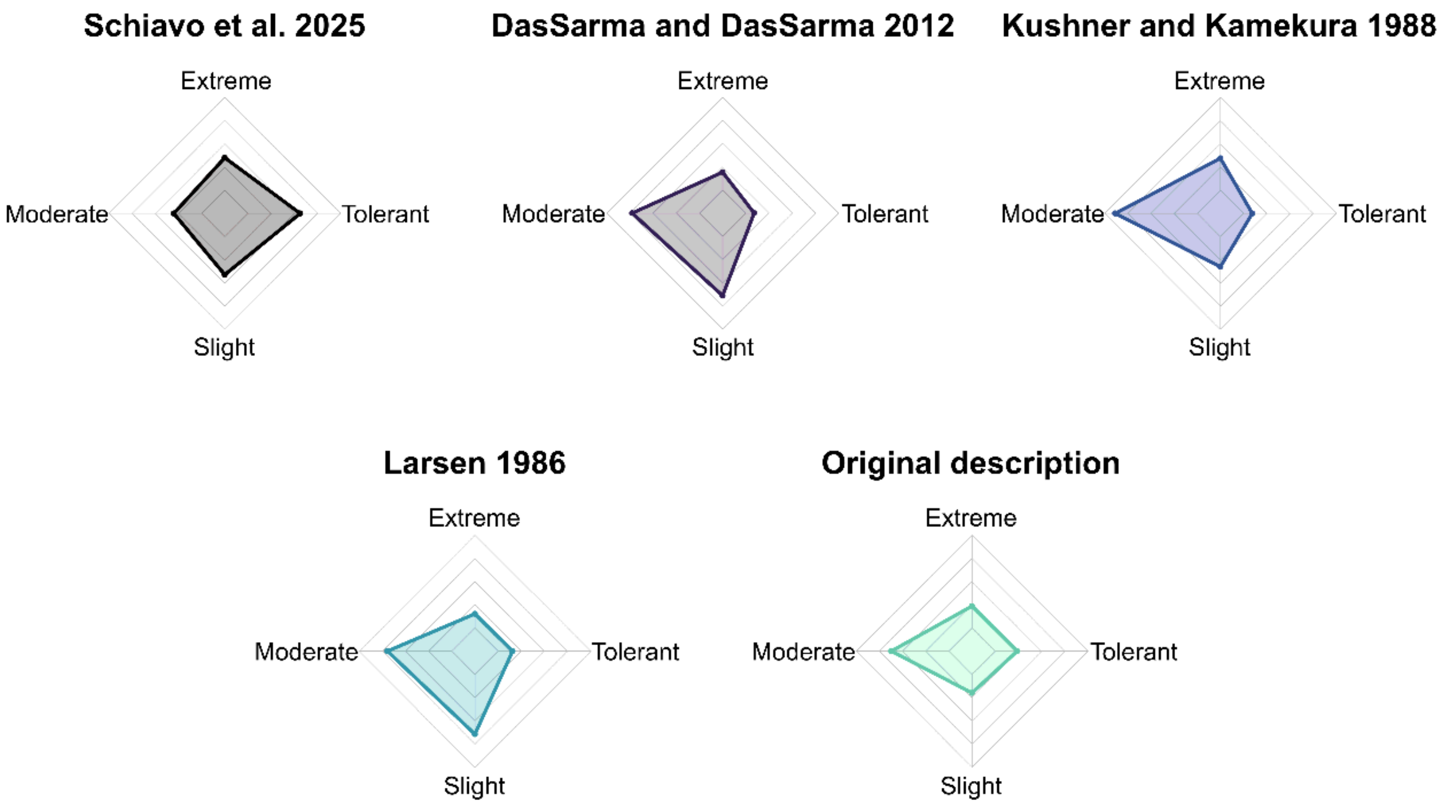

Figure 4

Radar chart of categorization of halophilic and halotolerant microorganisms, each interval between two lines corresponds to of 175 species. “Schiavo et al. 2025” refers to the halophile classification system proposed in this work and “Original description” refers to the category given by authors in species describing papers

Supplementary Files

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