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Halophilic bacteria in biotechnology: A seven-decade scientometric analysis of global research trends, knowledge gaps, and emerging applications (1955–2024)

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ABSTRACT

Halophilic bacteria have been a focal point of biotechnology research over the past seven decades due to their exceptional adaptability to extreme environments and broad application potential. This study aimed to analyze the evolution of halophilic research from 1955 to 2024 through a scientometric approach to identify trends, challenges, and future opportunities. The methodology involved analyzing 1,227 Scopus-indexed publications using bibliometric tools (VOSviewer and Biblioshiny). Data were categorized into distinct time periods (1955–1999, 2000–2009, 2010–2019, and 2020–2024). Analyses encompassed publication distribution and growth, journal productivity and impact, author contributions, citation metrics, thematic foci, collaboration networks, and emerging research trends. The findings revealed three evolutionary phases: a characterization phase (1955–1999), dominated by taxonomic and physiological studies; an application phase (2000–2019), emphasizing bioremediation and biopolymer production; and an engineering phase (2020–2024), marked by omics-driven approaches and synthetic biology. Cluster analysis identified three key research domains: cellular adaptation mechanisms, industrial applications, and metabolic engineering. Persistent challenges include limitations in genetic toolkits and scalability hurdles. Critical recommendations for advancing halophilic biotechnology include: (1) engineering microbial consortia for hypersaline industrial waste remediation, (2) exploring bioactive compounds from halophilic archaea, and (3) developing halophilic biosensors for real-time environmental monitoring. These interconnected avenues hold synergistic potential to drive sustainable biotechnology innovations leveraging halophilic resources.

Keywords: bibliometric analysis, bioremediation, biotechnology, extremozyme, VOSviewer.

INTRODUCTION

The exploration of halophilic bacteria has garnered significant attention in biotechnology due to their unique adaptive capabilities and broad application potential. Their defining characteristic is an obligate requirement for high salt (NaCl) concentrations for growth and survival. However, this requirement exists within a specific range, both insufficient salinity and excessively high salinity levels can inhibit growth or be lethal. Understanding these physiological limits on salt tolerance is crucial for harnessing their potential in biotechnology, particularly when considering the environmental conditions of target applications. These microorganisms thrive in hypersaline environments, positioning them as invaluable candidates for diverse industrial, agricultural, and pharmaceutical applications (Bawane et al., 2024). Their metabolic versatility and stability under high-salinity conditions further enhance their appeal as biotechnological resources. Their adaptability extends beyond salinity tolerance to include resilience to temperature fluctuations, pH extremes, and osmotic stress, making them ideal for challenging industrial processes (Rekadwad et al., 2023; Kurniawan et al., 2024).

Recent studies have highlighted the remarkable biotechnological potential of halophiles, particularly in producing bioactive compounds and enzymes. Specific strains have been identified as sources of stable enzymatic proteins, such as halocins, which exhibit antioxidant and antimicrobial properties (Martínez-Espinosa, 2024). These compounds show promise not only in therapeutic applications but also in agriculture, where they inhibit pathogenic fungal growth (Zhang et al., 2018; Far et al., 2019; Lach et al., 2021). Additionally, their enzyme stability under extreme conditions makes them suitable for industrial processes, including biosynthetic solute production and biofuel generation (Ruginescu et al., 2020; Ali et al., 2024).

Advances in omics technologies such as genomics, metagenomics, and proteomics—have driven recent breakthroughs in halophilic research, enabling deeper insights into their adaptive mechanisms and biotechnological potential (Rawat et al., 2024). For instance, genomic sequencing of *Halomonas boliviensis* has elucidated metabolic pathways linked to polyhydroxyalkanoate (PHA) synthesis (Enuh and Aytar-Çelik, 2023). Genetic manipulation and genomic characterization of such organisms have paved the way for advanced biotechnological applications. Biosynthetic gene clusters identified in halophilic strains may facilitate the discovery of novel biomolecules with unique biological activities (Boujida et al., 2018; Kumar and Nivetha, 2024). Furthermore, genetic engineering of *Halomonas* species is enhancing enzyme optimization for industrial use (Kaitouni et al., 2020; Ren et al., 2023). Innovations in metabolic engineering and synthetic biology now enable the design of halophilic strains for high-value compound production with enhanced efficiency. Despite these advances, most research remains exploratory, with limited translation to large-scale applications.

Critical knowledge gaps persist in halophilic research. First, studies on halophilic microbial consortia for bioremediation are scarce, despite their potential to degrade complex pollutants like hydrocarbons and heavy metals more efficiently. Second, optimizing halophilic enzyme production and stabilization for industrial scalability remains underexplored. Third, the biodiversity of halophiles in extreme environments (e.g., polar regions, traditional salt ponds) is poorly characterized, limiting the discovery of novel strains and enzymes.

This article addresses these gaps by presenting the first scientometric analysis mapping halophilic bacteria research trends in biotechnology. Using Scopus data and tools like VOSviewer and RStudio, we provide a comprehensive review of current advancements, challenges, and opportunities. Our findings aim to guide future research directions and foster academia-industry collaborations to accelerate real-world applications. By bridging scientific and practical perspectives, this work contributes to both academic discourse and the sustainable development of halophilic-based biotechnology.

MATERIALS AND METHODS

Data collection and search strategy

Bibliometric metadata were retrieved from the Scopus database on March 2025, using the following query string: (TITLE-ABS-KEY (“halophilic” OR “halophiles”) AND TITLE-ABS-KEY (“biotechnology” OR “industrial” OR “biodegradation” OR “bioactive compounds” OR “bioremediation”)). Scopus (Elsevier) was selected as the primary data source for this bibliometric analysis due to its extensive coverage of interdisciplinary fields relevant to halophilic prokaryotes (microbiology, extremophile studies, environmental biotechnology), capturing key journals and conference proceedings on hypersaline environments. Both Archaea and Bacteria were included in the search strategy to reflect the domain-integrated nature of hypersaline research, but analysis was confined exclusively to bacterial studies to align with the research objectives. Scopus’s robust bibliometric tools (citation tracking, author disambiguation) were essential for accurately mapping global trends and collaborations within this specialized bacterial biotechnology domain. The search spanned documents published between 1954 and 2024. Non-journal document types (e.g., conference papers, book chapters) were excluded, resulting in 1,674 initial records. To ensure a focused analysis of research trends, only original research articles were retained, and review articles were excluded using the AND NOT field code. Additional filters restricted the search to English-language journal articles, yielding 1.239 documents.

The final dataset was exported in CSV format for VOSviewer analysis and BibTeX format for RStudio processing. Metadata cleaning was performed in RStudio to remove duplicates and standardize formatting, resulting in 1,227 refined documents. These records included citation data, bibliographic information, abstracts, keywords, and metadata. Figure 1 illustrates the overall research framework. A key limitation of this study is its reliance on Scopus-indexed publications, which may exclude relevant works not indexed in the database.

Data analysis

The refined dataset was analyzed to assess the distribution, growth, and thematic evolution of halophilic biotechnology research. Key metrics included subject area trends, productive countries, authors, and journals, quantified using the Biblioshiny and Bibliometrix packages in RStudio v2024.12.0-467 (BibTeX file processing). Statistical graphs were generated to visualize publication trends and productivity patterns.

For bibliometric network mapping, the CSV file was imported into VOSviewer v1.6.18 (Leiden University, Netherlands). Keyword co-occurrence analysis was conducted with a minimum threshold of 5 appearances per term to ensure significance. Synonyms were consolidated into unified terms (in Supplementary 1). The resulting network maps visualized keyword relationships based on average publication year and link strength. Nodes (circles) represented keywords, with their size and proximity reflecting term prominence and interconnections. Link strength values quantified the frequency of co-occurrence, with higher values indicating stronger thematic associations. This approach identified emerging research fronts and predicted future trends in halophilic biotechnology.

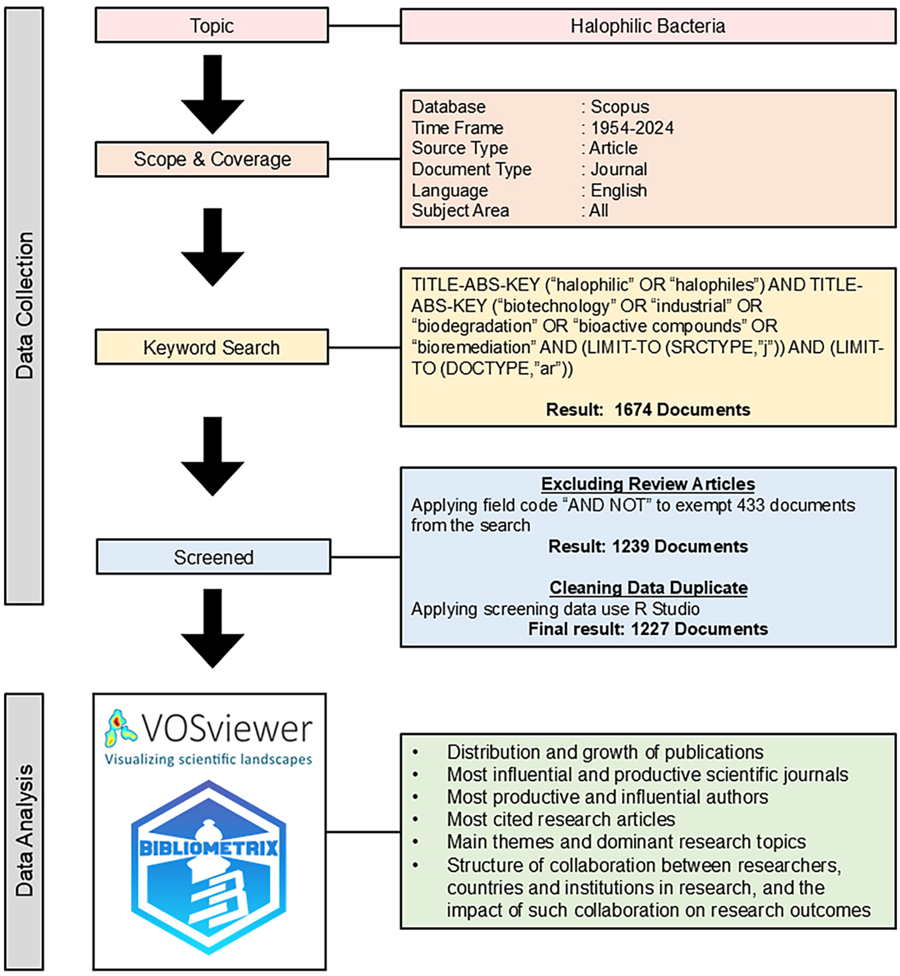

Figure 1. Research framework

Publications were categorized into four chronological periods (1954–1999, 2000–2009, 2010–2019, and 2020–2024) to trace thematic evolution. This temporal segmentation enabled analysis of keyword dynamics, highlighting dominant research topics and shifts in focus over time. The methodology not only provides a comprehensive overview of the current research landscape but also reveals historical trajectories and future-oriented trends.

RESULT AND DISCUSSION

Distribution and growth of publications

Analyses conducted with Biblioshiny and Bibliometrix in RStudio revealed a marked increase in halophilic bacteria-related publications (Figure 2). The figure illustrates annual and cumulative publication trends over time. While annual publication counts fluctuated, the cumulative count demonstrated exponential growth, rising sharply from 1999 to reach a total of 1,227 papers by 2024.

During the initial period (1954–1980), both annual and cumulative publication numbers were exceptionally low, with annual counts rarely exceeding 10 documents. Growth remained stagnant until the 1990s, when a gradual surge in annual outputs emerged. This trend accelerated exponentially in the 2000s, with annual publications surpassing 100 documents by 2010. A pronounced peak occurred between 2010 and 2020, likely reflecting heightened research interest or technological advancements in the field. By 2024, cumulative publications exceeded 1,200 documents. Collectively, the data highlight a clear trajectory research on halophilic bacteria has expanded dramatically, particularly over the last two decades. The cumulative publication curve underscores sustained growth, with progressively higher annual contributions driving the field forward.

Subsequent analysis pertains to countries with publications on halophilic bacteria (Figure 3). The figure displays the number of scientific publication documents on halophilic bacteria from the top 10 contributing countries. China leads significantly with 575 documents, followed by India (358 documents) and the United States (214 documents). Iran ranks fourth with 166 documents, while Japan, Spain, South Korea, Germany, Italy, and Saudi Arabia contribute between 90–150 documents. These data indicate that Asia (particularly China and India) and the United States are the primary hubs for halophilic bacteria research.

China's dominance in scientific publications on halophilic bacteria can be attributed to several key factors. First, China has made substantial investments in research and development (R&D),

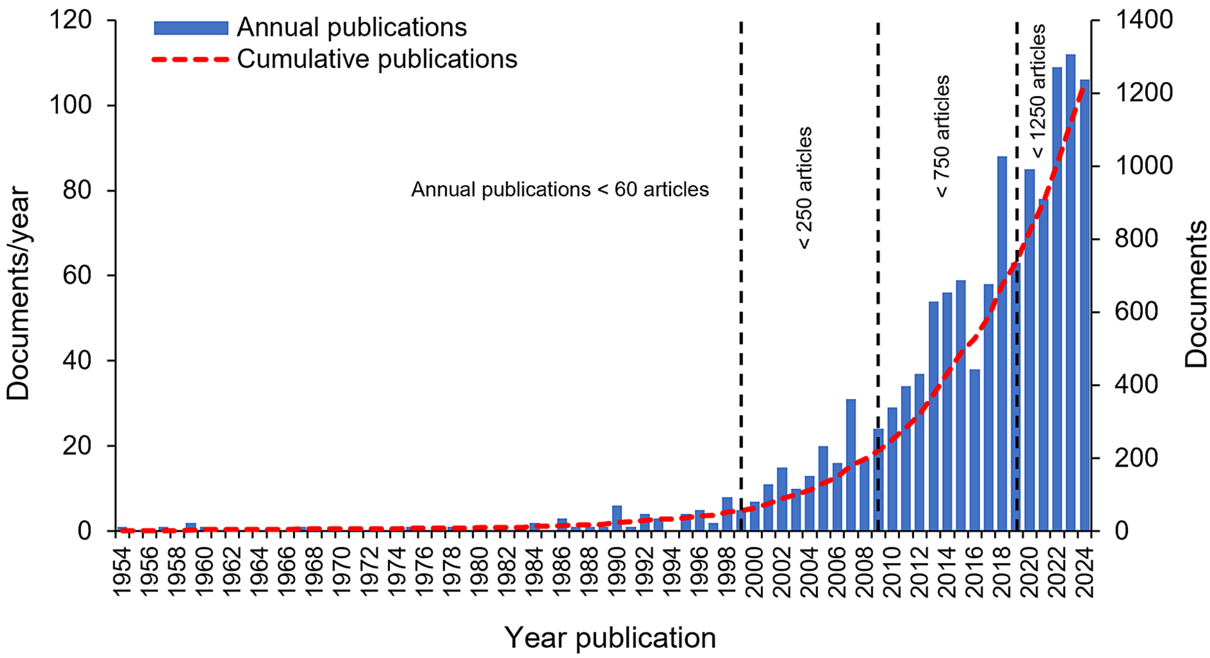

Figure 2. Annual scientific production

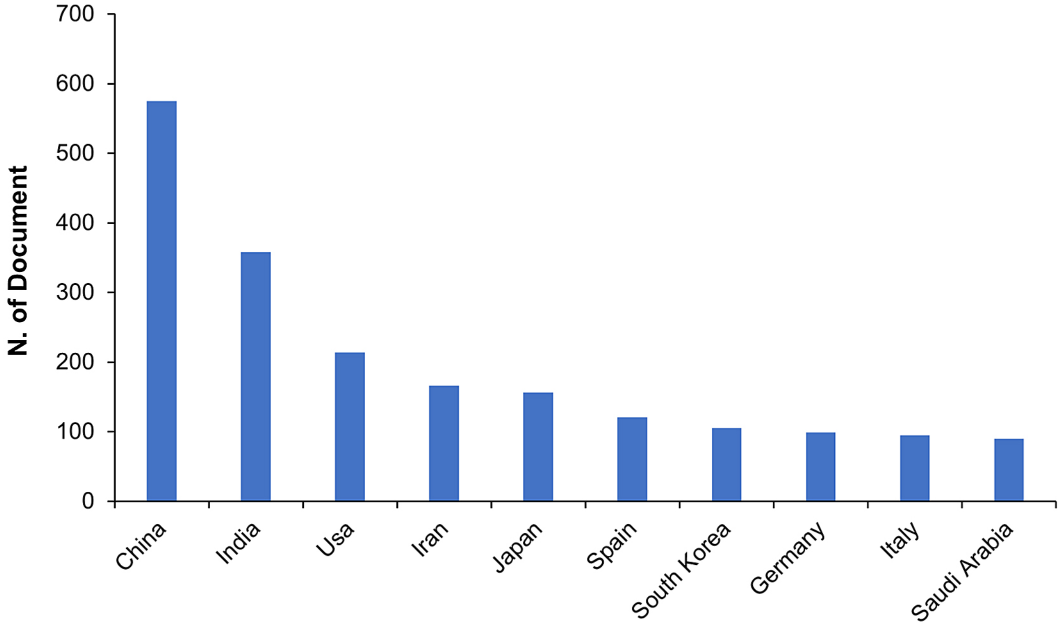

Figure 3. Countries' scientific production

with its research expenditure reaching 3.6 trillion yuan (USD 493 billion) in 2024, making it the second-largest research spender globally after the U.S. (National Bureau of Statistics of China, 2025). Second, the Chinese government has strategically prioritized industrial biotechnology in its Five-Year Plans, including research on extremophilic microorganisms such as halophilic bacteria, which hold critical applications in bioremediation and industrial enzyme production (Zhu et al., 2020). Advanced research infrastructure and international collaborations through initiatives like the Belt and Road Initiative have further accelerated scientific publication growth (Liu and Bennett, 2022). Additionally, China's natural environment, rich in saline lakes and salt-affected soils such as Qinghai and Lop Nur Lake, provides ideal habitats for halophilic bacteria research (Mianping et al., 2016). Incentive policies for researchers publishing in international journals also drive high scientific productivity (Xu et al., 2021).

Meanwhile, India secures the second position with approximately 600 documents, propelled by rapid growth in its biotechnology sector and government support through programs like the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR) (Kannan, 2024). The United States is recognized for superior research quality but exhibits lower publication volumes, likely due to its focus on more complex, multidisciplinary projects. Countries such as Iran, Spain, and Saudi Arabia also contribute significantly, supported by natural environments suited to halophilic research, including desert regions and salt lakes (Rodríguez-Núñez et al., 2020; Sacco et al., 2021; Al-Azzawi and Flowers, 2022).

The most influential and productive scientific journals

Analysis of scientific journals based on productivity level and influence is presented in Table 1. The data display the top 10 most productive and influential scientific journals in halophilic bacteria research publications. Based on quantitative and qualitative indicators such as publication count, H-index, G-index, and total citations, key journals serving as primary references in this field can be identified.

In the category of most productive journals, Extremophiles ranks first with 41 publications (3.34% of the total), followed by Bioresource Technology and Applied Microbiology and Biotechnology (38 publications, 3.10%) and Frontiers in Microbiology and International Biodeterioration and Biodegradation (37 publications, 3.02%). Temporal patterns indicate that journals such as Extremophiles and Journal of Industrial Microbiology and Biotechnology have been active since the late 1990s, demonstrating long-term contributions to the field. Meanwhile, Bioresource Technology and Frontiers in

Table 1. Most relevant sources and impact

No	Sources	N. of publication	H-index	G-index	M-index	Total citations	First year	Total publication (%)
1	Extremophiles	41	23	38	0.82	1507	1998	3.34
2	Bioresource Technology	38	27	38	1.35	2913	2006	3.10
3	Applied Microbiology and Biotechnology	37	24	37	0.96	2325	2001	3.02
4	Frontiers in Microbiology	33	13	21	1.08	464	2014	2.69
5	International Biodeterioration and Biodegradation	32	22	32	0.786	1556	1998	2.61
6	International Journal of Biological Macromolecules	18	18	18	0.621	1915	1997	1.47
7	Journal of Industrial Microbiology and Biotechnology	18	14	18	1.273	415	2015	1.47
8	Applied and Environmental Microbiology	17	13	17	0.565	782	2003	1.39
9	Journal of Biotechnology	17	10	17	0.333	325	1996	1.39
10	Journal of Microbiology and Biotechnology	17	10	17	0.455	312	2004	1.39

Microbiology exhibit high productivity consistency with an average of 2–3 publications per year, supported by their broad topical coverage in microbial biotechnology.

Regarding scientific influence, Bioresource Technology stands out with the highest H-index (27) and total citations (2.913), reflecting its substantial impact within the research community. Extremophiles and Frontiers in Microbiology also demonstrate strong performance, with H-indices above 20 and total citations exceeding 1,500, indicating consistent scientific article quality. In terms of relative temporal impact, Applied and Environmental Microbiology has the highest M-index (1.273), signifying rapid research influence despite its relatively low publication count (18), while International Journal of Biological Macromolecules (M-index 1.08) excels in recent research, particularly since 2014.

Trend analysis reveals several notable patterns. Journals such as Extremophiles and Bioresource Technology tend to focus on industrial applications of halophilic bacteria, including extremozymes and bioremediation, whereas Frontiers in Microbiology emphasizes microbial diversity and ecology. In terms of collaboration, journals with high G-indices like Bioresource Technology and Extremophiles (G-index 38) reflect extensive author collaboration networks, frequently involving researchers from China, the U.S., and Europe. However, gaps exist in journals such as International Journal of Microbiology and Biotechnology, which show progressive productivity but low impact (H-index 10–13), likely due to narrow specialization or limited global visibility.

Extremophiles, Bioresource Technology, and Frontiers in Microbiology emerge as the three most influential and productive journals for halophilic bacteria research, combining ideal publication volume, scientific impact, and temporal consistency. Selecting these journals for publication or literature review provides access to high-quality research and current trends. Recommendations based on these data include: researchers should prioritize publishing in Bioresource Technology or Extremophiles to achieve high visibility, while readers should utilize Frontiers in Microbiology as a primary resource for multidisciplinary studies.

Subsequent analysis of publication distribution by subject area over specific time periods is presented in Figure 4. The data illustrate the percentage distribution of scientific publications on halophilic bacteria categorized by primary research fields and publication timeframes. In terms of research fields, Biochemistry, Genetics and Molecular Biology emerges as the dominant discipline, followed by Immunology and Microbiology and Agricultural and Biological Sciences. This reflects the multidisciplinary nature of halophilic bacteria research, with primary emphasis on microbial biochemical and genetic aspects, agricultural applications, and medical potential. Fields such as Chemical Engineering, Chemistry, and Environmental Science also demonstrate significant contributions, indicating the application of halophilic

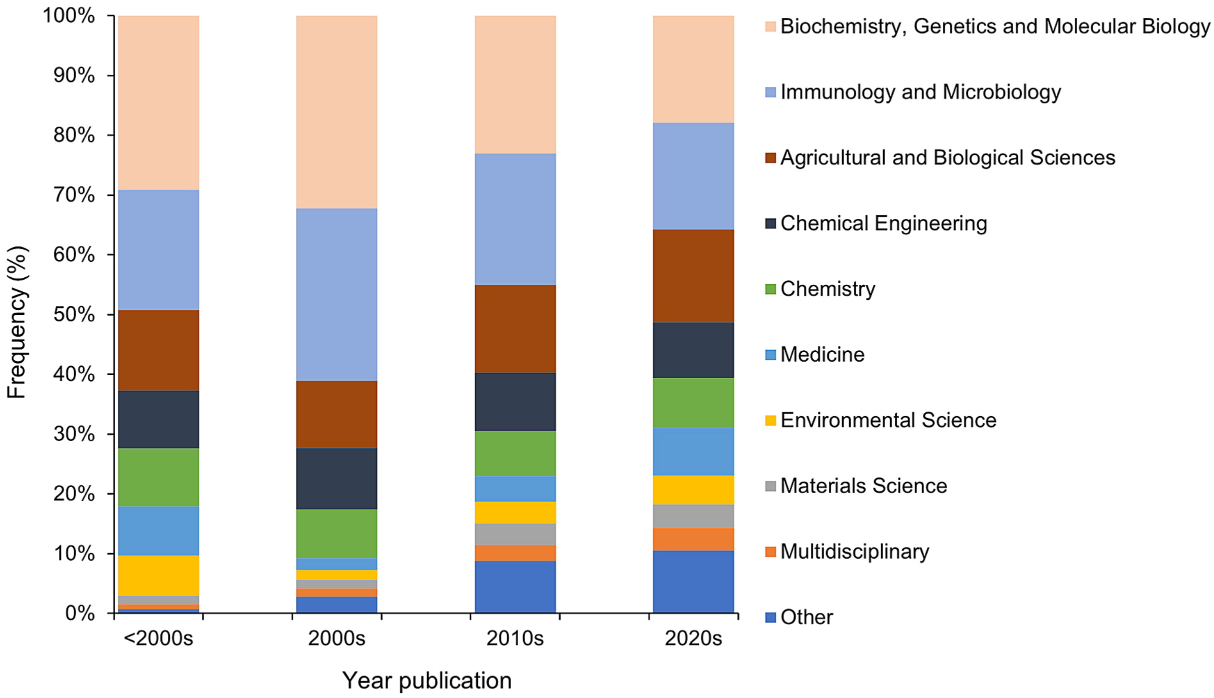

Figure 4. Publication distribution by subject area over the past decade

bacteria in bioprocessing, material chemistry, and bioremediation.

Temporally, the data reveal a significant increase in publication volume from the 2000s to the 2020s. The 2010–2020 period marks an era of accelerated research in this field, driven by advancements in DNA sequencing technology and growing interest in sustainable biotechnology. This trend persists into the 2020s, during which multidisciplinary research has become increasingly dominant, reflecting the necessity for integrated approaches to explore the potential of halophilic bacteria. Deeper analysis uncovers that the dominance of biochemistry and molecular genetics correlates with efforts to characterize extremophilic enzymes stable under extreme conditions, while the rise in publications in environmental science and chemical engineering over the past decade highlights a shift in focus toward industrial and environmental applications. This distribution further indicates that research on halophilic bacteria has evolved from foundational studies of microbial physiology to broader explorations of applied potential.

These findings underscore the importance of collaborative approaches across disciplines in halophilic bacteria research, while emphasizing the need for publication platforms capable of accommodating the multidisciplinary nature of contemporary studies in this field. These developments also signal promising prospects for industrial biotechnology and environmental applications in the coming decade.

The most productive and influential authors

The results of the analysis of the most productive and influential authors can be seen in Table 2. The data display the top 10 most leading researchers who have made significant contributions to halophilic bacteria research. Mohammad Ali Amoozegar from the University of Tehran emerges as the most productive author with 23 publications, followed by Wang Huan from Tsinghua University with 20 publications. In terms of scientific influence, Amoozegar also leads with an H-index of 15 and total citations of 1.334, reflecting the broad impact of his research within the scientific community. This achievement is supported by his consistent contributions since 2005, demonstrating sustained research productivity and relevance.

Several notable patterns emerge from these data. Researchers from Spain, such as Emilia Quesada (Universidad de Granada) and Antonio Ventosa (University of Sevilla), though having fewer publications (13–15), exhibit strong influence with total citations nearing 1.000 and H-indices of 13. This indicates that the quality and impact of their research are highly recognized, despite lower

Table 2. Most productive authors and impact

No	Author	ID Scopus	Affiliation	N. of publication	H-index	Total citations	First year
1	Amoozegar, Mohammad Ali	56742208100	University of Tehran	23	15	1334	2005
2	Quesada, Emilia	7007144031	Universidad de Granada	13	13	946	1995
3	Ventosa, Antonio	7006170495	University of Sevilla	15	13	991	2004
4	Wang, Huan	57191166781	Tsinghua University	20	13	635	2009
5	Chen, GuoQiang	7406536843	Tsinghua University	13	10	801	2011
6	Liu, Yan	57359371900	Yunnan Normal University	13	10	266	2014
7	Li, Xiangjin	58724995000	Henan University	11	9	527	2011
8	Llamas, Inmaculada	6603406366	Universidad de Granada	9	9	493	2000
9	Mellado, Encarnación	7003532603	University of Seville	9	9	727	2004
10	Pugazhendi, Arulazhagan	16833577500	King Abdulaziz University	13	9	307	2017

output volume. Quesada active since 1995, is the most experienced researcher on this list with contributions spanning over two decades.

Geographically, the data reveal diverse distribution, with strong representation from Iran (Amoozegar), Spain (Quesada, Ventosa, Llamas, Mellado), China (Wang, Chen, Liu, Li), and Saudi Arabia (Pugazhendi). Chinese researchers, though relatively recent in activity (active since 2009–2017), have demonstrated impressive productivity, particularly Wang Huan, who achieved 20 publications within a short timeframe. However, Chinese researchers tend to exhibit lower citation-to-publication ratios compared to their Spanish counterparts, potentially due to differences in research duration or topical focus. These findings highlight critical insights: high productivity does not always directly correlate with scientific impact, as seen in the comparison between Iranian and Spanish researchers. Additionally, long-term research experience tends to yield more sustained influence, as evidenced by Quesada and Ventosa.

The most cited research articles

The results of the analysis of the most-cited scientific articles reveal notable insights (Table 3). The data display the ten most influential articles in halophilic bacteria research based on citation counts. The scientific article by Oren (2002), published in the Journal of Industrial Microbiology and Biotechnology, ranks first with 632 citations, followed by the work of Margesin and Schinner (2001) with 592 citations. Notably, despite being published in 2002, Oren's article maintains an average of 26.33 citations per year, demonstrating sustained relevance in the field. This work discusses the physiological characteristics and biotechnological potential of extreme halophilic bacteria, serving as a critical foundation for subsequent studies. Significantly, 80% of the most-cited articles were published before 2015, suggesting that recent research may require more time to accumulate citations.

Key findings from these top-cited articles warrant emphasis. Margesin and Schinner (2001) in Applied Microbiology and Biotechnology revealed applications of halophilic enzymes in industry, while Asad (2007) in Bioresource Technology explored the use of halophilic bacteria for hypersaline environmental bioremediation. Strikingly, the article by He et al. (2017) in International Biodeterioration & Biodegradation, though relatively recent (published in 2017), has already garnered 324 citations with an average of 36 citations per year—the highest annual impact rate. This study investigates the capacity of halophilic bacteria to degrade hydrocarbons in polluted marine environments, a topic increasingly relevant to oceanic oil contamination issues.

Temporal patterns indicate that classic articles (1995–2002) tend to have higher total citations due to time advantages, while recent articles (2010–2017) exhibit higher annual citation rates, reflecting growing interest in halophilic bacteria research over the past decade. For instance, Duan et al. (2015) on halophilic bacteria-mediated bioplastic production and Tan et al. (2011) on biohydrogen production, both in Bioresource Technology, illustrate how research focus has shifted from foundational studies toward practical biotechnological applications.

Table 3. Most global cited documents

No.	Paper	DOI	Total citations	Total cited / year
1	Oren A, 2002, Journal of Industrial Microbiology and Biotechnology	10.1038/sj/jim/7000176	632	26.33
2	Margesin R, 2001, Applied Microbiology and Biotechnology	10.1007/s002530100701	592	23.68
3	Asad S, 2007, Bioresource Technology	10.1016/j.biortech.2006.08.020	410	21.58
4	He H, 2017, International Biodeterioration & Biodegradation	10.1016/j.ibiod.2016.10.007	324	36.00
5	Duan J, 2015, Bioresource Technology	10.1016/j.biortech.2014.12.057	310	28.18
6	Woolard CR, 1995, Water Research	10.1016/0043-1354(94)00239-4	294	9.48
7	Tan D, 2011, Bioresource Technology	10.1016/j.biortech.2011.05.068	293	19.53
8	Quillaguamán J, 2010, Applied Microbiology and Biotechnology	10.1007/s00253-009-2397-6	248	15.50
9	Sauer T, 1998, Biotechnology and Bioengineering	10.1002/(SICI)1097-0290(19980205)57:3%3C306::AID-BIT7%3E3.0.CO;2-L	241	8.61
10	Schwibbert K, 2011, Environmental Microbiology	10.1111/j.1462-2920.2010.02336.x	231	15.40

In terms of journals, Bioresource Technology and Applied Microbiology and Biotechnology dominate the list, each contributing three articles, solidifying their status as leading publication outlets for applied halophilic bacteria research. These findings not only reflect the historical progression of the field but also highlight current trends emphasizing environmental and industrial applications of halophilic bacteria, particularly in bioremediation, bioenergy production, and high-value compound synthesis. These articles serve as essential references for researchers seeking to understand the evolution and future trajectory of halophilic bacteria research.

Main themes and evolution research topics

Analysis of research keyword frequency data reveals that "halophiles" appears 105 times, ranking first as the most researched topic (Figure 5). The term "halophilic bacteria" follows with 77 occurrences, indicating a strong focus on basic characterization of halophilic microorganisms. However, the high frequency of "biodegradation" (77) and "bioremediation" (73) reflects a significant shift in research trends. A meta-analysis by Lasani et al. (2023) highlights an increase in publications on environmental applications since 2015, with applied research primarily focusing on utilizing halophilic bacteria for bioremediation of contaminated areas and heavy metals.

Keyword frequency analysis shows "Halomonas" appears 57 times, significantly higher than "haloarchaea" (33 occurrences). This disparity underscores researchers' preference for halophilic bacteria in applied studies. García-Roldán et al. (2024) report that 72% of applied research favors halophilic bacteria due to their ease of cultivation. Conversely, halophilic archaea have gained attention following a pivotal discovery by Wang et al. (2024), whose study demonstrated that enzymes from halophilic archaea exhibit 40% higher thermal stability compared to bacterial counterparts. This advantage opens new opportunities for their use in pharmaceutical and food processing industries.

The prominence of the keyword "bioremediation" underscores the growing importance of environmental recovery research. Venkatesan et al. (2023) successfully developed a halophilic consortium with exceptional efficiency, achieving 85% oil degradation under extreme salinity conditions (20%). This breakthrough offers critical solutions for addressing oil spills in coastal regions. Over the past decade, halophilic bacteria research has prioritized environmental applications, with recent findings validating their immense potential in resolving pollution challenges in hypersaline environments.

The keyword "salinity", appearing 37 times, remains relevant in contemporary research. Advances in omics techniques have revitalized halophilic studies. Wang et al. (2024) conducted comprehensive metagenomic mapping of Tibetan salt lakes, identifying novel halophilic microbial species previously unreported. Notably, their study revealed that 60% of salt resistance-related genes remain underutilized. These multi-omics

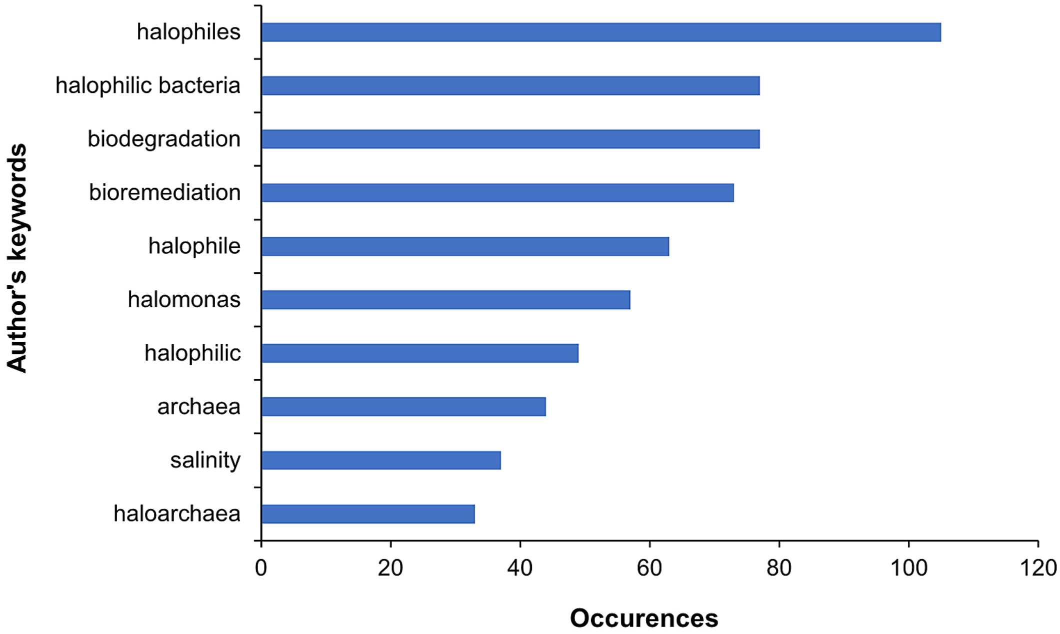

Figure 5. Most frequent keywords

approaches not only deepen our understanding of microbial diversity but also unlock new avenues for exploring their biotechnological potential. The integration of multi-omics techniques is pivotal in unraveling the mysteries of halophilic microbes.

Keyword co-occurrence network data reveals the complexity and strong interconnections among various halophilic research topics (Figure 6). Three distinct clusters are clearly identified: a blue cluster dominated by bioremediation and industrial application topics such as hydrocarbon degradation and exopolysaccharide production; a red cluster focused on extreme adaptation and physiology, encompassing studies on psychrophiles and thermophiles; and a green cluster comprising genomic and taxonomic investigations. This pattern illustrates the evolution of halophilic research from foundational studies toward practical applications across diverse fields. Inter-cluster connections indicate an increasingly intensive multidisciplinary approach to exploring the potential of halophilic microorganisms.

The blue cluster demonstrates a strong association between halophilic bacteria and hydrocarbon degradation capabilities. Research by Zhang et al. (2023) reports degradation efficiency reaching 85% under high-salinity conditions. These findings align with the high co-occurrence of crude oil and exopolysaccharides, suggesting the role of halophilic bioactive compounds in bioremediation. Gan et al. (2024) further show that exopolysaccharides produced by halophilic microbes enhance oil emulsification by 70%, establishing them as critical components in bioremediation processes. These bioactive compounds not only facilitate hydrocarbon breakdown but also protect microbial cells under extreme conditions. These results are corroborated by multiple studies highlighting the significant potential of halophilic microbial consortia in addressing environmental pollution (Hussain et al., 2024). Their application is increasingly relevant given rising oil spill incidents in high-salinity coastal regions (Sharma et al., 2024).

The red cluster elucidates unique molecular mechanisms enabling halophilic microbes to survive under diverse extreme conditions. Aziz and Masmoudi (2023) demonstrate the simultaneous activation of cold shock protein genes and osmolyte synthesis in environments with extreme temperature and salinity fluctuations. Halophilic microbes also employ sophisticated quorum sensing systems for intercellular communication under stress. Notably, enzymes from thermophilic halophiles exhibit potential antimicrobial activity against resistant pathogens (Amoozegar et al., 2017), opening avenues for novel antibiotic development and industrial enzymes stable under extreme conditions.

The green cluster reflects rapid advancements in genomic approaches to studying halophilic diversity. Single-cell genomics techniques

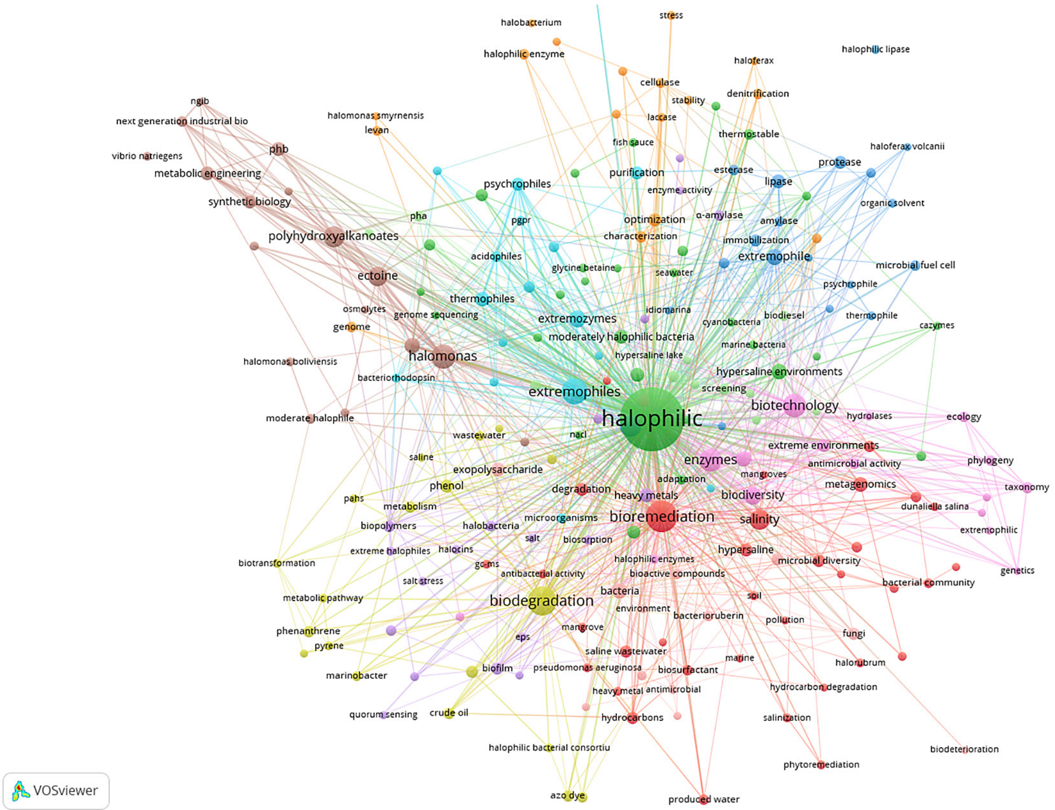

Figure 6. Network co-occurence keyword

have uncovered hundreds of previously unidentified species (Fromm et al., 2024). Phylogenetic analyses reveal complex evolutionary relationships among halophilic groups. However, research by Gadd et al. (2024) also identifies potential biodeterioration risks posed by extremophilic microbes on industrial materials. Integrating metagenomic data with physiological characterization has become a critical approach for comprehensively understanding halophilic microbial ecology. These developments drive the standardization of classification methods and nomenclature for extremophilic microbial taxa.

The development of halophilic bacteria research demonstrates a significant thematic shift from 1954 to 2015, culminating in 2020 (Figure 7). In the initial period (1954–1999), research primarily focused on the basic characterization of halophilic microorganisms, with dominant keywords such as halophilic bacteria, hypersaline, and archaea. Studies during this era predominantly addressed the identification and classification of microbes in extreme environments, as exemplified by Kushner (1983) pioneering work on cellular adaptation to high salinity. This period also marked early explorations into the simple biotechnological potential of halophilic microbes, though applications remained limited.

Entering the 2000–2015 period, a dramatic expansion of research themes occurred. Keywords like biodegradation, polyhydroxyalkanoates (PHA), and halotolerance began to dominate, reflecting a shift toward practical applications. Research by Margesin and Schinner (2001) became a cornerstone in utilizing halophilic bacteria for bioremediation of polluted environments. Concurrently, advances in response surface methodology enabled optimization of high-value compound production, such as PHA and halophilic enzymes. Oren (2002) also made significant contributions by elucidating the metabolic diversity of halophilic microbes through early genomic approaches.

Several key findings emerged between 2000–2015. First, studies on exopolymers and emulsifying activity revealed the potential of halophilic bioactive compounds for food and cosmetic industries. Second, the emergence of the term saline wastewater treatment highlighted growing environmental applications. John et al. (2020) successfully demonstrated the use of halophilic consortia in treating textile industrial wastewater

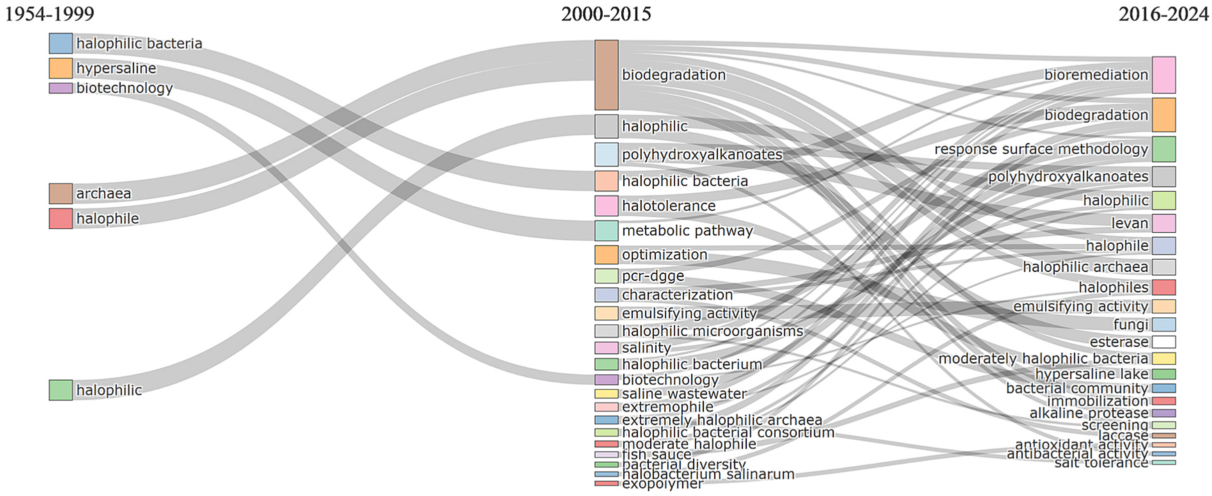

Figure 7. Thematic evolution

with 90% efficiency. Third, research on alkaline proteases and esterases from halophilic archaea paved the way for developing industrial enzymes stable under harsh conditions.

A comparison of halophilic research periods reveals three significant evolutionary patterns. First, a shift occurred from traditional taxonomic approaches toward active compound bioprospecting. Early studies focused on microbial classification (Kushner et al., 1983) evolved into explorations of bioactive compounds like exopolysaccharides and extremozymes (Narayan et al., 2024). Second, research methodologies transitioned from single-microbe isolation to complex bacterial community analyses, as evidenced by metagenomic studies of hypersaline environments (Galisteo et al., 2024), which uncovered previously undetected microbial interactions. Third, the rise of terminology such as optimization and metabolic pathway marked a transition from descriptive research to metabolic engineering approaches, exemplified by the engineering of Halomonas strains for PHA production (Koller, 2023).

Post-2015 research projections indicate three potential directions. Genetic engineering is predicted to underpin enhanced bioactive compound production, following CRISPR-Cas9 successes in Halobacterium modification (Swaminathan et al., 2024). Multi-omics approaches (genomics, transcriptomics, proteomics) are expected to advance understanding of microbial community dynamics in hypersaline environments, as pioneered in Tibetan salt lake studies (Rawat et al., 2024). Halophilic exopolysaccharides are also gaining attention for biomedical applications (Kiran et al., 2024).

Structure of collaboration

Analysis of international collaboration networks reveals distinct patterns in halophilic bacteria research from 1954 to 2024 (Figure 8). China emerges as the dominant hub within the global collaboration network, exhibiting robust linkages to multiple major research-producing nations. Data indicate intensive Chinese collaborations with India (42 joint publications), primarily on bioprospecting of salt pond microbes; with the United States (38 joint publications), focused on industrial enzyme engineering; and with Iran (29 joint publications) for hypersaline lake ecology studies. Secondary collaboration patterns are observed between Japan and South Korea (25 joint publications) in marine biotechnology, and between Spain and Italy (18 joint publications) for Mediterranean biodiversity studies. China's dominance in research collaboration is driven by strategic investments through the China Ocean Mineral Resources R&D Association (COMRA) (Yu et al., 2025).

These findings carry several critical implications for global research policy. First, diversification of partnerships by developing nations is imperative through initiatives such as enhanced India-Brazil collaboration. Second, establishing multilateral consortiums for unique salt ecosystems, such as an Asian hypersaline environment network, could strengthen regional cooperation. Third, standardization of cross-border sample exchange protocols requires attention to facilitate more effective collaboration. Network visualization maps reveal a clear collaboration structure, with China as the central node and region-specific interconnected clusters. These results indicate a robust community structure within the global halophilic research network. The findings not only delineate current collaboration patterns but also provide critical guidance for strengthening future research networks, particularly in addressing global challenges related to harnessing halophilic microbes for sustainable applications.

The countries' collaboration world map (Figure 8) reveals significant geographic disparities in halophilic bacteria biotechnology research. While the high output from Asia, Europe, and North America signifies strong research focus and capacity, the notably lower contribution from Africa and South America highlights a critical gap. These continents host diverse hypersaline ecosystems (China) representing vast reservoirs of unexplored halophilic diversity. Bridging this gap through collaborative initiatives and targeted funding is essential not only for equitable scientific progress but also for developing locally adapted biotechnological solutions. Harnessing the unique adaptations of halophiles native to these understudied regions holds immense promise for addressing pressing environmental challenges like salinity intrusion affecting agriculture and treatment of hypersaline industrial wastewater prevalent there.

Analysis of institutional contributions in halophilic bacteria research was conducted by extracting author affiliation data from Scopus (Figure 9a). A total of 9 top institutions were identified based on publication count, with normalization against name variants. The results reveal the distribution of the most productive institutions in halophilic research, with Islamic Azad University (Iran) and the University of Sevilla (Spain) as primary contributors. A notable pattern emerges in the dominance of developing nations, where 6 out of 9 institutions originate from Iran, India, and Russia. Regional specializations are evident, such as the University of Sevilla's focus on Mediterranean microbial ecology and Islamic Azad University's contributions to bioremediation applications.

Analysis of halophilic bacteria research contributors was performed by extracting author names from the Scopus dataset (Figure 9b). Name normalization was applied to account for writing variants. Results identify Amoozegar, Ventosa, Wang, and Zhang as authors collaborating extensively with numerous other researchers. This collaboration drives higher scientific publication

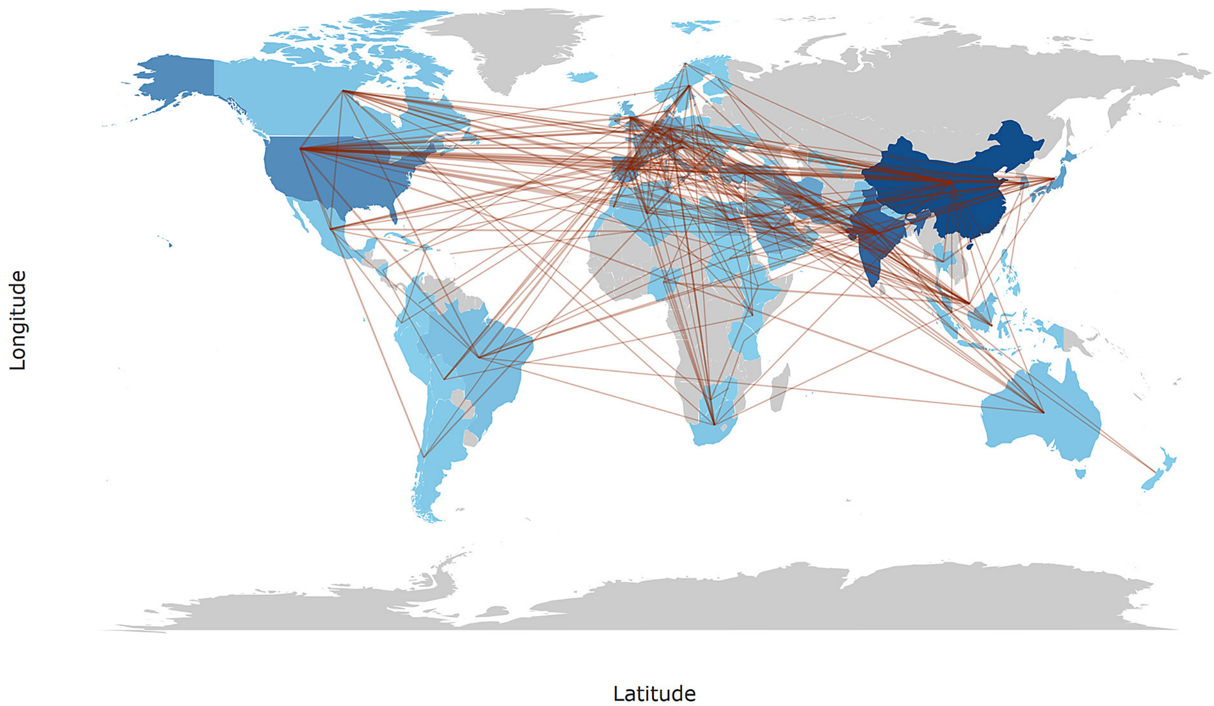

Figure 8. Countries' collaboration world map (The difference in color gradient indicates the number of publications)

Figure 9. Network of collaboration: (a) By institutions, (b) By authors

outputs on halophilic bacteria. The emergence of new strengths from China and Saudi Arabia in the past decade reflects shifting dynamics in the global halophilic research landscape. For future research, collaborations between experienced Spanish researchers and prolific Asian scientists could serve as a promising strategy to synergize research quality and quantity.

The future trends and challenge research

Bibliometric analysis using VOSViewer reveals rapidly evolving research on halophilic bacteria. Keyword data from recent studies highlight several major trends that will shape the future of halophilic research (Figure 10). First, azo dye degradation by halophilic bacteria emerges as a sustainable solution for textile industrial waste, with recent studies demonstrating that consortia of Halomonas and Haloferax can degrade dyes under high salinity (Hussain et al., 2024; Anabtawi et al., 2024). However, a key challenge lies in the stability of these consortia in complex waste containing heavy metals. Research on azo dye degradation using halophilic bacteria faces complex challenges in maintaining microbial consortium stability within heterogeneous industrial waste environments. Although halophilic bacterial consortia show degradation efficiencies of 100–400 mg/L under laboratory conditions (Rostami and Shahsafi, 2018; Tian et al., 2021), field applications are hindered by heavy metal interference and salinity fluctuations that disrupt key enzyme activities such as azoreductase. Additional research challenges include microbial consortium instability when exposed to volatile organic compounds and the formation of carcinogenic intermediates like aromatic amines. Another challenge is the need for real-time monitoring systems to optimize degradation conditions. Potential solutions include engineering genetic circuits that integrate heavy metal resistance genes with azo dye degradation pathways.

Next, antioxidant production from halophilic exopolysaccharides, such as levan from *Halomonas elongata*, exhibits radical-scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) equivalent to vitamin C (Mouro et al., 2024), but production costs remain higher than conventional antioxidants. The development of halophilic exopolysaccharide-based antioxidants like levan from *Halomonas elongata* faces several challenges. First, high production costs due to specialized hypersaline media requirements. Second, structural complexity of polysaccharides complicating characterization and standardization. Third, reduced functional stability under non-hypersaline conditions. Recent studies show DPPH radical-scavenging activity declines when applied in physiological buffer systems (John et al., 2019; Rathod et al., 2023; Rana et al., 2024). Metabolic engineering approaches to modify levan functional groups and in situ extraction systems are under development to address these issues.

Antibacterial activity of halophilic enzymes such as lipases and proteases against multidrug-resistant pathogens (e.g., MRSA) has gained attention, with unique mechanisms involving cell membrane destabilization (Rampasath et al., 2021; Huang et al., 2024). A critical limitation is the frequent loss of this activity under non-hypersaline conditions. Applications of halophilic enzymes like lipases and proteases as antibacterial agents face fundamental constraints in action mechanisms and stability. Despite efficacy against Methicillin-resistant *Staphylococcus aureus* (MRSA) via membrane destabilization (Hamdan et al., 2023), these enzymes lose 80–90% activity at physiological salinity. Further challenges include susceptibility to host proteases and narrow action spectra. Protein engineering solutions, such as fusion proteins with antimicrobial peptides and structural rigidification of domains, are under investigation. However, results remain suboptimal, with stability improvements of only 2–3-fold.

PAHs (polycyclic aromatic hydrocarbons) degradation by halophilic communities in coastal sediments shows 70–80% efficiency for compounds like pyrene (Lee et al., 2024; Pandya et al., 2024), but applications are hindered by slow degradation of heavy compounds like benzo[a]pyrene. PAHs degradation research faces challenges such as limited degradation mechanisms for heavy compounds like benzo[a]pyrene. Nutrient competition among microbial consortium members and accumulation of toxic intermediates also pose obstacles. Jiang et al. (2022) revealed that pyrene degradation efficiency (70–80%) does not linearly correlate with heavy PAHs due to the lack of specific dioxygenase enzymes. Multi-omics and machine learning approaches are being developed to predict metabolic interactions within PAHs-degrading consortia (Venkatesan et al., 2023; Wang et al., 2023).

Recent advances in metabolic engineering and synthetic biology have unlocked significant opportunities to harness halophilic bacteria as cell-based natural resources for high-value compound production. Patel et al. (2023) successfully engineered *Dunaliella salina* using a modified CRISPR-Cas9 system, achieving a 4-fold increase in β-carotene production by inactivating competing biosynthetic pathways and enhancing key gene expression with salt-inducible promoters. Zhao et al. (2023) demonstrated proof-of-concept for using *H. cupida* J9 as a halophilic chassis for in situ bioremediation via synthetic biology. *Halomonas cupida* J9 was engineered with strong promoters (P15/P8KT) to degrade PNP up to 200 mg/L and survive in high-salinity environments. 13C isotope analysis confirmed the strain's ability to convert PNP to CO2 in saline media, achieving degradation efficiency of 25 mg/L within 6 hours in seawater. Synthetic biology development in halophiles is constrained by the absence of comprehensive genetic toolboxes, difficulties in precise gene expression control due to ionic fluctuations, and limitations in high-throughput screening under hypersaline conditions. Quorum-sensing systems adapted from mesophilic bacteria often fail at salinities > 15%. These challenges underscore the need for novel approaches to advance affordable synthetic biology in halophilic bacteria.

Based on current trend analysis, three major research opportunities emerge in harnessing

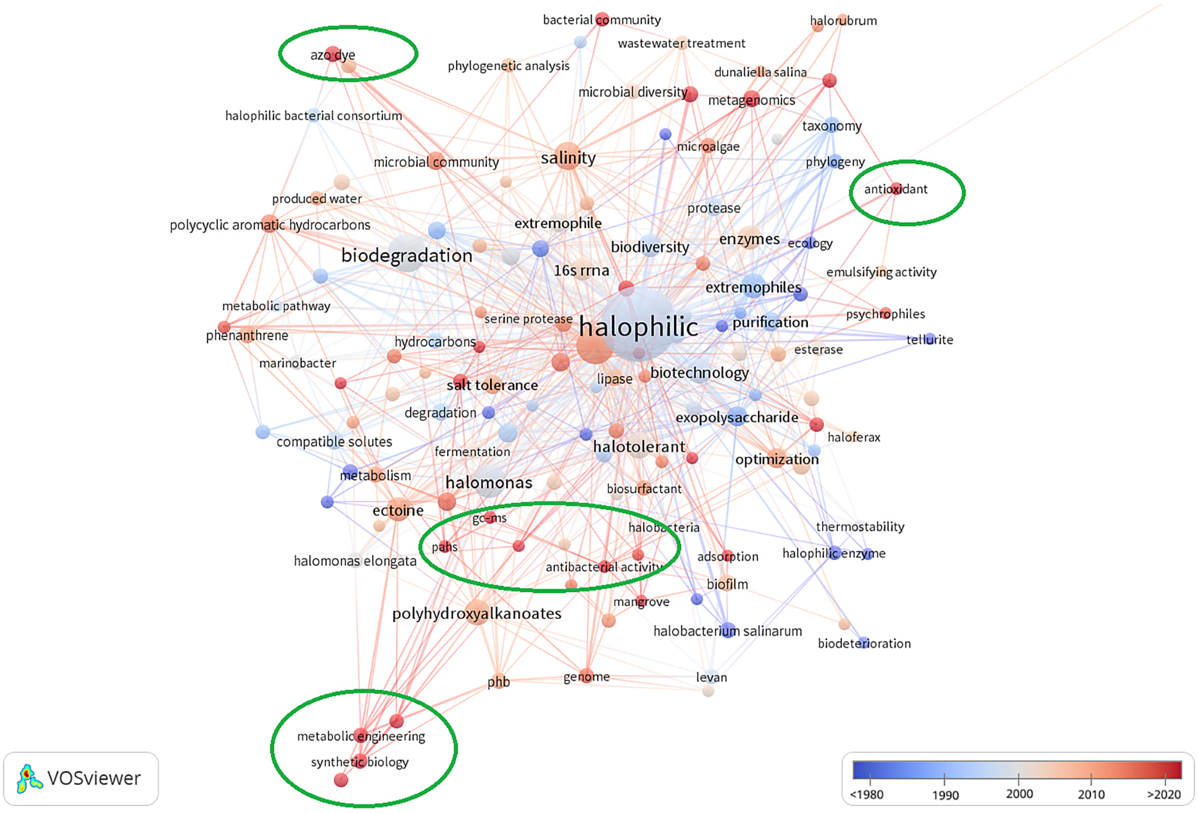

Figure 10. Analysis future trends and challenge research (1954–2024)

halophilic bacteria for biotechnology. First, engineering microbial consortia for complex industrial waste remediation is a promising field, particularly for treating mixed pollutants like heavy metals and aromatic compounds under hypersaline conditions. This approach requires designing microbial consortia that integrate specific degradation capabilities with salinity fluctuation resilience. Second, exploring untapped bioactive compounds from halophilic archaea offers significant potential, as only 15% of extremophilic archaeal diversity has been biotechnologically exploited. Research could focus on identifying antitumor compounds and thermostable enzymes from species thriving in extreme hypersaline environments (> 25% NaCl). Third, developing halophilic biosensors for real-time environmental monitoring provides an innovative solution, especially for rapid contaminant detection in coastal areas and salt-processing industries. This technology could leverage natural halophilic stress-response systems combined with visual genetic reporters. These three research areas are complementary and form the foundation for sustainable biotechnology development based on halophilic resources.

CONCLUSIONS

Based on the analysis results using VOSviewer and Biblioshiny, a comprehensive scientometric analysis has mapped the evolution of halophilic bacteria research over 70 years. Key findings demonstrate a transition from basic research to advanced biotechnological applications, with significant acceleration in the last decade in metabolic engineering and synthetic biology. Future research directions emphasize the exploitation of untapped potential from halophilic archaea, environmental biosensor development, and microbial community engineering for complex remediation applications. Although halophilic bacteria research has shown significant progress, major challenges continue to hinder broader biotechnological application development. Two critical issues requiring focus include developing more stable and efficient genetic toolboxes for hypersaline conditions and optimizing production processes from laboratory to industrial scales. Resolving these two challenges will be pivotal in unlocking the full potential of halophilic bacteria for diverse sustainable applications in the future.

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