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Ectoine from Halophilic Bacteria: Biosynthesis, Diversity, and Industrial Applications

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Abstract

Halophilic bacteria produce ectoine quite effectively, a vital osmoprotectant and compatible solute, helping them survive extremely high salinity and osmotic shock. Cyclic amino acid garners considerable industrial attention owing mainly to protective properties in various biotechnological, medical, and some cosmetic applications. Microorganisms thriving in salt pans and hypersaline lakes alongside marine ecosystems produce ectoine naturally under extremely salty conditions. Deeper insight into genetic and biochemical mechanisms underlying ectoine biosynthesis, namely ectA, ectB, and ectC gene cluster, has enabled a significant boost in production via metabolic engineering improvements. Although fermentation-based production is still the major technique, other strategies, including synthetic biology, bacterial milking, and adaptive evolution, are becoming viable substitutes. Ectoine is extracted and purified industrially utilizing advanced techniques such as solvent extraction, chromatographic procedures, and membrane-based filtering; research is continuously being conducted to find more economical and environmentally friendly approaches. This review provides a summary of the microbial diversity of ectoine producers, biosynthetic processes, and the most recent technical advancements in its synthesis and industrial applications.

Keywords: Ectoine, Halophilic bacteria, Salt, Biosynthesis, Applications

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Introduction

Halophilic microorganisms are salt-loving organisms that can survive in hypersaline habitats. There are Prokaryotic and Eukaryotic halophilic organisms that can balance the osmotic pressure of the environment and resist salt. [1]. Halophiles are found in hypersaline environments all over the world, and many are found in natural hypersaline brines in arid, coastal, and deep seas, as well as artificial salterns [2]. Halophiles can be divided into two groups: extreme halophiles and moderate halophiles [3]. They can undergo different physiological adaptive mechanisms to survive in high-salt environments. This helps them to produce products that are suitable for applications in many industries, which are done under saline conditions.

In order to cope with hyperosmotic stress, halophilic bacteria that inhabit environments with high ionic strength modify the composition of membrane lipids and control the intracellular concentration of low molecular weight solutes [4]. The latter response enables the cells to sustain appropriate osmotic balance in the face of hyperosmotic stress, which is essential to keep the cell from leaking water and preventing irreversible plasmolysis and dehydration, as well as to produce turgor pressure within ranges required for growth [5]. Cells regulate internal osmolarity by accumulating compatible solutes. These compatible solutes are very soluble, have low molecular weight, and are mostly either uncharged or zwitterionic organic molecules gathered in the cytoplasm.

Compatible solutes mainly include sugar (such as sucrose, trehalose, and so forth), polyols (such as glycerol, glycosyl-glycerol, mannosyl-glycerol, arbitol, and mannitol). Also, their derivatives (proline, glycine, betaine, ectoine, and hydroxyectoine) are widely distributed in nature [6]. These compatible solutes not only act as osmoprotectants but also stabilize cellular structures such as proteins, ribosomes, and membranes, protecting them from denaturation due to osmotic and thermal stresses [7]. Additionally, many halophiles exhibit specialized membrane lipid adaptations, such as the presence of diether and tetraether lipids in archaeal halophiles, which confer higher stability and low permeability under extreme salinity and temperature conditions [8]. Furthermore, some halophiles synthesize salt-stable enzymes with highly acidic surfaces that retain activity under high salt, making them important candidates for biotechnological applications like saline wastewater treatment, bioremediation of polluted saline environments, and biosynthesis under industrial saline conditions.

Studies have shown that moderate halophiles, such as *Halomonas elongata*, can accumulate ectoine up to 20% of their dry cell weight under high salt stress, making them

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attractive for industrial scale ectoine production [9]. Moreover, halophiles play vital roles in nutrient cycling in saline ecosystems by mediating carbon, nitrogen, and sulfur cycles through their unique metabolic pathways [2]. For example, halophilic archaea produce bacteriorhodopsins and halorhodopsins that convert light energy into biochemical energy, contributing to primary production in hypersaline ecosystems [2].

The unique ability of halophilic microorganisms to withstand multiple stressors (high salinity, UV radiation, temperature extremes) is increasingly explored for astrobiology, as analogues for potential life forms in extraterrestrial saline environments such as Mars and Europa [10]. Thus, understanding their osmoregulation mechanisms, compatible solute accumulation, and membrane adaptations is fundamental not only for biotechnological exploitation but also for evolutionary and ecological studies of life in extreme environments [11].

Recent metagenomic and proteomic studies have revealed extensive genetic diversity among halophiles, indicating the presence of novel genes encoding salt-tolerant biomolecules, extremozymes, and stress-protectant pathways. Further exploration and bioprospecting of halophilic microorganisms are expected to discover new extremozymes and enzymes with superior industrial relevance in the coming years [12].

Ectoine is a common industrial extremolyte which was first isolated and obtained from a halophilic bacterium, *Ectothirhodospira halochloris*. Ectoine (1,4,5,6-tetrahydro-2-methyl-4- pyrimidine carboxylic acid) (**Figure 1**) is a heterocyclic amino acid that is derived from aspartate with a zwitterion structure [13]. It maintains the osmotic balance of microbial cells without interfering with their ability to carry out their internal metabolic functions. The cytoplasm's ionic strength is barely affected by the zwitterionic ectoine of the bacterium. Hydroxyectoine (HE) (5-Hydroxy-2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) (**Figure 1**) is a hydroxylated form of ectoine. It is biochemically produced from ectoine-by-ectoine hydroxylase activity, and natural extremophiles frequently co-occur with two derivatives. Additionally, HE is a target of commercial interest because it may be used to defend against heat stress and desiccation and has an even higher stabilizing capacity than Ectoine [14]. Ectoine has drawn a lot of interest in its uses in the biotechnological, cosmetic, and pharmaceutical sectors because of its stabilizing qualities [15]. The economic potential of ectoine has been highlighted by recent studies that have concentrated on optimizing its production through metabolic engineering and large-scale bioprocessing. The variety of halophilic bacteria that produce ectoine, the biosynthesis processes and genetic regulation involved, and the growing industrial uses of this useful extremolyte are all examined in this review.

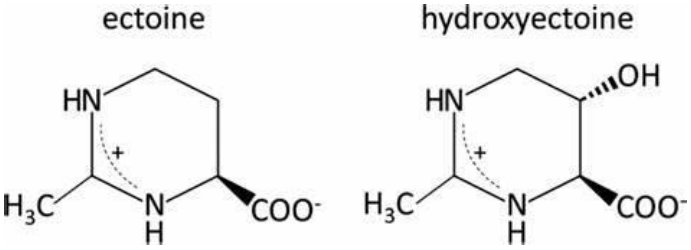

Figure 1: Chemical structures of Ectoine and Hydroxyectoine

Diversity of Ectoine-producing strains

Microorganisms producing ectoine encompass diverse genera like *Halomonas* and *Chromohalobacter,* thriving remarkably well in salinity environments ranging from hypersaline lakes and salt mines to oceanic habitats [16]. *Halomonas elongata* has become quite valuable for industrial biosynthesis owning largely owing to its high potential for ectoine production through bacterial milking [17]. *H. elongata*'s inherent ability gets exploited thoroughly in this process. Ectoine synthesis and secretion occur in *H. elongata* under diverse saline conditions, allowing massive production sans genetic modification. *Chromohalobacter salexigens* synthesizes ectoine and hydroxyectoine, quite remarkably enabling existence in harsh saline environments by maintaining a rather delicate osmotic balance. Moderately halophilic bacteria, like *Marinococcus halophilus* and *Pseudomonas stutzeri*, accumulate ectoine as a primary osmoprotectant, allowing them to survive environments of varying salinity [18].

Extremophilic bacteria, e.g., *Halorhodospira halophila* and *Salinivibrio* species, thrive in extremely salty environments like salt flats under very harsh conditions. Bacteria produce ectoine as an osmoregulation strategy to cope with high salt and extreme alkalinity under harsh conditions usually found in saline environments [19]. Novel ectoine-producing strains, namely *Sinobaca* species, were isolated from saline soils adjacent to a salt mine in fairly recent studies. Ectoine biosynthesis range has expanded significantly, highlighting evolutionary significance in halophilic and halotolerant bacteria, remarkably now. Additional genera of ectoine-producing bacteria include *Virgibacillus* and *Bacillus* species isolated from saline and alkaline soils, which accumulate ectoine as their primary compatible solute [20]. *Virgibacillus*

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pantothenticus has shown significant ectoine production under high osmotic stress, suggesting its utility in biotechnological applications requiring moderate halophiles [21]. Moreover, *Planococcus* species isolated from saltern soils have demonstrated ectoine accumulation as an adaptive mechanism to withstand combined salinity and desiccation stresses [22]. Members of the genus *Alkalibacillus*, isolated from soda lakes with high pH and salt concentrations, also synthesize ectoine, highlighting their dual adaptation to alkalinity and hypersalinity [23].

Furthermore, marine Vibrio species isolated from shrimp farm sediments and mangrove ecosystems have been reported to produce ectoine, suggesting a role in osmoregulation under fluctuating salinity and temperature regimes typical of coastal ecosystems. Studies on *Thioalkalivibrio* species from hypersaline soda lakes revealed ectoine and hydroxyectoine production contributing to their resilience in haloalkaline environments. Recent metagenomic analyses of hypersaline microbial mats in solar salterns have detected ectoine biosynthesis genes affiliated with diverse uncultured bacterial lineages, underscoring the hidden diversity of ectoine producers in natural hypersaline ecosystems.

Ectoine production also occurs in certain *Cyanobacteria*, such as *Spirulina platensis*, under salt stress conditions. Though ectoine concentrations are lower compared to halophilic bacteria, cyanobacterial ectoine offers prospects for sustainable bioproduction using phototrophic systems. In addition, lactic acid bacteria like *Lactobacillus plantarum* have been engineered for ectoine production by introducing halophilic bacterial genes, demonstrating the potential for producing ectoine using food-grade hosts.

The discovery of ectoine biosynthesis in thermophilic bacteria such as *Thermus thermophilus* and *Geobacillus* species indicates that ectoine also functions in protection against thermal stress, expanding its ecological and physiological relevance beyond halophilic adaptation alone [24]. Comparative transcriptomic studies in these thermophiles have revealed upregulation of ectABC genes under combined heat and osmotic stress conditions [25].

Interestingly, recent reports suggest that certain fungi, such as *Aspergillus sydowii*, isolated from hypersaline environments, can accumulate ectoine, although at low concentrations, indicating horizontal gene transfer events across domains [26]. Such discoveries expand the phylogenetic distribution of ectoine producers, revealing evolutionary convergence in extremolyte biosynthesis pathways.

Ectoine production occurs widely among diverse bacterial taxa, highlighting broad significance in microbial adaptation under extremely salty conditions. Ectoine has garnered considerable attention for various biotechnological applications, largely owing to its protective effects against harsh osmotic stress and temperature fluctuations. Novel bacteria that produce ectoine are being researched pretty vigorously nowadays for potential applications in cosmetics and enzyme stabilization processes. Biosynthetic capacity of halophilic bacteria varies greatly across diverse ecological niches, providing opportunities for bioproduction and screening novel extremophilic ectoine-producing strains with enhanced capabilities quite sustainably.

Recent research has identified a broader phylogenetic range of ectoine-producing microorganisms beyond traditional genera. Archaea such as *Haloferax mediterranei* and *Halobacterium salinarum* have shown the capacity to accumulate ectoine under osmotic stress conditions [27]. Actinobacteria, including *Streptomyces chrysomallus*, not only produce ectoine but also its derivative hydroxyectoine, particularly under thermal and oxidative stress. Marine sediment-derived bacteria, such as *Alkalilimnicola ehrlichii* and *Marinobacter hydrocarbonoclasticus*, demonstrate ectoine biosynthesis in response to deep-sea pressure and salinity. This growing list of extremophiles expands the biotechnological reservoir for ectoine biosynthesis. Comparative genomics has revealed conserved ectABC operons in several genera, suggesting convergent evolution in osmotolerance mechanisms [28]. In below, **Table 1** shows the major ectoine- producing microorganisms are summarized, including their taxonomic affiliation, halophilic category, optimal salinity condition, and reported ectoine yields.

Biosynthesis and Regulation of Ectoine Production

The biochemical establishment and study of the Ectoine biosynthesis pathway were conducted in *H. halochloris, H. elongata DSM 2581* [31], and *H. elongata DSM 3103* [32]. According to[33] , the latter strain and *H. elongata ATCC 33174* were reclassified as the new species C.*salexigens DSM* 3043 and *ATCC 33174*, which belongs to the genus *Chromohalobacter*. Ectoine biosynthesis initiates with phosphorylation of L-aspartate. L-aspartate conversion into L-aspartate-phosphate gets initiated by aspartate kinase, and L-aspartate-semialdehyde dehydrogenase

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Table 1: Major ectoine-producing microorganisms

Microorganism	Domain	Halophilic category	Optimal salinity (% NaCl)	Reported ectoine yield*	Reference
Halomonas elongata	Bacteria	Moderate halophile	5- 15	Up to 20% DCW/ ~25 g L^{-1}	[9]
Chromohalobacter salexigens	Bacteria	Moderate halophile	5- 20	~8- 12 g L^{-1}	[29], [30]
Marinococcus halophilus	Bacteria	Moderate halophile	5- 10	~5- 8 g L^{-1}	[16]
Virgibacillus pantothenticus	Bacteria	Moderate-extreme halophile	5- 12	~3- 6 g L^{-1}	[21]
***Salinivibrio* spp.**	Bacteria	Moderate-extreme halophile	10- 25	Not quantified	[19]
Halorhodospira halophila	Bacteria	Extreme halophile	20- 30	Not quantified	[19]
Thermus thermophilus	Bacteria	Thermophile	5- 10	Low	[24], [25]
Haloferax mediterranei	Archea	Extreme halophile	20- 30	Low- moderate	[27]
Aspergillus sydowii	Fungi	Halotolerant	5- 15	Trace levels	[26]

***Reported yields vary with cultivation strategy and extraction methods.

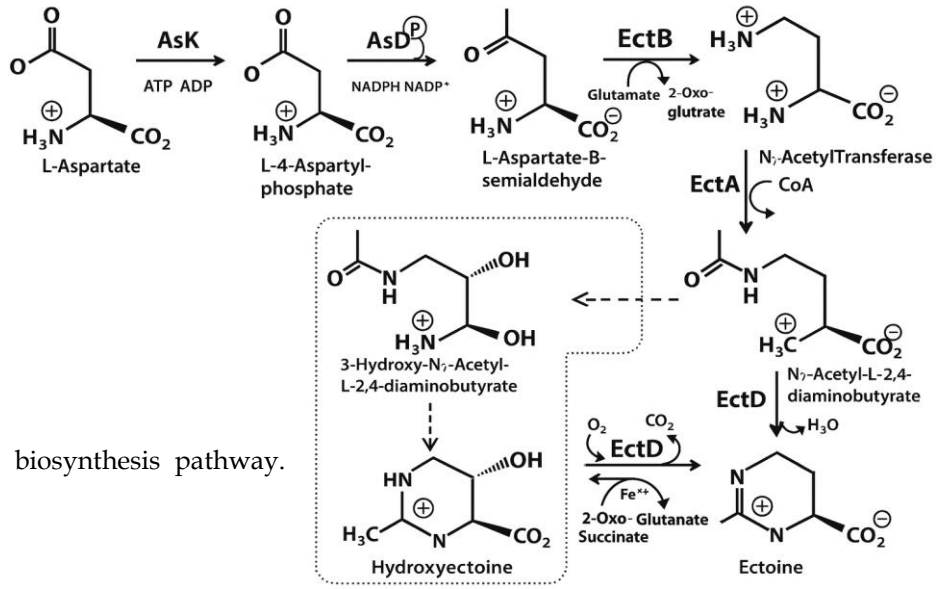

Figure 2: Ectoine biosynthesis pathway. Cited from [9]

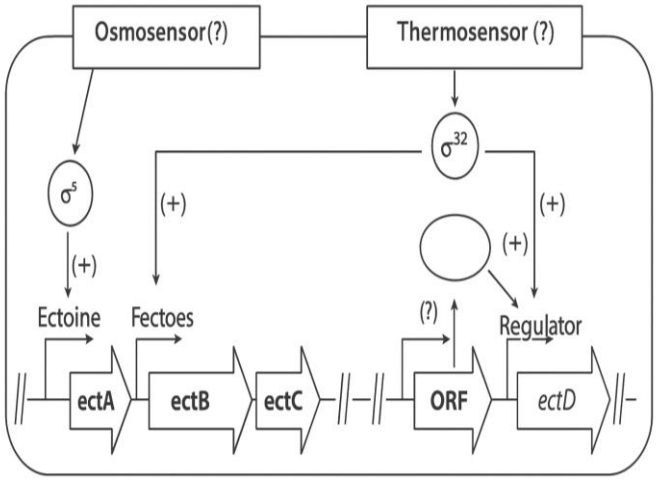

Figure 3. Regulatory pathway of ectoine. Cited from [41]

facilitates L-aspartate-phosphate conversion into L-aspartate-beta-semialdehyde immediately. L-2-4-diaminobutyrate transaminase (ectB) L-2-4-diaminobutyrate acetyltransferase (ectA), and ectoine synthase (ectC) catalyze biosynthesis of the aspartate family to produce ectoine [30], [34]. Enzyme L-diaminobutyric acid transaminase (ectB) converts L-aspartate-beta-semialdehyde into L-diaminobutyric acid, which L-2-4-diaminobutyrate acetyltransferase (ectA) subsequently acetylates quiet vigorously to N-y-acetyldiaminobutyric acid. Ectoine synthase (ectC) catalyzes cyclic condensation of NADA quite rapidly resulting in ectoine production during the final step. Ectoine hydroxylase has been identified in *Streptomyces chrysomallus* and *Chromohalobacter. Salexigens*

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hydroxylates ectoine as the primary biosynthetic pathway for hydroxyectoine production [29], [35].

The biosynthesis of ectoine occurs in halophilic bacteria through a highly regulated process operated by environmental stress factors and genetic regulation mechanisms. The production of ectoine, a compatible solute protecting bacterial cells from osmotic stress, occurs via the ect operon comprising ectA, ectB, and ectC genes [36]. Expression of this operon is induced in hyperosmotic environments, allowing cells to accumulate ectoine and maintain cellular integrity by stabilizing proteins and membranes [37]. Transcriptional regulation of the ect operon is controlled by alternative sigma factors such as σˆS (RpoS) and σˆB, which play significant roles in bacterial stress adaptation. In bacteria such as *Bacillus subtilis*, σˆB is involved in the general stress response, whereas in *Halomonas elongata*, there are specific transcription factors that control the response to osmotic stress [38]. In addition to transcriptional regulation, biosynthesis control of ectoine is also influenced by transport systems, responsible for the uptake and efflux of ectoine, depending on the osmotic status of the growth medium. Transporters OpuA and OpuC facilitate uptake of ectoine, enabling bacteria to collect ectoine from the surroundings as needed, while the TeaABC transport system exports excess ectoine to regulate intracellular concentrations within ideal ranges [39]. Dynamic regulation preserves harmonious ectoine concentrations to support cellular functions without triggering metabolic stresses.

On an enzymatic level, ectoine production is controlled by feedback inhibition mechanisms. The final step of ectoine biosynthesis, which is catalyzed by ectC, is feedback controlled, in which higher intracellular concentrations of ectoine may inhibit enzyme action to prevent unnecessary overproduction [40]. This is crucial for optimizing cellular energy investment, as ectoine biosynthesis requires significant metabolic expenditure.

In parallel to natural regulatory mechanisms, metabolic engineering approaches have been employed to enhance ectoine production for applications. Genetic modification of *Halomonas elongata* and *Corynebacterium glutamicum* has led to enhanced production of ectoine by optimization of biosynthetic pathways and overexpression of key biosynthetic genes. Recent advancements in CRISPR-Cas9 genome editing have made precise modifications in regulatory elements controlling the ect operon, and thus, strains with improved biosynthetic capability have been generated [42]. These discoveries have increased the biotechnological worth of ectoine to the point that it is currently a valuable compound with uses in pharmaceuticals and cosmetics. In general, ectoine biosynthesis is controlled by a complex system of transcriptional, post-transcriptional, and metabolic control mechanisms. Understanding such control networks is crucial to optimizing ectoine production from microbial cultures so that it can be utilized economically and sustainably in numerous industries.

Synthetic biology has unlocked new potential for scalable ectoine production. Heterologous expression of the ectABC cluster in non-halophilic hosts like *E. coli*, *Bacillus subtilis*, and *Corynebacterium glutamicum* has enabled high yields under low-salinity conditions [43], [44]. Codon optimization, promoter engineering, and modular cloning approaches have been used to enhance expression efficiency. CRISPR-Cas systems, especially CRISPRi, have facilitated repression of competing pathways, channeling more carbon flux towards ectoine biosynthesis [42]. Recent innovations include dynamic metabolic control and synthetic toggle switches to balance ectoine production and cell growth, improving overall yields [45].

Recent studies have explored the use of strong constitutive promoters and ribosome binding site libraries to enhance ectABC transcription and translation, increasing ectoine titers by over 30% in engineered strains [46]. Additionally, adaptive laboratory evolution (ALE) has been utilized to select for strains with higher osmotic tolerance and ectoine productivity under industrial fermentation conditions [47]. The integration of systems biology and computational metabolic modeling has enabled the identification of metabolic bottlenecks and optimal flux distributions, facilitating rational strain design for ectoine overproduction (61. Moreover, co-culture systems involving halophilic and non-halophilic bacteria have been tested to integrate ectoine biosynthesis with complementary bioprocesses, enhancing substrate utilization and product recovery [48].

The regulation of ectoine production also involves global regulatory proteins such as Crp and Fnr, which modulate ect operon expression in response to nutrient status and oxygen availability, indicating intricate environmental signal integration in biosynthetic control [49]. Furthermore, recent findings suggest post-translational modifications such as acetylation and phosphorylation of ectoine biosynthetic enzymes may fine-tune their activities under fluctuating osmotic and nutrient conditions [50].

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Bioprocess innovations have included fed-batch and continuous cultivation strategies to optimize growth rates and osmotic induction, achieving ectoine concentrations up to 25 g/L in Halomonas elongata cultures [38]. Two-stage fermentation strategies using osmotic upshock to trigger ectoine accumulation after biomass production have shown to enhance yields while reducing overall production costs [51]. In addition, membrane-based extraction and in situ product recovery methods have been implemented to continuously remove ectoine during fermentation, alleviating feedback inhibition and enhancing productivity [52].

The future of ectoine biosynthesis lies in integrating synthetic regulatory circuits that enable dynamic control of production pathways in response to real-time process parameters, ensuring maximum yields with minimal stress on host cells [47]. Such synthetic biology tools, coupled with improved downstream processing and purification methods, will make ectoine production more sustainable and economically viable for large-scale pharmaceutical, cosmetic, and agricultural applications.

Methods for Ectoine Extraction and Production

Microbial fermentation remains crucial for industrially producing ectoine largely accumulated by halophilic bacteria like *Chromohalobacter* and *Marinococcus* in saline environments naturally [9] Numerous factors affect the fermentation process, including temperature, pH, salinity, various carbon sou rces, and nitrogen availability. Fed-batch and continuous fermentation techniques are frequently chosen over batch culture because they greatly increase the efficiency of the yield naturally [53]. Recent research has investigated using inexpensive substances like agricultural waste and industrial byproducts for ectoine production [54].

Bacterial Milking: A Sustainable Approach to Ectoine Production

Ectoine extraction via bacterial milking emerges as a quite sustainable approach, allowing repeated harvesting without perturbation of bacterial cellular integrity [55]. Halophilic bacteria, like *Halomonas elongata*, grow in extremely saline conditions, triggering ectoine accumulation and subsequently undergo osmotic downshifts that force them to excrete intracellular ectoine into the external medium without cell lysis [56]. Once extracted, the bacteria can be returned to high-salinity conditions, which restores their ectoine levels and allows for multiple production cycles [54].

Bacterial milking is advantageous in various ways over conventional extraction methods. It significantly reduces biomass loss and the need for costly cell disruption procedures, hence making the process cost-effective and eco-friendly [57]. It also maintains bacterial viability, which allows for repeated or continuous ectoine production without the need for frequent reinoculation. Optimization of osmotic shock conditions is, nonetheless, crucial to realize efficient release of ectoine without damaging cell integrity. Genetic modification has been explored to enhance the excretion of ectoine and bacterial milking efficiency [53].

Recent studies have demonstrated that controlled osmotic downshock protocols, involving stepwise reductions in NaCl concentration, can increase ectoine excretion efficiency up to 80% per milking cycle [58]. Moreover, integrating bacterial milking with membrane filtration systems allows continuous removal of ectoine while recycling bacterial cells back to bioreactors, enhancing process sustainability [59]. Strategies such as overexpression of efflux transporters (e.g., TeaABC or EctP) in Halomonas elongata have been applied to facilitate ectoine secretion, reducing reliance on external osmotic shocks [38]. The use of immobilized bacterial cells on carriers like alginate beads or hollow fiber membranes is also under investigation to improve operational stability and ease of separation for repeated milking cycles.

The combination of bacterial milking with adaptive laboratory evolution has yielded Halomonas strains with enhanced osmotic tolerance and secretion efficiency. For example, evolved strains exhibited 1.5-fold higher ectoine secretion rates under repeated milking, with minimal viability loss after ten cycles [60]. Such approaches contribute to process intensification in industrial biotechnology. In addition, co-cultivation strategies involving ectoine-producing Halomonas and waste-consuming bacteria have been explored to integrate production with wastewater bioremediation, offering circular bioeconomy solutions[59] .

Advancements in Downstream Processing

Following fermentation or bacterial milking, ectoine is recovered by a combination of physical and chemical processes. The conventional techniques encompass cell lysis by osmotic shock, mechanical disruption, or sonication and solvent extraction by ethanol or methanol[61]. Novel separation techniques such as ultrafiltration, electrodialysis, and aqueous two-phase systems are also utilized in order to promote the efficiency of extraction and reduce the use of organic solvents[62]For high-purity purposes, e.g., pharmaceuticals and cosmetics, further purification

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stages by means of ion-exchange chromatography and HPLC become necessary [63]. Recent innovations in downstream processing include membrane-based integrated systems combining microfiltration, ultrafiltration, and nanofiltration for stepwise concentration and purification of ectoine with minimal thermal degradation [45]. Aqueous two-phase extraction systems using polyethylene glycol (PEG) and phosphate salts have shown partition coefficients above 10, enabling efficient primary recovery [64]. In situ product recovery (ISPR) strategies, such as adsorption of ectoine onto hydrophilic resins during fermentation, have been tested to continuously remove ectoine, preventing feedback inhibition and increasing productivity by up to 40% [64]. Electrodialysis has gained attention for its selective separation capabilities, reducing energy consumption compared to evaporation-based concentration methods [65]. Additionally, expanded bed adsorption chromatography enables direct processing of unclarified fermentation broths, reducing purification steps. Recent techno-economic analyses emphasize that coupling ISPR with bacterial milking could halve production costs by reducing downstream energy and resin regeneration requirements [66], [67].

Advances in green downstream processing, such as subcritical water extraction and deep eutectic solvents, are also under exploration for sustainable ectoine purification without harsh chemicals [67]. Implementation of continuous chromatography systems, including simulated moving bed (SMB) chromatography, promises high-purity ectoine production with reduced footprint and operational costs.

Future advances in ectoine production focus on the convergence of synthetic biology, adaptive laboratory evolution, and bioprocess optimization. Dynamic metabolic control, CRISPR-mediated gene editing, and co-cultivation of microbial consortia are some of the approaches being explored to further enhance yield and efficiency [68]. Besides, new recovery processes, for instance, in situ product extraction and membrane-based separation with continuous fermentation, can potentially reduce production costs and increase sustainability. Demand for ectoine grows steadily in the pharmaceutical and cosmetic industries, yet a gap remains for enhanced processing to facilitate large-scale, cost-effective production.

Synthetic Biology and Pathway Engineering for Ectoine Production

Synthetic biology approaches have revolutionized ectoine production by enabling heterologous expression of ectoine biosynthetic genes in fast-growing, easily engineered hosts such as Escherichia coli, Bacillus subtilis, and Corynebacterium glutamicum [46]. Codon optimization and promoter engineering have significantly improved ectABC expression levels, leading to ectoine titers exceeding 10 g/L in shake flask experiments [46]. Dynamic control systems utilizing synthetic riboswitches responsive to osmolyte concentrations have been designed to balance ectoine synthesis with host growth, optimizing overall productivity [69].

CRISPR-Cas genome editing technologies have allowed precise deletion of competing metabolic pathways and insertion of ectoine biosynthetic clusters at high-expression genomic loci, improving carbon flux toward ectoine production [70]. Moreover, CRISPRi (interference) systems have been applied to repress pathways competing for aspartate-semialdehyde, the key precursor of ectoine biosynthesis. Modular pathway optimization using Golden Gate assembly has facilitated rapid construction and testing of ectoine biosynthetic variants, accelerating strain engineering pipelines [71]. Recent studies have engineered Corynebacterium glutamicum for de novo ectoine production from lignocellulosic hydrolysates, integrating pentose and hexose utilization pathways with ectABC expression, enabling sustainable production from agricultural residues [72]. Cell-free synthetic biology approaches have also been explored for in vitro ectoine biosynthesis, eliminating cellular regulatory constraints and enabling process intensification. Future directions include the construction of synthetic microbial consortia where one strain produces precursors while another carries out ectoine synthesis, creating efficient division of labor systems.

Even though route engineering and synthetic biology have greatly improved ectoine synthesis, there are several drawbacks that must be considered in addition to the accomplishments that have been documented. High ectoine titers at low salinity conditions have been achieved by engineered non-halophilic hosts such as *Escherichia coli* and *Corynebacterium glutamicum*, which provide benefits like less corrosion, easier process control, and energy efficiency [44]. However, during long-term culture, heterologous expression of the ectABC pathway frequently places a significant metabolic strain on the host, resulting in decreased growth rates, pathway instability, and increased vulnerability to genetic alterations [34]. Under industrial fermentation conditions, host-specific regulatory networks may also

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Table 2. Comparison of Ectoine production strategies

Production method	Host system	Principle	Advantages	Limitations	Industrial relevance	References
Bacterial milking	*Halomonas elongata*	Osmotic upshift/ downshift induces ectoine release	Cell refuse, no genetic modification, sustainable	Salt cycling stress, scalability issues, and high operational cost	Commerciall y established	[53], [56]
Conventional fermentation	Halophilic bacteria	Intracellular accumulation under high salinity	High natural titer, robust producers	Cell destruction required, salt corrosion	Widely used	[49]
Engineered microbial hosts	*E. coli, C. glutamicum*	Heterologous expression of ectABC	Low salt, easier scale up, lower corrosion	Metabolic burden, regulatory approval	High future potential	[21], [44]
Adaptive laboratory evolution	Halophiles	Selection for high tolerance and productivity	Improves robustness and yields	Time consuming	Process optimization	
Cell- free systems	Enzyme systems	In vitro ectoine synthesis	No cellular regulation	Low yield, high cost	Experimenta l	[42]

disrupt pathway expression, leading to varied productivity.

Natural halophilic producers, on the other hand, have strong osmoadaptive regulation and high genetic stability, but they need high-salt conditions, which raise operating costs and complicate downstream processing [42].

Natural halophilic producers, on the other hand, have strong osmoadaptive regulation and high genetic stability, but they need high-salt conditions, which raise operating costs and complicate downstream processing [56]. New options, such as cell-free systems, do away with salt needs and cellular regulatory restrictions, but their use is now constrained by poor yields, expensive enzymes, and issues with system stability and scalability. Table 3 summarizes the yields, salt needs, benefits, and drawbacks of natural halophiles, designed hosts, and cell-free systems. To establish commercially feasible ectoine production, this study illustrates the trade-offs between various production platforms and emphasizes the necessity of integrated techniques integrating synthetic biology, adaptive evolution, and process optimization[38], [53].

Table 2 highlights the advantages, limitations, and industrial relevance of different ectoine production methods, illustrating the trade-offs between process sustainability, scalability, and operational complexity. As mentioned above, the comparative analysis presented in **Table 3** underscores the complementary roles of natural and engineered systems, suggesting that future industrial ectoine production may rely on hybrid or integrated approaches.

Table 3: Natural halophiles Vs. Engineered hosts Vs. Cell- free systems for ectoine production

Parameter	Natural halophiles	Engineered non-halophiles	Cell-free systems
Salt requirements	High	Low	None
Typical yields	High	Moderate-high	Low
Process complexity	Moderate	High (genetic engineering)	Very high
Scalability	Proven	Developing	Limited
Metabolic burden	Low	High	Not applicable
Genetic stability	High	Variable	Not applicable
Regulatory acceptance	Established	Require approval	Experimental
Industrial maturity	High	Medium	Low
Reference	[49]	[34], [44]	[21], [42]

Industrial and Biotechnological Applications

The unique properties of Ectoine, such as stabilizing biomolecules, have led to its extensive applications in biotechnology, cosmetics, pharmaceuticals, and industrial processes. Ectoine serves as a naturally occurring compatible solute useful in drug research and enzyme stabilization, protecting biological macromolecules from harsh environmental stressors.

Ectoine in Enzyme Stabilization and Industrial Biocatalysis

The ability of ectoine to stabilize enzymes and increase catalytic performance ranks high among its most promising applications in industrial bioprocesses. Ectoine increases lipase activity and lifetime significantly, and this enzyme plays a vital role in

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biodiesel generation [66]. Ectoine increases methylester output by 20.9% remarkably in a solvent-free methanolysis system using cottonseed oil. The capacity of ectoine maintains the structural integrity of immobilized lipase under harsh reaction conditions, avoiding denaturation and loss of enzymatic activity effectively [66].

Moreover, ectoine plays a protective role in other enzymatic processes by reducing the impact of freezing, heating, drying, and proteolysis induced by proteases like trypsin and trypsinogen [49]. Because of this, ectoine is a useful ingredient in industrial biotechnology, especially in fields like food processing, pharmaceutical synthesis, and biofuel generation that depend on enzyme-based reactions.

Beyond lipases, ectoine has been shown to stabilize proteases, amylases, and cellulases, enhancing their thermal stability and activity in high-salt and organic solvent-rich environments common in industrial reactions [73]. Ectoine protects nitrile hydratase in acrylamide production and extends the half-life of alkaline proteases used in detergent formulations [74]. Its kosmotropic nature influences water structure, thus maintaining enzyme conformation and active site geometry under stress [75]. Recent research demonstrates that ectoine enhances β-galactosidase activity in lactose hydrolysis for dairy processing by protecting enzyme subunit interactions at elevated temperatures. Application of ectoine in enzymatic esterification reactions has increased yields by stabilizing enzyme–substrate complexes in non-aqueous media. Industries are now testing ectoine as an additive to preserve immobilized enzyme columns for continuous bioreactors, reducing operational costs through extended enzyme reuse cycles [56]. Thus, ectoine's role in enzyme stabilization can revolutionize industrial biocatalysis by enhancing enzyme tolerance to temperature, solvents, and mechanical shear stress, ensuring robust bioprocessing systems for large-scale production.

Ectoine in Cosmetic and Dermatological Applications

Ectoine has a remarkable capacity to preserve human skin cells, and its growing use in skincare formulations has been rapidly adopted by the cosmetic industry. Ectoine shields skin from environmental stresses, including ultraviolet radiation (UV), wind, and drastic temperature fluctuations [49]. Quenching singlet oxygen species prevents oxidative damage, lowering the risk of photocarcinogenesis and photodermatoses, and premature skin aging [76]. Ectoine maintains skin hydration effectively and works amazingly beneath the skin surface. Ectoine prevents trans epidermal water loss and enhances skin moisture retention capabilities, making it a valuable ingredient in anti-aging skincare products. Research indicates that ectoine-based creams significantly enhance skin barrier function and hydration levels, mostly in people with dry skin [77].

Ectoine is used to treat atopic dermatitis (AD), a chronic inflammatory condition characterized by excessive dryness and compromised skin barrier function. According to [78] developed an ectoine-based topical treatment for AD that showed superior therapeutic effects compared to traditional treatment methods. Furthermore, it has been observed that the patients who used ectoine-containing creams have improved skin hydration, reduced inflammation, and enhanced barrier repair. This makes ectoine a novel and potent treatment for AD and other skin conditions.

Recently, researchers have found that ectoine can disperse keratin bundles in the human stratum corneum [79]. This demonstrates the further expansion of ectoine in dermatological applications.

Additionally, ectoine has been formulated in anti-pollution skincare products to protect against particulate matter-induced oxidative stress and inflammation [80]. Its ability to stabilize cell membranes and repair damaged proteins contributes to anti-inflammatory and anti-irritant properties, benefiting sensitive skin formulations [81]. Clinical trials report that sunscreens containing ectoine exhibit enhanced photoprotective effects by reducing UV-induced erythema compared to conventional formulations. Moreover, ectoine-based eye creams effectively alleviate periorbital dryness and irritation, making them suitable for use in ophthalmic dermatology. Nanoencapsulation of ectoine in lipid carriers improves its skin penetration and bioavailability, opening avenues for advanced dermatological product development [82]. Patents have been filed for ectoine-containing microneedle patches targeting hyperpigmentation and wrinkle reduction via dermal hydration and protection of collagen networks [83]. Thus, ectoine's multifunctional roles in moisturization, UV protection, and anti-aging substantiate its expanding relevance in high-value cosmeceutical markets.

Ectoine in Pharmaceutical and Medicinal Applications

In addition to cosmetics, ectoine has been investigated for possible uses in pharmaceutical and medical industries. Microorganisms that survive in harsh environments create them as stabilizers of cellular activity [84].

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Compatible solutes influence the hydration layer of macromolecules to promote their stability and functionality because of their unique characteristics in aqueous solutions [85]. According to this data thus far, ectoine can prevent cell stress reactions in the airways and is well tolerated by cells, even at high concentrations. This suggests that ectoine may be used to treat human respiratory disorders linked to chronic neutrophilic lung inflammation. Studies have shown that ectoine-based inhalation treatments can alleviate symptoms of chronic obstructive pulmonary disease (COPD) and allergic rhinitis by reducing inflammation in the respiratory tract [86]. Recently, ectoine has been used in clinical trials to treat seasonal allergic rhinoconjunctivitis, an eye condition. Some studies have shown that in a well-established mouse model, ectoine can considerably reduce the hallmark pathologies associated with dry eye disease, suggesting that it could be a promising treatment option for human conditions [87].

Moreover, ectoine is used to treat inflammatory bowel disease (IBD), which is chronic inflammation in the gastrointestinal (GI) tract [88]. Studies have shown that ectoine and hydroxyectoine could be used as effective supplemental medications for IBD to prevent relapses and thus increase periods of remission, by stabilizing the intestinal barrier [89].

Further pharmacological applications include using ectoine in mouth rinses for oral mucositis management in cancer patients undergoing chemotherapy, where its membrane-stabilizing effect reduces inflammation and promotes healing [90]. Research is exploring ectoine as an excipient in protein and peptide formulations to enhance stability and shelf-life of biopharmaceuticals [91]. Intranasal ectoine sprays have shown efficacy in treating rhinosinusitis and postoperative nasal mucosa healing by reducing ciliary damage and mucosal dryness [92]. Additionally, ectoine eye drops have demonstrated superior tolerability in patients with contact lens-induced dry eye compared to traditional lubricants [93]. Preclinical studies suggest ectoine mitigates renal tubular injury and oxidative stress in models of acute kidney injury. Its immunomodulatory and cytoprotective properties are now being evaluated for applications in neuroinflammation and retinal degenerative disorders[94]. Thus, ectoine's versatile therapeutic potential is driving its integration into diverse medicinal and pharmaceutical products globally.

Ectoine in Agricultural and Veterinary Applications

Ectoine has been explored as a bio-stimulant in agriculture to enhance plant resistance to drought, salinity, and temperature extremes. Studies indicate that foliar sprays of ectoine improve chlorophyll content, relative water content, and root elongation in crops such as wheat and tomato under saline stress [66]. In veterinary science, ectoine supplementation has been shown to reduce heat stress in broiler chickens and dairy cows, improving feed intake and productivity [67]. Ectoine-based eye drops are also under trial for veterinary ophthalmology, particularly for treating dry eye syndrome in dogs and horses [68].

Recent greenhouse experiments demonstrate that ectoine-treated rice seedlings exhibit enhanced photosynthetic efficiency and reduced electrolyte leakage under salt stress, supporting their resilience during early growth stages [51]. In horticulture, ectoine foliar applications have improved flowering rates and fruit set in bell pepper and cucumber under greenhouse saline irrigation. Seed priming with ectoine enhances germination rates and seedling vigor in barley and maize exposed to drought and salinity. In veterinary applications, ectoine-based oral supplements reduce respiratory tract irritation in horses exposed to stable dust [95]. Feed additives containing ectoine have been tested in aquaculture, showing improved stress tolerance and growth rates in shrimp and tilapia cultured in brackish water [47]. Additionally, topical ectoine gels are being trialed for the treatment of bovine digital dermatitis and canine atopic dermatitis, with promising outcomes in reducing inflammation and enhancing skin barrier function. Thus, ectoine's bio-protective properties extend into sustainable agriculture and veterinary health to improve productivity and welfare under stress conditions.

Conclusion

Ectoine represents a biologically significant compound industrially, with vast untapped potential in various pharmaceutical fields and somewhat obscure biotechnological applications. Halophilic bacteria flourish in super saline environments, serving as natural ectoine reservoirs, with prominent producers hailing from the genera *Halomonas* and *Marinococcus*. Biosynthetic pathways of ectoine are now better understood, paving the way for genetic modifications and metabolic engineering strategies, enhancing yield productivity. Microbial fermentation remains dominant but alternative approaches like bacterial milking and

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heterologous expression in non-halophilic hosts offer promising, cost-effective, sustainable solutions. Ectoine extraction and purification keep evolving rapidly with a strong emphasis on developing scalable, eco-friendly methods. Future research should prioritize optimizing bioprocesses vigorously and exploring novel microbial sources extensively to enhance ectoine production efficiency significantly across various industries. Ectoine can be further leveraged as a key bio-based compound, having broad scientific applications and surprisingly profitable commercial potential in various industries nowadays.

The integration of omics technologies, including genomics, proteomics, and metabolomics, can accelerate the discovery of novel ectoine-producing strains and elucidate regulatory networks for pathway optimization [96]. Advances in synthetic biology offer opportunities to engineer non-halophilic industrial hosts like E. coli or yeast for high-yield ectoine production without high-salt cultivation limitations [46]. Downstream processing innovations such as membrane-based extraction and crystallization techniques can improve purity and reduce environmental impact compared to traditional solvent extraction methods (90). Furthermore, combining ectoine production with wastewater treatment using halophilic bacteria offers sustainable biorefinery models. Expanding ectoine utilization as a protective agent in vaccines, probiotic formulations, and novel biomaterials could unlock new market avenues [97]. Overall, ectoine stands as a model extremolyte bridging environmental microbiology with industrial biotechnology, supporting future innovations aligned with global demands for stress-protective, sustainable, and health-promoting compounds.

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