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# Microbial production of extremolytes — high-value active ingredients for nutrition, health care, and well-being

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Extremolytes are small organic molecules, which protect cells under extreme, virtually inhabitable conditions. Their exceptional properties can be translated into health-promoting and therapeutic activities, which open an avenue of opportunities for the cosmetic, medical, and food industries. Supported by powerful approaches from systems and synthetic biology and systems metabolic engineering, the bio-industry becomes more and more attracted to exploit this 'goldmine'. In addition to the well-established flagship ectoine, several novel extremolytes have emerged in the past years and high-efficiency cell factories have been created for bio-based extremolyte production. Here, we review recent prominent examples and success stories in the field.

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## Introduction

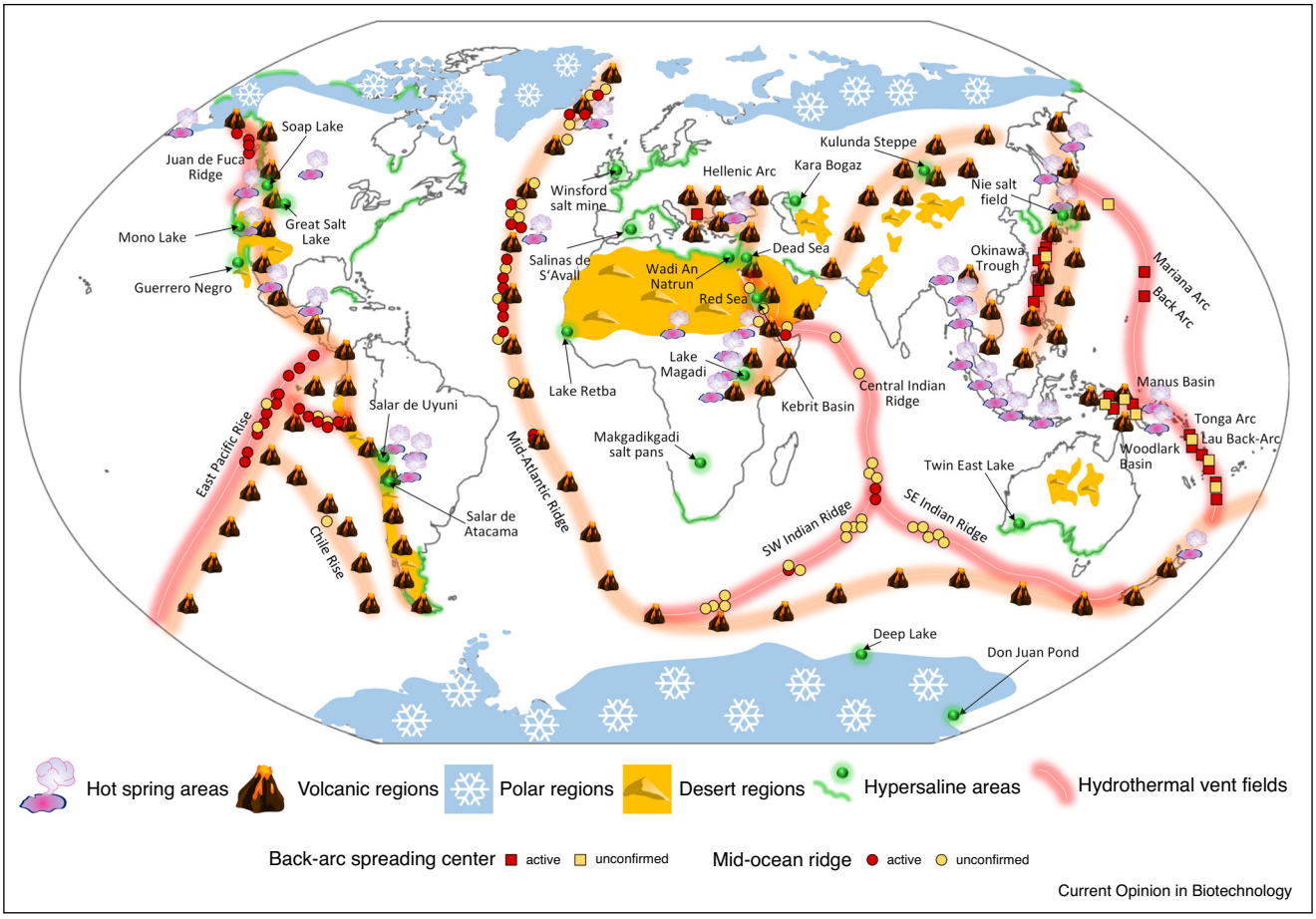
Extremophilic microbes thrive in virtually inhabitable environments on Earth. They love it hot, sour, or salty and have broken many records in tolerance to extreme conditions. Over the years, isolates with thrilling properties have been collected from hot geysers, deep water hot springs, salt lakes, dry deserts, volcanic areas, and the polar ice (Figure 1) [1–4]. Moreover, prominent extremophiles have been isolated from rather extreme niches of industrial processes, including highly basic pulp mill effluents [5] and evaporating ponds of solar salt facilities [6,7]. With systems and synthetic biology techniques getting better and better, industry is becoming more and more interested in these exotic microorganisms. Over the past four decades, pioneering studies have discovered a set of unique organic molecules — so called extremolytes — that accumulate inside extremophilic bacteria and archaea (either synthetized *de novo* or taken up) and are obviously crucial to enable their life style [8,9]. Chemically, microbial extremolytes are low molecular weight organic molecules (Figure 2) and mainly include sugars, polyols, heterosides, amino acids, and their derivatives. Prominent examples are ectoine [8,10], hydroxyectoine [8,10], proline [11], mannitol [12], glycine-betaine [13], and trehalose [14] often found in halophiles. Hyperthermophilic microbes often bear heterosides, such as glucosyl-glycerol (GG, glycoin) [15,16], glucosyl-glycerate (GGA) [15,17], mannosyl-glycerate (MG, firoin) [18•,19,20], and mannosyl-glyceramide (MGA, firoin-A) [8]. In addition, phosphorylated compounds such as di-*myo*-inositol 1,1-phosphate (DMIP) [8,20,21], α-diglycerol phosphate (DGP) [8,21], and cyclic diphosphoglycerate (cDPG) [8,13,22] are broadly distributed in such microbes. Moreover, UV light-scavenging compounds of complex chemical nature such as scytonemin, mycosporine-like amino-acids (MAAs), bacterioruberin, and melanin [23–26] have been isolated from radiation-resistant bacteria.

Most extremolytes (then also called compatible solutes or chemical chaperones) protect macromolecules and cell structures of extremophiles from their hostile habitats by forming and stabilizing protective water layers around them [27], for example, under salt and temperature stress or in the presence of cytotoxins [8] (Figure 3). Others are efficient chemical scavengers and prevent cells and their structures from being damaged from UV radiation and oxidative stress [23,28]. It is interesting to note that many extremolytes work against various stresses at the same time, making them multi-functional agents [10,13]. Their striking properties have triggered a set of tests over the recent years, which demonstrate protecting effects on isolated proteins [29,30], DNA [31], food [32,33], feed [13], human cells [8,34], and human tissue [35]. Accordingly, industry has spotlighted extremolytes as an 'unexploited gold-mine' with great opportunities for the cosmetic, the medical, and the food industry, where the protection of sensitive biomolecules and biostructures is a key to success.

### Ectoine

Today, the compatible solute ectoine (2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) is the flagship of industrial extremolytes. The non-proteinogenic amino acid was originally discovered in *Halorhodospira* (formerly *Ectothiorhodospira*) *halochloris*, an extremely halophilic

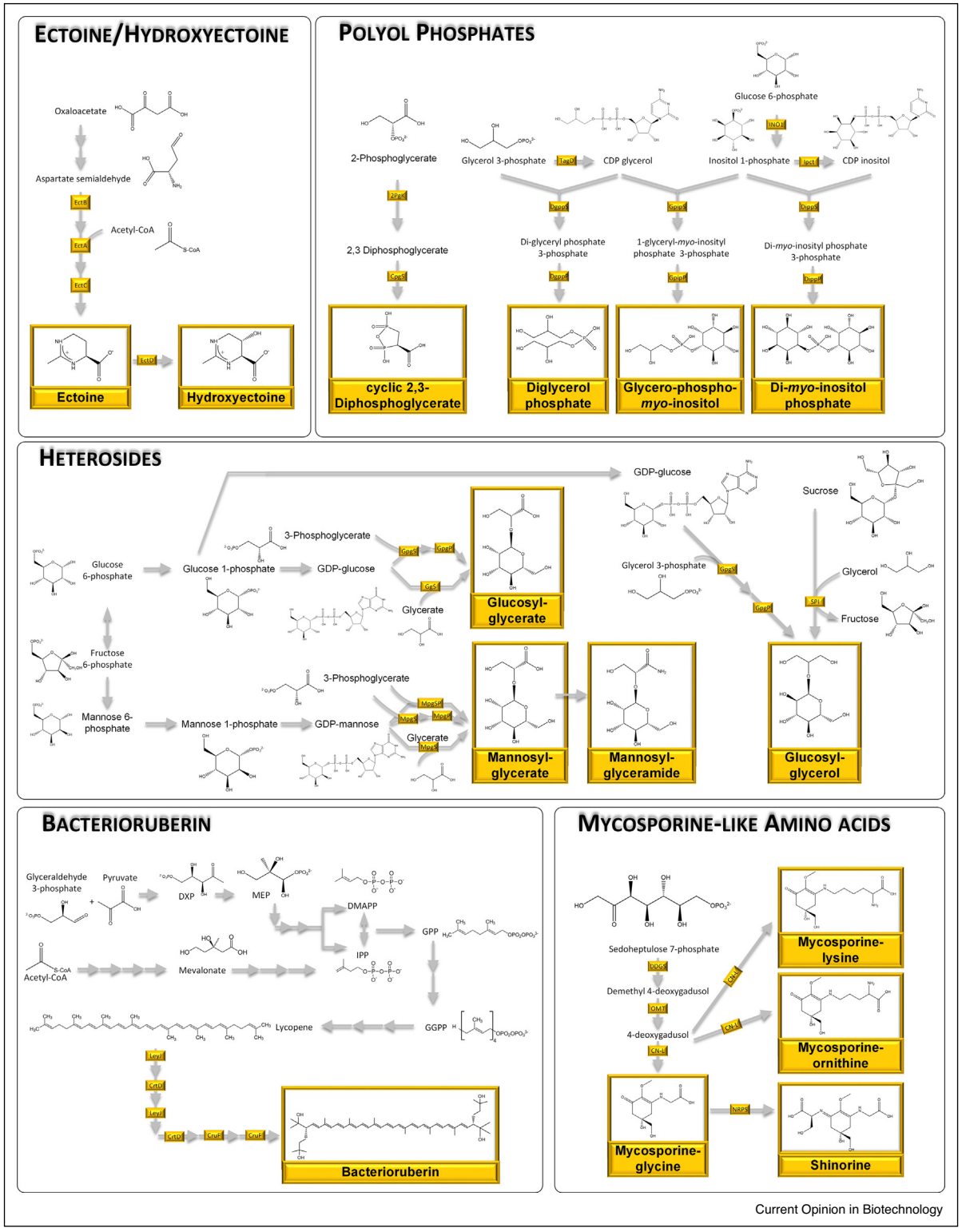
**Figure 1**




Global distribution of extreme habitats such as deserts, salt lakes, polar ice, volcanic regions, and hydrothermal vent fields as a rich natural source for extremophilic microbes and extremolytes [4,100,101]. Prominent examples comprise *Rhodothermus marinus*, isolated from a submarine hot spring in Iceland [2], *Halorhodospira* (formerly *Ectothiorhodospira*) *halochloris* and related species, isolated from the alkaline soda lakes of the Wadi Natrun [37], and *Halobacterium* (*Haloferax*) *volcanii*, discovered in the Dead Sea [3].

phototrophic eubacterium, isolated from the Wadi Natrun, Egypt [36,37] (Figure 1). Biochemically, ectoine is formed from L-aspartate-β-semialdehyde (ASA) through a three-step pathway catalyzed by L-2,4-diaminobutyrate transaminase (EctB), L-2,4-diaminobutyrate acetyltransferase (EctA), and ectoine synthase (EctC) (Figure 2) [10,38]. Ectoine exhibits cosmotrophic properties, that is, it is a co-solvent and contributes to the stability and structure of water–water interactions [10,27]. Because of this property, ectoine shields proteins, cell membranes, and human tissues from allergens, UV light, heat, and dryness and consequently has become a desired ingredient of skin and hair care products such as lotions, sprays, and crèmes [8,13] (Table 1). Besides cosmetics, ectoine is used in over-the-counter (OTC) medical devices, such as nasal sprays [35] and lung inhalation fluids [35]. The world market is in the scale of several tons per year. Estimated sales values of approximately 1000 US$ $kg^{-1}$ [10,39] and a likely attractive CGAR (calculated annual growth rate), inferred from the many novel commercial products (Table 1) create an interesting multimillion US$ market with promising perspectives. The world leader for ectoine manufacturing is the German company bitop AG. The bitop process relies on fermenting a non-genetically modified organism (non-GMO) strain of the halophilic bacterium *Halomonas elongata* [35]. Initially, ectoine has been obtained by so-called 'bacterial milking': ectoine accumulates inside cells of *H. elongata* in a high-salt fermentation (15%) and is then released into the broth upon an osmotic downshock to 3% [40]. More recently, 'superleaky' mutants of *H. elongata* have been isolated, which beneficially excrete ectoine during high-salt production [35]. The downstream process consists of micro/ultrafiltration, electro dialysis, chromatography, drying and crystallization to remove cells and other impurities and recover enantiomerically pure ectoine. According to market estimates, bitop covered more than 90% of the global

**Figure 2**




Biochemical and metabolic diversity of extremolytes and their biosynthetic pathways. 2PgK: 2-phosphoglycerate kinase, CN-L: CN-ligase, CpgS: cyclic 2,3-diphosphoglycerate synthase, CrtD: carotenoid 3,4-desaturase, CruF: C50 carotenoid 2″,3″-hydratase, DDGS: dimethyl 4-deoxygadusol synthase, DgppS: di-glyceryl phosphate 3-phosphate synthase, DgppP: di-glyceryl phosphate 3-phosphate phosphorylase; DMAPP: di-methylallyl pyrophosphate, DippP: di-*myo*-inosityl phosphate 3-phosphate phosphorylase, DippS: di-*myo*-inosityl phosphate 3-phosphate synthase, DXP: 1-deoxy-xylulose 5-phosphate, EctA: L-2,4-diaminobutyrate acetyltransferase, EctB: L-2,4-diaminobutyrate transaminase, EctC: ectoine synthase,

production in 2016. Meanwhile other companies such as the Chinese enterprise Bloomage Biotechnology Corporation have entered the market. To be mentioned here, chemical ectoine synthesis is also possible, but suffers from a low stereo-selectivity.

As the industrial high salinity medium is corrosive to conventional stainless-steel fermenters and connected equipment, and adds extra costs to wastewater treatment, current research aims at low salt ectoine production. Recent attempts engineered halophiles for production at reduced salt level and provide at least a proof of concept, although the achieved performance so far is low and needs additional improvement: a mutant of *Halomonas hydrothermalis* Y2, which lacked the $Na^+/H^+$ antiporter Mrp, formed 11 g $L^{-1}$ ectoine at 6% salt, whereas the parent strain required 10% salt and produced only 3 g $L^{-1}$ [41].

In addition, pioneering studies used *Escherichia coli* [42] and *Corynebacterium glutamicum* [38] to provide ectoine at the gram scale. Both strains optimally grow at salt levels of 0.5%, 30-fold less than required for *H. elongata* [40]. As example, *C. glutamicum* ECT-2 expressed the *ectABCD* cluster from *Pseudomonas stutzeri* and formed 4.5 g $L^{-1}$ ectoine from glucose [38]. In recent years, low salt ectoine production has advanced further. The recombinant strain *E. coli* Ect05 expressed a codon optimized *ectABC* cluster from *H. elongata* and formed 25 g $L^{-1}$ ectoine at a yield of 0.11 g $g^{-1}$ [43]. Unfortunately, strain engineering eliminated the biosynthetic pathways for L-threonine, L-isoleucine, and L-methionine so that the mutant required expensive medium ingredients. A more recent study systematically investigated the impact of nutrient composition and process temperature on ectoine production in *E. coli* [44]. The titer was highest at approximately 2% salt and 20°C but remained low (<1 g $L^{-1}$) even for the identified optimum. In addition, *C. glutamicum* was modified for enhanced supply of the ectoine precursor aspartate-semialdehyde ($pyc^{P458S}$, $hom^{V59A}$, $lysC^{T311I}$), de-repressed glucose metabolism (*ΔsugR*) and elimination of lactate secretion (*ΔldhA*) [45]. The obtained strain *C. glutamicum* Ecto-5 achieved an ectoine titer of 22 g $L^{-1}$ but formed L-lysine as a by-product in significant amount (6 g $L^{-1}$) limiting performance.

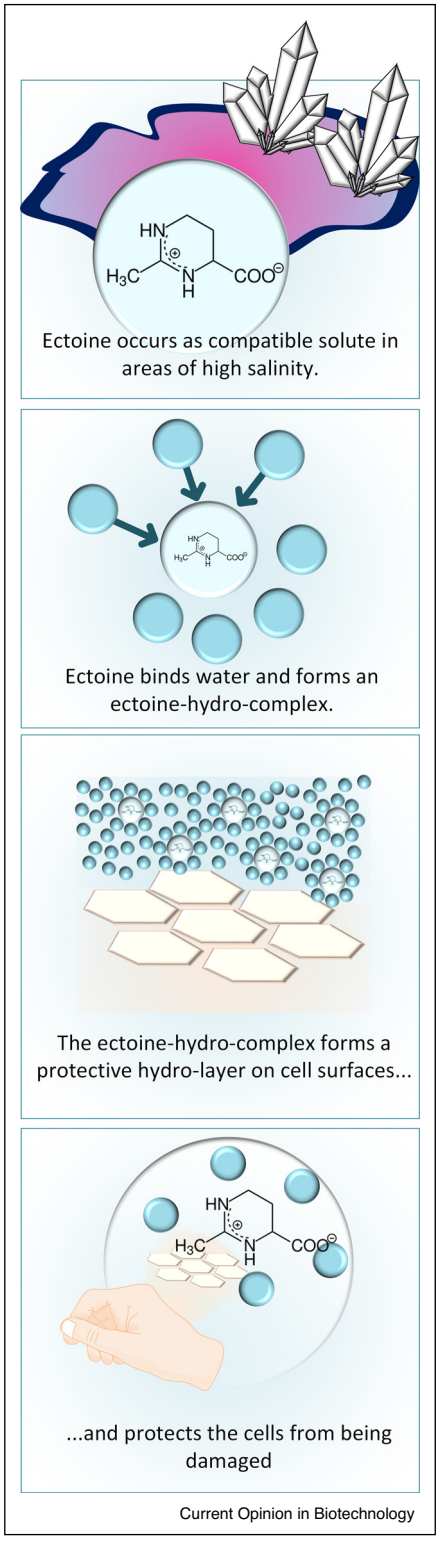
A seminal study recently maximized the ectoine flux in recombinant *C. glutamicum* by a fine-tuned balancing of the ectoine pathway [46••]. The conventional polycistronic *ectABC* cluster was replaced by a monocistronic design to individually control the expression level of each of the three ectoine genes. Randomized assembly of the genes with 19 different promoters and 3 different linkers resulted in a library of synthetic pathways with 185,193 possible variants. The library was transformed into a chassis strain, which expressed a feedback resistant aspartokinase ($lysC^{fbr}$) and lacked the L-lysine exporter (Δ*lysE*), crucial to avoid carbon loss via L-lysine secretion [47,48]. Miniaturized screening of hundreds of the pathway mutants for ectoine production, previously established in microtiter plate scale [49], identified several hyper producers, which shared two crucial features: the protein level of EctB was above a certain threshold and significantly higher than that of EctA [46••]. The best strain *C. glutamicum* $ectABC^{opt}$ accumulated 65 g $L^{-1}$ ectoine within 55 hours and sets a milestone toward industrial ectoine production at low salt level, when compared to previous developments (Figure 4).

## Hydroxyectoine

Hydroxyectoine (HE, 5-Hydroxy-2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid) is a hydroxylated form of ectoine. Biochemically, it is formed from ectoine by the activity of ectoine hydroxylases (Figure 2) and the two derivatives often occur together in natural extremophiles [50]. HE is also a target of economic interest for the cosmetics, the food and the medical industry, due to its applicability to protect against heat stress and desiccation, and an even greater stabilization capacity than ectoine [51,52]. Biotechnological production appears most straightforward due to the low stereo-selectivity of chemical synthesis. HE production was demonstrated for the heterologous hosts *C. glutamicum* [38], and *E. coli* [53], where titers of 0.4 g $L^{-1}$ and 1.2 g $L^{-1}$ were achieved. The best HE fermentation process to date is based on a natural isolate of *Halomonas salina*, which formed 2.9 g $L^{-1}$ in an optimized fermentation process with addition of iron and α-ketoglutarate [54]. Admittedly, all processes so far only provide mixtures of ectoine and HE and it appears economically unattractive to purify HE from such a process, given the high chemical similarity of the two compounds. An interesting concept recently used biotransformation with *E. coli*, expressing the ectoine hydroxylase encoding gene *ectD* from *P. stutzeri* [55]. The biocatalyst converted the supplied ectoine into 2.4 g $L^{-1}$ HE within a few hours. Given the high ectoine price and the need to purify HE from the biotransformation it remains open, if such a set-up can provide viable economics. *De novo* HE synthesis from cheap substrates at

**(Figure 2 Legend Continued)** EctD: ectoine hydroxylase, GGPP: geranylgeranyl pyrophosphate, Ggs: glucosylglycerate synthase, GpgP: glucosylglycerol phosphate phosphorylase, GpgS: glucosylglycerol phosphate synthase, GpipP: 1-glyceryl-*myo*-inositol phosphate 3-phosphate phosphorylase, GpipS: 1-glyceryl-*myo*-inositol phosphate 3-phosphate synthase, GPP: geranyl pyrophosphate, GpsP: glucosylglycerate phosphate phosphorylase, GpsS: glucosylglycerate phosphate synthase, INO1: *myo*-inositol 1-phosphate synthase, Ipct: CTP: *myo*-inositol 1-phosphate cytidylyltransferase, IPP: isopentenyl pyrophosphate, LeyJ: bifunctional lycopene elongase and 1,2-hydratase, MEP: 2C-methylerythritol 4-phosphate, MgpP: mannosylglycerate phosphate phosphorylase, MpgS: mannosylglycerate phosphate synthase, MpgSP: bifunctional mannosylglycerate phosphate synthase/phosphorylase, NRPS: non-ribosomal peptide synthase, OMT: O-methyl-transferase, SPI: sucrose phosphorylase, TagD: CTP:glycerol 3-phosphate cytidylyltransferase.

**Figure 3**




Cell-protecting mode of action of the extremolyte ectoine. Ectoine binds water due to its cosmotrophic properties and forms an ectoine-hydro-complex. The complex covers the cell surface with a protective hydro-layer serving as shield against external stress.

high selectivity appears more attractive on long term. Recent success in systems metabolic engineering of high-performance extremolyte producers [46••] and increasing knowledge on ectoine hydroxylases [10,55] will hopefully drive future development.

### Pipecolic acid

Pipecolic acid (piperidine 2-carboxylic acid, PA) is a key compound for the pharmaceutical industry and serves as chiral building block for therapeutics [56]. Only recently, its value as compatible solute has been proven for *C. glutamicum* [57•]. When grown under hyper-osmotic stress conditions, PA is accumulating in the cells and released upon an osmotic down shock, similarly to the ectoine bacterial milking process. Metabolically, PA is derived from the amino acid L-lysine, providing an ideal basis for biotechnological production of PA by exploiting well-known L-lysine overproducing chassis strains [57•,58] or biotransformation from L-lysine [56,59].

### Homoectoine

Homoectoine (4,5,6,7-tetrahydro-methyl-1H-[1,3]-diazepine-4-carboxylic acid) is a synthetic 7-ring analogue of ectoine. It can be chemically derived from ortho-trimethyl acetate and ornithine, using the so-called Koichi route for cyclic amidines [60•], however, at limited stereoselectivity. Initial tests revealed moderate beneficial effects [52], but recent studies demonstrate that homoectoine protects mice against colitis, which suggests future application as a dietary supplement for patients, suffering from inflammatory bowel diseases, a widely distributed disorder [61]. It is interesting to note that (because of certain degree of promiscuity) ectoine hydroxylases accept homoectoine as a substrate in addition to their natural substrate ectoine [60•]. This can be used to derive hydroxylated homoectoine, that is, *trans*-5-hydroxyhomoectoine.

### Mannosyl-glycerate and mannosyl-glyceramide

As ionic solute from thermophilic microbes, mannosylglycerate (MG, fiorin, 2-*O*-α-mannosyl-D-glycerate) protects proteins against heat stress [19]. In addition, it protects membranes, cells and tissues against radiation, stabilizes liposomes (because of its structural similarity with glycolipids), acts a penetration enhancer for cosmetic active ingredients [62] and can be derivatized into immunostimulating agents [63] making it attractive for the cosmetic and medical industry. The substance is formed in a range of (mainly marine) thermophilic microbes through a two-step cascade from GDP-mannose and 3-phosphoglycerate (Figure 2) [64,65]. As an exception, the thermophilic bacterium *Rhodothermus marinus* forms MG directly from GDP-mannose and D-glycerate (Figure 2) [66]. Initial attempts extracted MG from extremophilic microbes at low efficiency [67]. Successful expression of

**Table 1**

**Selected commercial cosmetics and health-care products using extremolytes as active ingredients**

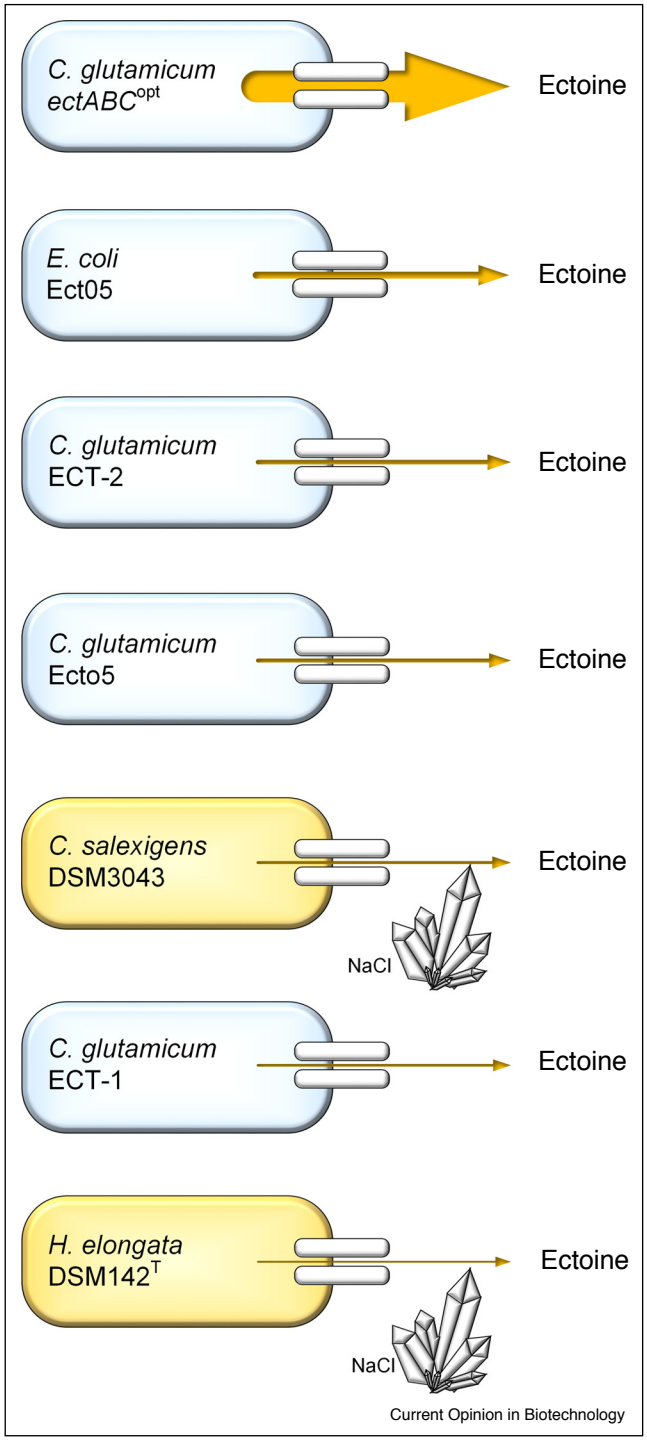
| Product | Application | Active ingredient | Supplier |
|---|---|---|---|
| Olynth | Nasal spray | Ectoine | Johnson & Johnson |
| Ectoine Rhinitis | Nasal spray | Ectoine | bitop AG |
| Ectoine Mouth & Throat Spray | Mouth & throat spray | Ectoine | bitop AG |
| Vividrin | Eye drops | Ectoine | bitop AG |
| Pari ProtECT | Mouth & throat spray<br>Nasal spray<br>Pastilles | Ectoine | Pari GmbH |
| Ectoine Allergy | Eye drops<br>Nasal spray | Ectoine | bitop AG |
| Ectoine Sicca | Eye drops | Ectoine | bitop AG |
| Ectoine dermatitis | Salve | Ectoine | bitop AG |
| Syxyl MedEctoine crème | Salve | Ectoine | bitop AG |
| Ectoine Anti-Aging fluid | Skin care | Ectoine | DADO-cosmed GmbH |
| Anti-Aging Eye Cream-Gel | Skin care | Ectoine | bitop AG |
| Revitalizing Face Cream | Skin care | Ectoine, Glycoin | bitop AG |
| Soothing Hand Cream | Skin care | Ectoine | bitop AG |
| Foaming Face Wash | Skin care | Ectoine, Glycoin | bitop AG |
| EUBOS Kinder Haut Ruhe EctoAkut forte | Salve | Ectoine | Dr Hobein (Nachf.) GmbH, med. Hautpflege |
| After Sun Serum | Skin care | Ectoine | Börlind Gesellschaft für kosmetische Erzeugnisse mbH |
| Glycoin Extremium | Skin care | Glycoin | Jan Dekker |
| Glycoin natural | Skin care | Glycoin | bitop AG |

the MG pathway in *E. coli* and *Saccharomyces cerevisiae* demonstrated the feasibility of recombinant production [68]. A nice work, recently metabolically engineered *S. cerevisiae* to enhance MG production [18•]. In batch culture, strain MG01 expressed MG synthase and MG phosphatase from *Dehalococcoides mccartyi* (*Dehalococcoides ethenogenes*), isolated from sewage sludge [69], and accumulated 6 (mg MG) $g^{-1}$ from glucose. A second-generation mutant (MG02) pushed a fraction of the glycolytic flux toward MG biosynthesis (through overexpression of the upstream pathway enzymes mannose 6-phosphate isomerase (PSA1) and GDP-mannose pyrophosphorylase (PMI40), which resulted in an increased accumulation of 16 (mg MG) $g^{-1}$. The authors carefully investigated the physiology of the producers and could reduce the massive secretion of glycolytic byproducts in glucose-grown batch cultures (ethanol, acetate, and glycerol) by continuous processes at low dilution rate, although MG production dropped under these conditions. Admittedly, the overall performance is still rather weak even in the best producer, and the non-secretion of MG remains as crucial drawback. However, the study is an important proof-of-concept and will likely lead to more efficient producers given additional rounds of strain and process engineering. Mannosyl-glyceramide (MGA) is a rare neutral amide derivative of MG. It has only been observed in the thermophilic microbe *Rhodothermus*, where it replaces MG at salt levels close to the maximum tolerated. Despite its proven efficacy as active ingredient [62], the enzyme catalyzing the amidation of MG into MGA is so far unknown [70] and biotechnological production has not been attempted so far.

### Glucosyl-glycerol and glucosyl-glycerate

In nature, the heteroside glucosyl-glycerol (GG, glycoin, R-2-*O*-α-D-glucopyranosyl-glycerol) is primarily found in marine cyanobacteria, and also occurs as re-activating substance in the resurrection plant *Myrothamnus flabellifolia* [15,16]. GG is well known to protect proteins, cells, and tissues against different types of stress and has a great potential, for example, as healthy food supplement and therapeutic agent [16,71]. Two biotechnological strategies have been proposed to derive GG. A one-step enzyme catalytic process utilizes sucrose phosphorylase from *Leuconostoc mesenteroides* [72]. The enzyme uses glycerol as glucosyl acceptor and transfers the glucosyl moiety from sucrose into the 2-*O*-position of glycerol (Figure 2), forming enantiomerically pure GG. The process benefits from simple, low price raw materials: sucrose and glycerol. Today, the company bitop uses this biocatalytic process to produce GG at industrial scale. The heteroside is very hygroscopic and therefore marketed as aqueous solution. The industrial product is named Glycoin Natural (by the manufacturer bitop) and Glycoin Extremium (by the distribution partner Jan Dekker International) and applied as anti-aging ingredient in cosmetics [16]. More recently, a two-step biosynthetic GG pathway from *Synechocystis* sp. PCC 6803, which forms the product from ADP-glucose and glycerol 3-phosphate, was exploited for production. In the natural producer, GG accumulation in the medium was achieved by elimination of GG re-uptake in osmotically stressed cells [73]. Additional deletion of *ggpR*, encoding a repressor of the GG pathway, enabled GG production under low-salt conditions and increased production under salt stress. Agar-bases gel encapsulation allowed

**Figure 4**




Ectoine production performance in various microbial producers (adapted from Ref. [46••]). The arrow width represents the specific ectoine production rate for *Corynebacterium glutamicum ectABC*$^{opt}$ (120 mg $g^{-1}$ $h^{-1}$) [46••], *C. glutamicum* ECT-1 (10 mg $g^{-1}$ $h^{-1}$) and ECT-2 (17 mg $g^{-1}$ $h^{-1}$) [38], *C. glutamicum* ecto5 (15 mg $g^{-1}$ $h^{-1}$) [45], *Escherichia coli* Ect05 (26 mg $g^{-1}$ $h^{-1}$) [43], *Chromohalobacter salexigens* DSM3043 (11 mg $g^{-1}$ $h^{-1}$) [102] and *Halomonas elongata* DSM 142 (7 mg $g^{-1}$ $h^{-1}$) [40]. The data are related to the cell dry mass.

semi-continuous GG production over a period of 32 days (4 cycles of periodic salt-stress and hypoosmotic shock), achieving a final titer of 1.64 g $L^{-1}$. The *Synechocystis* pathway was also introduced into *C. glutamicum* [74•]. Interestingly, GG was only formed in osmotically stressed cells. Subsequent elimination of ADP-glucose consuming routes toward glycogen and trehalose and a shift to nitrogen limitation provided 2 g $L^{-1}$ of GG. The structurally related solute glucosyl-glycerate (GGA, R-2-*O*-α-D-glucopyranosyl-glycerate) has similar protective properties as GG, but is not as common in nature [15]. It has not been produced so far, despite the biochemistry of GGA synthesis is known (Figure 2) [17,66,75].

## Chemical scavengers

Microbes from environmental areas, which are exposed to extensive radiation, have developed natural sunscreens for protection. These compounds are potent UV light absorbers that dissipate the radiation without the release of aggressive free radicals. Prominent examples comprise carotenoids [76–78] and mycosporine-like amino acids [79,80]. The carotenoid bacterioruberin has 13 conjugated double bonds (Figure 2), which confer a specifically high scavenging activity thus enabling high tolerance of haloarchaea toward strong light, gamma and UV irradiation, and $H_2O_2$ [76]. Though exploitation of haloarchaea for biotechnological production is challenging [76], bacterioruberin has been successfully extracted from *Haloferax volcanii* and applied as protectant for increasing the viability of sperm cells [1]. As the biosynthetic pathway has been elucidated [78,81], the door for overproduction of bacterioruberin in promising heterologous hosts has been opened [82–84].

Mycosporine-like amino acids (MAAs) comprise another efficient sunscreen compound of extremophilic organisms [85]. MAA biosynthesis originates from the pentose phosphate pathway intermediate sedoheptulose 7-phosphate (S7P) and involves dimethyl 4-deoxygadusol (DDG) synthase and *O*-methyltransferase to form the central MAA core 4-deoxygadusol (Figure 2). Elucidation of the shinorine pathway and identification and cloning of the encoding gene cluster of *Actinosynnema mirum* enabled heterologous shinorine production in *Streptomyces avermitilis* [80]. The specific production of 13.9 mg $g^{-1}_{dcw}$ was much more efficient than that of natural producers (0.03–0.98 mg $g^{-1}_{dcw}$) [80], pointing out the relevance of heterologous systems as production platform for extremolytes. Shinorine production was also established in *C. glutamicum* as heterologous host recruiting the *A. mirum* gene cluster [86•]. The basic producer was optimized in an iterative manner by improving the supply of S7P. First, S7P consumption by transaldolase was eliminated by deletion of the encoding *tal* gene; second, S7P supply was improved by overexpression of *gnd*, encoding 6-phosphogluconate dehydrogenase [86•]. Other successful

examples for heterologous MAA production comprise mycosporine-lysine (Figure 2) and mycosporine-ornithine (Figure 3) production with *E. coli* [79].

## Conclusion

The past years have shown a tremendous progress in the biotechnological production of extremolytes, driven by more and more opportunities to apply the compounds in cosmetics and more recently also in medical and nutritional products. From our viewpoint, three points appear important for future development. First, the immense progress in extremolyte biotechnology has been enabled by excellent fundamental studies, which have discovered novel extremolytes, and elucidated their genetics, biochemistry, and mode-of-action [27,28,31,61,81]. We need more of such studies in the future to drive the field. For sure, we have only touched the top of the iceberg and there likely wait multiple of unknown bacteria and archaea with great potential in nature's goldmine. Second, it seems that (given a few years of extra development) native extremophilic microbes will largely become inferior to well-established workhorses in fermenting extremolytes for industry. Metabolically engineered *E. coli* [87,88], *C. glutamicum* [89,90], *and S. cerevisiae* [91,92] meanwhile provide a spectrum of interesting extremolytes and promise further potential upon optimization by systems metabolic engineering [88,91,93,94]. Their low nutritional requirement, and capacity to produce chemicals at high titer and yield [48,95,96] from second and third generation renewables [97,98], together with a proven robustness [99] will enable simple manufacturing processes all over the world. Exceptions to this might hold, for example, for the cosmetic market, which prefers natural (non-GMO) extremolytes and is probably willing to pay higher prices from an eventually more expensive manufacturing.

## Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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