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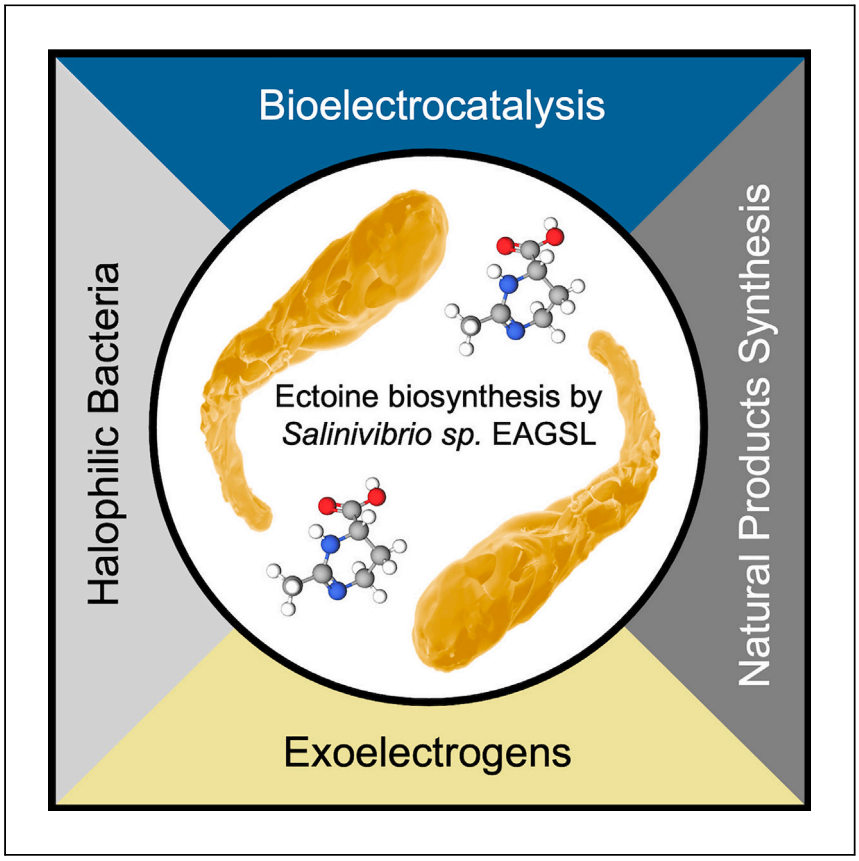
Salinivibrio sp. EAGSL as a halophilic and ectoine-producing bacteria for broad microbial electrochemistry applications



Salinivibrio sp. EAGSL is an electrogenic halophile, recently isolated from the Great Salt Lake in Utah, that synthesizes ectoine. Here, Guynn and Beaver et al. examine how environmental salt concentrations impact ectoine synthesis and create a salt-tolerant microbial fuel cell that could eventually be used for bioenergy, bioremediation, and natural product electrosynthesis.

Isaac P.A. Guynn, Kevin Beaver, Erin M. Gaffney, Ana Bonizol Zani, Ashwini Dantanarayana, Shelley D. Minteer

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Highlights

Biosynthesis of valuable product ectoine is established in novel halophile

Ectoine synthesis in *Salinivibrio sp.* EAGSL is affected by sodium chloride concentration

Power densities of ~2 W m^{-3} are generated in salt-tolerant microbial fuel cells

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Salinivibrio sp. EAGSL as a halophilic and ectoine-producing bacteria for broad microbial electrochemistry applications

Isaac P.A. Guynn,[1,3] Kevin Beaver,[1,3] Erin M. Gaffney,[1] Ana Bonizol Zani,[1] Ashwini Dantanarayana,[1] and Shelley D. Minteer[1,2,4,*]

SUMMARY

***Salinivibrio sp.* EAGSL (*S.* EAGSL) is an extremophile that was isolated from the Great Salt Lake (UT, USA) in 2017, and this strain has since been the focus of promising research in the field of microbial electrochemistry. Namely, *S.* EAGSL is an organism with both halotolerance and electroactivity, giving this microbe the unique ability to bridge the gap between power output and halotolerance in microbial fuel cells. While studying the genome, a biosynthetic gene for ectoine was identified. Ectoine is an osmolyte that is deemed a value-added chemical due to its ability to stabilize proteins and other biomolecules in varying conditions, proving its importance for the biochemical and cosmetic industries. Other halophilic bacteria, including *Halomonas elongata*, have been previously used for industrial production of ectoine. Herein, we evaluate the ectoine production from *S.* EAGSL, demonstrate proof of concept for *S.* EAGSL-based microbial fuel cells, and offer discussion for future electrosynthesis applications.**

INTRODUCTION

Bioelectrocatalysis is the cooperation of electrocatalysis and biocatalysis. Electrocatalysis seeks to convert chemical energy into electrical energy by driving redox chemistry with an electrode. Biocatalysis uses biological entities to catalyze desired reactions, which may be carried out by enzymes *in vitro*.[1] Under mild conditions for optimal cell growth, whole-cell catalysis provides protection to the enzymes and allows them to operate under their natural conditions. Bioelectrocatalysis introduces enzymes into the system as a catalyst for redox chemistry and allows integration of these systems with an electrode. Due to the specificity and efficiency enjoyed by enzymes, the combination of these two disciplines allows for the creation of reliable energy harvesting methods that can be tailored to specific conditions.[2]

Microbial fuel cells (MFCs) are an exciting application of bioelectrocatalysis that are being developed as a source of renewable energy as well as a wastewater treatment technology. MFCs seek to harness the metabolism of electroactive bacteria to produce storable electrical energy. Electrons produced by bacterial metabolic processes are transferred to an anode, thereby generating an electric current. While MFCs do not currently produce power on the scale of other renewable forms of energy, their power generation can be coupled with other properties of the MFC to create a self-powered system. Biosensing represents an exciting application of MFC power generation. Given the increasing rates of pollution in global water supplies, identifying and quantifying contaminates in an efficient and cost-effective way

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is of paramount importance. In the presence of certain contaminants, including heavy metals and organic materials, MFCs will generate a current proportional to the substrate concentration, allowing for the analytical measurement of a contaminate in a self-powered, cheap, and highly selective system.[3] The same selectivity that makes MFCs effective biosensors also allows for their use in wastewater treatment. As stated above, MFCs can metabolize organic materials and generate power. Given enough time, an MFC can exhaust a certain substrate in wastewater, removing the contaminant while simultaneously generating electricity.[4]

In addition to power generation, biosensing, and wastewater treatment, MFCs can produce value-added products. While redox chemistry is driven by the electroactivity of an MFC, microorganisms' metabolic pathways remain intact. As a result, microorganisms live and produce the metabolites generally dictated by their environment. Therefore, depending on the microorganism selected for use in the MFC, different metabolites may be targeted as value-added products and extracted from the system.[5] One value-added product that has garnered significant attention is the compatible solute ectoine.

Generally produced by extremophilic bacteria, ectoine allows microorganisms to survive high-stress environments with unfavorable temperatures, pH, or salinity. Halophiles, for example, can withstand the mostly sterile waters of the world's saltiest lakes. Furthermore, soil-dwelling bacteria, as well as those living in brackish environments, face constant fluctuations in the salt content of their environment due to rainfall and washout.[6] These microorganisms face a common threat to their viability: osmotic stress. Like all organisms, halophiles possess a semipermeable membrane; water can flow in and out of the cell, leaving them vulnerable to osmotic fluctuations. To combat this, halophiles have evolved different strategies that allow them to temper these fluctuations. Compatible solutes, such as ectoine, allow the cell to modulate their internal osmotic potential in an effort to react to external osmotic stress. When external osmotic potential is high, cells can synthesize compatible solutes to prevent the exodus of cytoplasmic water. Conversely, when external osmotic potential drops off, compatible solutes can be excreted to prevent water from entering and lysing the cell.[7]

In addition to controlling aqueous fluxes across an organism's membrane, compatible solutes like ectoine provide cells with cytoprotective capabilities. Specifically, they can stabilize proteins under the inhibitory conditions that accompany a stressed cell.[8] By suppressing the unfolding of proteins, compatible solutes allow the cell structures of microorganisms to withstand stressors that accompany high osmotic stress, as well as other factors, like temperature and pH, that threaten to denature vital cell structures. Ectoine is one of the most widely synthesized compatible solutes, with a gene cluster that is conserved across numerous bacterial species, a limited number of archaea,[9] and, surprisingly, some halotolerant eukaryotic organisms.[10]

Because of ectoine's ability to protect cell structures from environmental stress, it has demonstrated therapeutic value in cosmetic products. In various clinical studies, ectoine has been shown to treat epithelial inflammatory conditions. Moreover, due to its low toxicity, ectoine can be used with minimal side effects.[11] These properties have garnered medical interest, establishing the osmolyte as a value-added product. Furthermore, because of these properties, ectoine can be used to stabilize MFCs in stressful environments. Specifically, MFCs run into a unique challenge under the harsh conditions of hypersaline wastewater. Due to their reliance on thriving

biofilm, MFCs generally perform poorly under hypersaline conditions, as many electroactive species struggle to grow in the presence of high osmotic stress. Given that hypersaline wastewater comprises roughly 5% of global effluents,[12] developing MFCs that are able to thrive under these conditions is an intriguing area of research, and one that can look to the production and integration of ectoine into MFCs as a means of increasing output.

Researchers have investigated two strategies to improve salt tolerance of MFCs: exogenous ectoine production and endogenous ectoine production. The former relies on flora uninvolved in the bioelectrochemistry of the MFC. Rather, this method uses ectoine-producing organisms to excrete ectoine into the MFC. This, in theory, could transfer salt tolerance to the non-halophilic bacteria of the MFC. Tan et al. have demonstrated that the addition of ectoine to an MFC under saline conditions can improve its output by roughly 60%.[13] Furthermore, Zeng et al. used *Halomonas* strains to biosynthesize ectoine, which improved MFC performance under saline conditions by about 55%.[14]

Endogenous ectoine production, on the other hand, relies on electroactive microorganisms that can simultaneously synthesize ectoine. *Salinivibrio sp.* EAGSL (*S.* EAGSL) is a microorganism that fits these criteria. A halophilic bacteria isolated from Utah's Great Salt Lake in 2017, *S.* EAGSL has been used in hypersaline MFCs to generate a moderate power output as well as an effective biosensing tool in hypersaline wastewater treatment.[15] Combined, these two properties suggest that *S.* EAGSL MFCs may be developed into biosensors that are powered by their own metabolism and the organic material present in hypersaline wastewater. This exciting technology could significantly reduce the high operational costs of current analytical techniques involved in hypersaline wastewater treatment.[5]

While *S.* EAGSL is electroactive and halotolerant, the underlying mechanisms for these properties and the relationship between them remain relatively unexamined. In this work, we outline the development of a protocol that seeks to demonstrate the existence of ectoine production in *S.* EAGSL, as well as quantify intracellular pools of ectoine. Furthermore, the relationship between culture salinity and ectoine production is examined. To relate these findings to bioelectrochemical applications, MFCs with *S.* EAGSL-based bioanodes and air-breathing cathodes were designed to understand the relationship between salinity, ectoine biosynthesis, and electrochemical output.

RESULTS AND DISCUSSION

Ectoine biosynthetic pathway of *S.* EAGSL

As shown in Figure 1, the biosynthesis of ectoine in *S.* EAGSL begins with the metabolite L-aspartate-β-semialdehyde (L-ASA). L-2,4-diaminobutyrate-2-oxoglutarate transaminase (EctB; EC 2.6.1.76) catalyzes the pyridoxal phosphate (PLP)-dependent transamination of L-glutamate to make 2-oxoglutarate and L-2,4-diaminobutyrate (DAB). L-2,4-diaminobutyrate acetyltransferase (EctA; EC 2.3.1.178) catalyzes the acetylation of DAB via acetyl-CoA to make CoA and N-γ-acetyl-L-2,4-diaminobutyrate. Finally, N-γ-acetyl-L-2,4-diaminobutyrate is converted into ectoine by ectoine synthase (EctC; EC4.2.1.108).

EctA, EctB, and EctC are part of the EctABC biosynthetic gene cluster, present in organisms that synthesize the compatible solute ectoine. Often contained in an operon, EctABC can be activated during periods of high stress, including

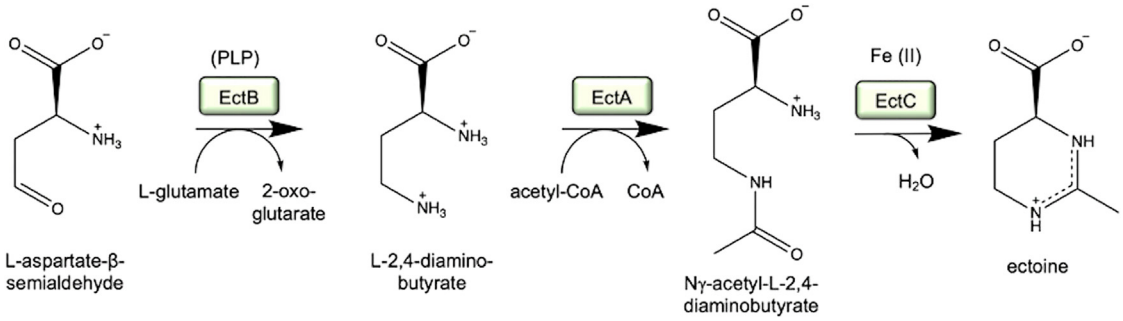

Figure 1. The ectoine biosynthetic pathway

The biosynthetic synthesis of ectoine utilizes enzymes EctB, EctA, and EctC from the EctABC gene cluster.

fluctuations in temperature, salinity, and pH. *S.* EAGSL's ability to produce ectoine was the basis of this experiment. While *S.* EAGSL is halophilic in nature, an indicator of ectoine production, additional verification was necessary to begin the experiment. *S.* EAGSL's genome has been sequenced and is available through the National Center for Biotechnology Information (NCBI).[16] The annotation files and Basic Local Alignment Search Tool (BLAST) were used to confirm the presence of the EctABC biosynthetic gene cluster in *S.* EAGSL's genome (NCBI: JABWMG000000000). Using the reference EctABC sequence for *Salinivibrio* spp. (NCBI sequence ID: WP_021024033.1), protein BLAST revealed 100% sequence similarity for EctB and EctC in *S.* EAGSL. In the EctA protein in *S.* EAGSL, there is a single substitution from glycine to serine at amino acid 36 (G36S) achieving 99.46% sequence similarity to the *Salinivibrio* spp.

Quantification of intracellular ectoine production by *S.* EAGSL

Ultra-performance liquid chromatography-coupled mass spectrometry (UPLC-MS) was used to quantify the ectoine production of *S.* EAGSL in varying saline conditions. The UPLC-MS analysis of each culture extract returned the following results, which are depicted in Figure 2: the 25, 50, 75, 100, and 150 gL^{-1} NaCl culture extracts had average ectoine concentrations of 21 $\pm$ 2, 190 $\pm$ 10, 300 $\pm$ 10, 790 $\pm$ 10, and 1,140 $\pm$ 30 μM, respectively. Further, the purified cell extract produced a mass spectrum resembling that of commercially produced ectoine dissolved in 70% ethanol, shown in Figure S1, demonstrating the purity of the ectoine fraction and confirming the efficacy of the extraction procedure. UPLC-MS analysis of each cell extract produced results that suggest that *S.* EAGSL makes more ectoine as salinity increases. This suggests that *S.* EAGSL is more effective in a value-added product context in wastewater that approaches 100 gL^{-1} NaCl. Wastewater from most tanning processes, for example, contains up to 80 gL^{-1} NaCl.

Ectoine production, however, can increase due to a variety of factors. While individual cells can modulate their ectoine production, the success of each culture, measured by their optical density at 600 nm (OD600), can also affect the total concentration of ectoine in solution. Because it lives in the brackish waters of the Great Salt Lake, typically concentrated at 120 to 160 gL^{-1} NaCl,[17] *S.* EAGSL cultures higher than 100 gL^{-1} NaCl tended to have higher OD600s than those lower saline, as shown in Figure 3. Because OD600 increases with salinity, while ectoine production undergoes a corresponding increase, the relationship between these three factors is difficult to discern.

Even though cell density of *S.* EAGSL cultures is roughly correlated with salt concentration, shown in Figure S2, OD600 hits a threshold when salinity is 100 gL^{-1} and

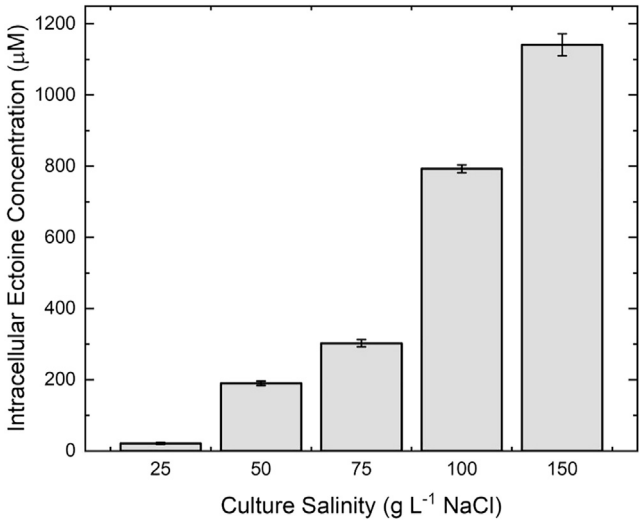

Figure 2. Ectoine production from *S.* EAGSL

S. EAGSL cultures at varying salinities (25, 50, 75, 100, and 150 gL^{-1} NaCl) were studied for their ectoine production. Bars represent mean ectoine concentration, while error bars indicate standard deviation (N = 3).

above. The implications of these data are 2-fold. First, they strengthen the suggestion that *S.* EAGSL is most effective as a value-added product producer at higher salinity. Furthermore, they show that while ectoine production may increase with OD600, it independently increases with salinity. Comparing the 100 and the 150 gL^{-1} NaCl cultures illustrates this relationship; cell densities of the 100 and 150 gL^{-1} cultures are comparable (1.78 $\pm$ 0.04 and 1.80 $\pm$ 0.04, respectively), while the 150 gL^{-1} NaCl culture produces significantly more ectoine (1,140 $\pm$ 30 μM compared with 790 $\pm$ 10 μM). Similarly, the lower salinity cultures of 50 and 75 gL^{-1} NaCl show similar optical densities (0.92 $\pm$ 0.03 and 0.93 $\pm$ 0.03, respectively), but the cells grown at 50 gL^{-1} NaCl produced significantly less ectoine than the cells at 75 gL^{-1} NaCl (190 $\pm$ 6 μM compared with 300 $\pm$ 10 μM). Therefore, the increases in ectoine concentration are not directly a result of more cells being present in solution. It is also not reliable to normalize ectoine biosynthesis results to a given cell density because measuring the optical density or weighing the biomass does not account for dead cells or precipitated salt crystals.

Due to electrochemical applications being the target biotechnologies of *S.* EAGSL, MFCs were designed to understand the relationship between ectoine biosynthesis and electronic output. Specifically, the bioanodes of the MFC used the oxidative metabolism of *S.* EAGSL to generate an electric current that passed to an air-breathing cathode membrane electrode assembly (MEA), where the oxygen reduction reaction (ORR) occurred (Figure S3).

After conducting slow scan rate linear sweep voltammetry (LSV), power was calculated as the product of current and potential at a given point (Figure 4). Maximum power densities (Figure S4) were calculated according to the volume of the carbon felt anode and were not significantly different between MFCs operating at 50 (1.9 $\pm$ 0.3 Wm^{-3}), 100 (2.4 $\pm$ 0.3 Wm^{-3}), or 150 gL^{-1} NaCl (2.1 $\pm$ 0.2 Wm^{-3}). The maximum current densities (Figure S5) achieved by the MFCs were also recorded for each salinity: 50 (13 $\pm$ 2 Am^{-3}), 100 (15 $\pm$ 1 Am^{-3}), and 150 gL^{-1} NaCl (15 $\pm$

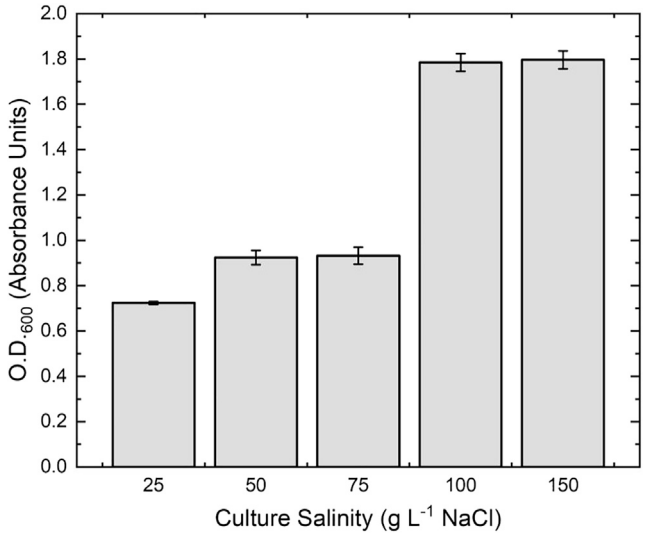

Figure 3. *S.* EAGSL cell growth at varying salinities

S. EAGSL culture cell densities at varying salinities (25, 50, 75, 100, and 150 gL^{-1} NaCl) were measured to ensure cell growth was not influencing ectoine production. Bars represent mean absorbance at 600 nm, while error bars indicate standard deviation (N = 3).

2 Am^{-3}). Like maximum power density, salinity did not significantly affect the maximum current density of the MFCs.

In fuel cell applications, open circuit potential (OCP) represents the maximum operating voltage of the system, which is the voltage where current is zero. Mean OCP was determined for each MFC (Figure S6), and increasing salinity was correlated with decreasing OCP. Specifically, the MFC at 50 gL^{-1} NaCl (0.90 $\pm$ 0.03 V) had a significantly higher OCP than the MFC at 150 gL^{-1} NaCl (0.81 $\pm$ 0.02 V), while the MFC at 100 gL^{-1} NaCl (0.86 $\pm$ 0.06 V) had an intermediate OCP.

Future outlooks and applications for ectoine production from *S.* EAGSL

As an electroactive organism with a demonstrated capacity for ectoine production, *S.* EAGSL offers exciting new directions of investigation in the field of microbial electrochemistry. One direction of study involves the EctB enzyme. This step of ectoine biosynthesis is PLP dependent and is therefore redox active. It has previously been shown that redox active steps in bacterial metabolisms can be manipulated via potentiation in the presence of a particular electron carrier.[18] If transferable to the ectoine biosynthetic pathway, ectoine production could be modulated inside an MFC.

Furthermore, using *S.* EAGSL in an MFC can add an additional focus to the fuel cell due to its capacity for ectoine production, which is a value-added chemical product. Using an extraction method similar to the one used in this experiment, ectoine can be extracted from MFCs and purified into the valuable product that is pure ectoine. Bacterial milking methods, like modulating the salt concentration of each culture, could increase ectoine production and secretion, improving yield and making extraction unnecessary.[17]

Finally, finding a reliable way to stimulate ectoine secretion in *S.* EAGSL in the context of an MFC can provide an exciting application in the form of a co-culture. Known electroactive strains, for example *Geobacter* spp. or *Shewanella* spp.,[19]

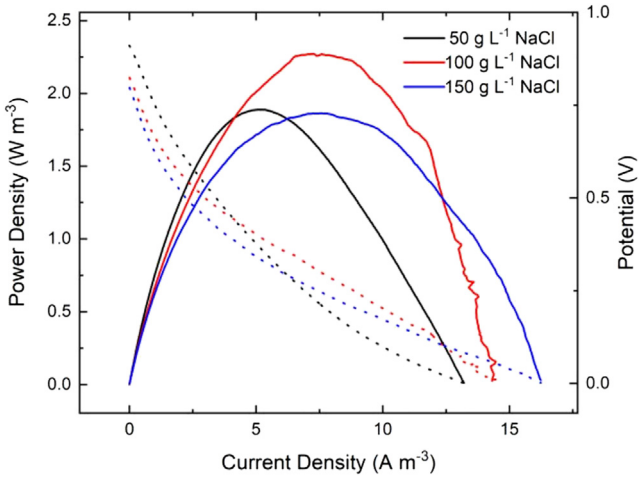

Figure 4. Power curves and polarization curves for *S.* EAGSL MFCs

Representative power curves (solid lines) were calculated from LSV and plotted for *S.* EAGSL MFCs at varying salinities. Dotted lines depict the polarization curves showing the current produced at each potential, scanned from OCP to 0 V.

struggle to tolerate hypersaline solutions. An external source of ectoine, however, could transfer some degree of osmoprotection to these highly electroactive species, rendering them useful in the context of hypersaline MFCs.[14] An MFC with a co-culture comprised of a non-halotolerant microbe like *Geobacter* spp. or *Shewanella* spp., along with *S.* EAGSL, represents one application of this idea. It will be important to determine efficient methods of isolating and measuring extracellular fractions of ectoine in order to understand how these co-cultures will work and to characterize ectoine uptake mechanisms in the non-halotolerant strain. Electrochemical studies performed over a longer duration than 48 h would also contribute to understanding how these MFCs would function in long-term industrial applications. It is plausible that further experiments could be continued for time scales up to months or even years.

Through UPLC-MS analysis, the biosynthesis of ectoine in *S.* EAGSL cultures has been demonstrated. Furthermore, increasing the cell density of *S.* EAGSL cultures has been shown to increase ectoine production. However, once the cell density levels out above optimal salt concentrations, ectoine production undergoes additional increases that are positively correlated with salinity. These findings establish *S.* EAGSL as a value-added product producer and shed light onto *S.* EAGSL's robust halotolerance, opening opportunities for MFCs for saline wastewater that also produce ectoine as an important natural product.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Shelley D. Minteer (minteer@chem.utah.edu).

Materials availability

This study did not generate new unique materials.

Data and code availability

This study did not generate/analyze datasets/code other than the data presented in the paper and the Supplementary Material.

S. EAGSL growth curves and culture

To properly study the ectoine production levels of *S.* EAGSL, ectoine was isolated from cultures at different salt concentrations, each in biological triplicate. 9 mL sterile sulfur-reducing broth (SRB) media at 25, 50, 75, 100, 125, and 150 gL^{-1} sodium chloride were inoculated with 10 μL *S.* EAGSL from a frozen glycerol stock. The SRB media contained (for 1 L media) 2 g sodium acetate trihydrate, 6.38 g sodium citrate dihydrate, 1.75 g sodium lactate, 0.5 g potassium phosphate monobasic, 1 g ammonium chloride hexahydrate, 1 g sodium sulfate, 1.83 g magnesium chloride hexahydrate, 1 g yeast extract, 0.1 g ascorbic acid, 0.013 g sodium thioglycolate, 1 g calcium chloride dihydrate, 0.5 g iron sulfate heptahydrate, and the listed concentration of sodium chloride (pH 7.50). These pre-cultures were then incubated at 30°C and shaken at 140 rpm for 24 h in a 40 mL culture flask. The pre-cultures were then divided into 3 aliquots of 3 mL. Each aliquot was used as a 1:10 inoculation for a 30 mL culture and was therefore transferred to 27 mL sterile SRB at a corresponding salt concentration. These 30 mL cultures, now in biological triplicate at each desired salt concentration, were incubated at 30°C and shaken at 140 rpm at 24 h in a 40 mL culture flask. The OD600, a measurement typically used to determine cell density, was obtained for each 30 mL culture, after a 2-fold dilution, using an absorbance spectrometer (ThermoSpectronic Genesys 20 Model 4001/4) and a plastic cuvette.

In order to maximize culture density and ectoine production, each culture was aborted near the end of their exponential phase. In order to determine an appropriate time to stop each culture, *S.* EAGSL growth curves were set up in a 96-well plate reader. The plate was loaded with deionized (DI) water on the edges, and the remaining cells were filled with 180 μL SRB media at 100 gL^{-1} NaCl. Most of the wells were inoculated 1:10 with 20 μL *S.* EAGSL culture at 100 gL^{-1} NaCl, while some of the cells were left uninoculated for a sterile control. The plate was shaken continuously at 140 rpm and incubated at 30°C in the plate reader. Every 10 min, the plate reader took an absorbance reading at 600 nm to determine the OD600 of each well. Informed by the results shown in Figure S7, 24 h was deemed a suitable time to abort each *S.* EAGSL culture for ectoine isolation.

Ectoine isolation

After obtaining the OD600, the intracellular pool of ectoine was extracted from each culture based on a procedure from Zeng et al.[14] adapted to the growth conditions of *S.* EAGSL. Each of the 30 mL cultures of *S.* EAGSL at each salt concentration were transferred to a 50 mL centrifuge tube and centrifuged for 20 min at 4,500 rpm. The supernatant consisting of growth media and extracellular metabolites was collected and saved for future testing. To wash away any remaining supernatant from the condensed cells, the pellet was resuspended in 100 mM phosphate buffer (pH 7.4) and the same salt concentration as the culture. This solution was then centrifuged for 20 min at 4,500 rpm. The supernatant, consisting of 100 mM phosphate buffer, was then discarded, and the pellet was resuspended in 70% ethanol for 24 h to extract intracellular ectoine. After extraction, the tube was spun down under the same parameters, and the supernatant was filtered through a sterile syringe filter with 0.2 μm polyethersulfone membrane (VWR International) and submitted for UPLC-MS for analysis.

Identification of intracellular ectoine via UPLC-MS and quantification of ectoine via UPLC

The cell extracts in 70% ethanol were analyzed by UPLC-MS to separate the ectoine from other metabolites in solution and subsequently injected into a mass spectrometer to confirm the presence of ectoine in the fraction. The concentration of intracellular ectoine in each culture was calibrated to an integrated UPLC peak area with a calibration curve prepared from standard ectoine solutions. Stock solutions of ectoine were created by dissolving >95.0% HPLC-grade ectoine (Sigma-Aldrich) in 70% ethanol at concentrations of 10, 50, 250, 500, 750, 1,000, 1,250, and 1,500 μM. LC data were collected using a Waters ACQUITY H-class ultra-high pressure liquid chromatograph coupled to a Waters ACQUITY PDA detector. An ACQUITY UPLC BEH Amide column (2.1 × 50 mm) was used for separation and resolving samples. The compounds were eluted from the column using an isocratic method with 15% solvent A and 85% solvent B. Solvent A was 5 mM ammonium acetate (pH 7), and solvent B was acetonitrile. The solvent flow rate was 0.45 mL per minute. The peak area for each solution was plotted against the ectoine concentration, allowing for the determination of intracellular ectoine concentrations of each culture by comparing their peak area with the calibration curve.

MFCs for power generation and ectoine quantification

MFCs were created using H-cell half cells, with the cathode being comprised of an MEA, creating an interface between the liquid bacterial culture and air. Nafion-117 (Fuel Cell Earth) and 20 wt % Pt/C cloth (Fuel Cell Earth, 0.5 mg cm^{-2}) were hydraulically pressed at 2,000 psi and 130°C for 3 min to create the MEA, and the Pt/C cloth side was exposed to air. All MFC components were sterilized under UV light for 20 min before assembling the MFC. The anode of the fuel cell was a carbon felt electrode (Alfa Aesar, 99.0%) with dimensions of 2 × 2 × 0.318 cm secured to a nichrome wire (Electron Microscopy Sciences, 30G). A vented filter cap (Celltreat) was used to increase the amount of oxygen entering the MFC. Overnight liquid cultures of *S.* EAGSL in SRB (70 mL, OD600 = 1) were grown using the same 10% liquid transfer procedure as stated above. The SRB media containing 100 gL^{-1} NaCl was used to grow all cells for 18 h (30°C, 140 rpm) in vented 250 mL growth flasks. The cultures were centrifuged for 20 min at 4,500 rpm, then resuspended in 60 mL fresh SRB media containing 50, 100, or 150 gL^{-1} NaCl. The MFCs were assembled and filled with this suspension.

A Squidstat Prime (Admiral Instruments) potentiostat was used for all electrochemical tests, which were conducted at room temperature (20°C ± 2°C). The MEA cathode was the working electrode, and the felt anode was connected to both the counter and reference electrode leads. An external resistance of 1,000 Ω was applied to each fuel cell for 24 h, allowing biofilm to form on and within the carbon felt anode. It takes several hours for current measurements to stabilize in these systems, which is likely due to the wicking of the carbon felt and the gradual process of cells adhering to the electrode surface. After this stabilization period, power curves were generated from LSV experiments, where potential is scanned in the negative direction toward 0 V from open circuit voltage (OCP). These OCP readings represent the MFC voltage where there is zero current. LSV experiments scanned potentials from OCP to 0 V at a rate of 1 mV s^{-1}, and the measured current densities at these applied potentials were used to calculate power densities. From these power curves, maximum power density, maximum current density, and OCP measurements were obtained. The fuel cells were left to operate for another 24 h by applying a load of 1,000 Ω as in the previous stabilization period. After this 48 h period, the MFC

experiments were aborted, and ectoine was analyzed to ensure ectoine biosynthesis occurred (Table S1).

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrp.2023.101420.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

I.P.A.G. and E.M.G. conceived the study and designed the experimental setup. I.P.G., K.B., E.M.G., A.B.Z., and A.D. grew *S.* EAGSL and performed the ectoine isolation and quantification studies. K.B. and A.B.Z. designed MFC setups and conducted electrochemical experiments. I.P.A.G., K.B., E.M.G., and S.D.M. critically analyzed the results and wrote the manuscript, which was edited and approved by all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We support inclusive, diverse, and equitable conduct of research.

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