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MINI REVIEW

Marinobacter: A case study in bioelectrochemical chassis evaluation

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Abstract

The junction of bioelectrochemical systems and synthetic biology opens the door to many potentially groundbreaking technologies. When developing these possibilities, choosing the correct chassis organism can save a great deal of engineering effort and, indeed, can mean the difference between success and failure. Choosing the correct chassis for a specific application requires a knowledge of the metabolic potential of the candidate organisms, as well as a clear delineation of the traits, required in the application. In this review, we will explore the metabolic and electrochemical potential of a single genus, *Marinobacter.* We will cover its strengths, (salt tolerance, biofilm formation and electrochemical potential) and weaknesses (insufficient characterization of many strains and a less developed toolbox for genetic manipulation) in potential synthetic electromicrobiology applications. In doing so, we will provide a roadmap for choosing a chassis organism for bioelectrochemical systems.

INTRODUCTION

Thanks to many years of painstaking work, the ability of certain specialized microorganisms to transfer electrons directly to or from an electrode is finally reaching the point at which it can be manipulated and transferred to non-native hosts using the tools of synthetic biology. The ability to actively control electron transfer in organisms essentially allows us to send and receive messages from bacteria using electrical current, opening the door to an entirely new class of living electronics. Several recent reviews have covered the application of synthetic biology to bioelectrochemical systems (BES) (Bird et al., 2019, 2021; Li et al., 2018; Zhao et al., 2021).

When moving from this broad vision of living electronics to specific, working examples, choosing the correct chassis is vital for the rapid development and ultimate success of the application. The bulk of synthetic biology research and applications are based on a few well-established model organisms such as *Saccharomyces* species and *Escherichia coli*. However, as the imagined applications of synthetic biology have expanded from controlled laboratory and industrial conditions to field deployments (such as their use in biosensors (Saltepe et al., 2022), biomanufacturing and living surface coatings), there is a growing awareness of the need for environmentally relevant microorganisms that will thrive in field conditions where standard chassis may not (Adams, 2016).

When choosing a chassis for any synthetic biology application, there are three main factors to consider: first, the organism must be relatively easy to genetically manipulate, as synthetic biology by definition involves genetic engineering. Second, the organism must be well suited to the target environment, as an organism that struggles to survive when deployed will likely not fulfill its function effectively. Finally, the most complex requirement is that the chassis should have as many of

the required functionalities as possible already in place. A proposed application may require diverse functions and deciding which to engineer and which to look for as a native trait in the chassis can be tricky. Typically, the more complex function would be preferred as a native trait. For example, if the ability to grow in hypersaline or otherwise extreme conditions is required, it is likely better to use a chassis with this ability rather than attempting to add it to a non-halophile through genetic engineering.

In this minireview, we will focus on the potential of members of the *Marinobacter* genus to serve as chassis for BES in marine and other high salt environments. In doing so, we will discuss the various requirements that must be considered when creating a living electronic technology, such as genetic tractability, biofilm formation and native electroactive potential.

MARINOBACTER ENVIRONMENTS AND DIVERSITY

The *Marinobacter* genus includes over 70 validly named species and many more isolates. Members of the genus have been found throughout the world's oceans, from the Arctic to the Antarctic (Singer et al., 2011). Species and strains have also been isolated from saline soils, sand, hypersaline lakes, subsurface aquifers, cave complexes and oil and gas wells (Handley & Lloyd, 2013). As expected from their natural environments, they are halophilic and halotolerant to varying degrees. As discussed below, they display a wide range of metabolic abilities: members of the genus have been shown to consume a variety of hydrocarbons and dyes, making them good candidates for bioremediation projects (Handley & Lloyd, 2013). Some members of the genus can use nitrogen as an electron acceptor, while others are obligate aerobes. They also produce a variety of lipids (described below), making them potential biosynthesis chassis. A few are even reported as able to fix nitrogen (Al-Mailem et al., 2013). These attributes suggest a wide range of operational conditions for this genus, making them attractive chassis for applications in polluted and non-freshwater environments. It is also worth noting that they can survive and even grow under extremely low-carbon/energy conditions (Jain et al., 2021). This can serve as a potential advantage in field applications, when periods of starvation may occur.

EASE OF GENETIC MANIPULATION

Ease of genetic manipulation is of primary importance in a microbial chassis. Some ability to genetically engineer the microorganism in question is essential to developing a synthetic biology toolkit: however, the ease and speed with which this can be done falls on a spectrum and must be balanced against other traits. In this category, *Marinobacter* species fall somewhere in the middle. They grow moderately quickly on aerobic plates: *Marinobacter atlanticus* takes two days to form colonies (Bird et al., 2018), as do *M. adhaerens* (Kaeppel et al., 2012), *M. subterrani* (Bonis & Gralnick, 2015) and *M. nauticus* (Huu et al., 1999). They also display sensitivity to many common antibiotics, making them easier and faster to work with than many environmental isolates, which may not grow on plates at all. On the other hand, bacteria such as *E. coli* form colonies on plates overnight, making them twice as fast to engineer.

Research in the past decade has led to strategies for genetic manipulation of several species of *Marinobacter*. The initial genetic manipulation tools were demonstrated by Sonnenschein et al, (Sonnenschein et al., 2011), who first identified suitable resistance markers and tested different plasmids for their ability to replicate in *M. adhaerens*. They transferred the broad host range plasmids pBBR1MCS2 and pSUP106 to *M. adhaerens* via electroporation and conjugation and demonstrated transposon mutagenesis and gene-specific mutagenesis using homologous recombination. Several other researchers have since disrupted various genes in *Marinobacter* species: genes encoding for efflux pumps (Stahl et al., 2015), thioesterases (Lijewski et al., 2021), alkane uptake (Mounier et al., 2018) and flagellin proteins (Bonis & Gralnick, 2015) have all been genetically inactivated and the functionality of some of them restored following complementation in trans. More recently, Bird et al, (2018) developed a genetic system for *M. atlanticus* CP1, a strain with known electrochemical properties. In this study, conjugation and homologous recombination were used to create a double deletion mutant of two genes involved in wax ester production. Plasmid-based expression using a pBBR1-derived plasmid (Kovach et al., 1995) was also demonstrated (Bird et al., 2018). Strains deficient in wax ester synthesis were able to synthesize higher levels of phloroglucinol when the gene for the PhlD protein was expressed (Meyer, Saaem, et al., 2019). Finally, engineered *M. atlanticus* CP1 was used in a study visualizing real-time protein expression in electrochemically active biofilms (Phillips et al., 2020) using a plasmid-based system with an inducible promoter from the Marionette family of 12 small molecule sensors recently developed and optimized for low background and high response in *E. coli* (Meyer, Segall-Shapiro, et al., 2019). The use of promoters optimized in *E. coli* without major adjustments in *Marinobacter* is part of what makes it a promising chassis, as it means that previously developed genetic parts can be used in engineering its functions (Bird et al., 2022).

Taken together, the work described above indicates that *Marinobacter* species are amenable to both genome editing and carrying plasmid-based systems.

While these initial successes are promising and allow initial engineering projects to progress, there is a need for more advanced rapid manipulation and transformation tools to enable high-throughput screening and optimization of synthetic biology modules. Potentially useful techniques include recombination-mediated genetic engineering (recombineering) using the λ–red recombinase system (Datsenko & Wanner, 2000) or CRISPR-Cas (Jiang et al., 2013). While these systems have been widely used for *E. coli* and other bacteria, their application in *Marinobacter* has been limited in part due to differences in optimum growth temperature, requirement of selectable marker(s) and additional curing/excision steps. More recently, the INTEGRATE system has been developed, which combines the high efficiency, seamless integration of transposases with CRISPR-mediated targeting (Vo et al., 2021). All of these tools have the potential to increase the speed and throughput of *Marinobacter* genome engineering. This increase in efficiency may be particularly important because while some *Marinobacter* species have proven amenable to electroporation, others have not, making the transfer of DNA into the cells a potential bottleneck for which more efficient genetic techniques could compensate.

EXTRACELLULAR ELECTRON TRANSFER

The ability of a number *Marinobacter* species to oxidize and reduce metals like iron and manganese (Bonis & Gralnick, 2015; Singer et al., 2011; Wang et al., 2012) and metalloids like arsenic and sulphur (Handley et al., 2009a, 2009b; Rani et al., 2017b) has been known for years, including their ability to oxidize solid minerals (Muller et al., 2014). This ability suggests that they could be electroactive as well, since organisms that are able to oxidize or reduce solid metals can often generate current on electrodes in BES. The expectation that some *Marinobacter* species may be electroactive has been borne out in the enrichment of *Marinobacter* strains on electrodes (Erable et al., 2010; Rousseau et al., 2016; Wang, Leary, et al., 2015), and in several studies in which *Marinobacter* strains have been isolated and grown as electrotrophs from marine sediment cathode enrichments (Debuy et al., 2015; Rowe et al., 2014; Wang, Eddie, et al., 2015). Another recent study (Onderko et al., 2019) demonstrated that *M. atlanticus* CP1 could either accept or donate electrons when grown with succinate, depending on the potential of the electrode and the oxygen concentrations in the reactor. The current produced by *M. atlanticus* appears to be linked to mineral cycling, possibly iron or copper, based on evidence of differential gene expression (Eddie et al., 2021), as there are no genes known to be linked to extracellular electron transfer (EET), and no evidence of shuttle production in *M. atlanticus*. In all of these studies, the strains produced small but stable currents, with a maximum of around 0.1 mA/cm^2. Finally, genes linked to EET, though not present in *M. atlanticus*, have been found in some *Marinobacter* species (Baker et al., 2022), suggesting that these species may be adapted to growth using EET to access electron acceptors, though this has not been tested. Although many characterized strains of *Marinobacter* have not been tested for electrochemical activity, several of the sequenced and characterized strains listed in Table 1 have demonstrated or predicted EET activity or have demonstrated iron oxidation capabilities.

There are, of course, a number of bacterial species that are highly adapted to perform EET and will produce significant amounts of current (up to 1 A/m^2, and sometimes higher, depending on electrode materials and conditions) without genetic manipulation. These species, including well-studied members of the *Shewanella* and *Geobacter* genera, contain protein complexes that allow them to transport electrons from the inner membrane to the cell's exterior (for further discussion of the mechanisms of EET, the reader is referred to the many reviews on this topic, including [Shi et al., 2009; Thapa et al., 2022]). *Shewanella oneidensis* in particular has a well-defined set of EET proteins including the outer membrane Mtr complex that can be both genetically controlled in the native organism (Dundas et al., 2020) and successfully ported into *E. coli* (Jensen et al., 2016; TerAvest et al., 2014) and, more recently, into *M. atlanticus* (Bird et al., 2022). These naturally electroactive bacteria may be excellent chassis depending on the application; the best-studied species in *Shewanella* and *Geobacter* are freshwater and sediment-dwelling organisms, and *Geobacter* species are obligate anaerobes, making them unsuitable for high salt and oxygenated environments. EET gene complexes are also found naturally in the marine organism *Vibrio natriegens* (Baker et al., 2022; Conley et al., 2020). Although the EET properties of *V. natriegens* are less well studied, this species has also been investigated as a chassis (Ellis et al., 2019) and may turn out to be useful in some applications involving EET.

Paradoxically, however, high-functioning EET in a chassis can actually be a disadvantage when tight control of EET is one of the desired outcomes. Because the electrochemical activity of these organisms is so highly tuned and well integrated into the bacterium's metabolism, current production may vary due to environmental fluctuations, while a heterologously expressed system for current production can be more tightly controlled. For example, *S. oneidensis* achieves its comparatively high current density in part through the use of electron shuttles that interact with the Mtr complex to boost overall current production (Kotloski & Gralnick, 2013; Mevers et al., 2019). This can clearly be seen in media replacement

TABLE 1 Promising *Marinobacter* model chassis. Summary of the best-studied strains.

Strain	Genome	Genetic System[a]	KEGG models	Acetyl-coA acetyltransferase genes	MEP[b,c]	MVA[b,d]	Wax Ester production[a]	Electroactivity[a]	References
M. atlanticus CP1	CP011929	Y	mari	8	C	A	Y	Confirmed	Bird et al. (2018)
M. adhaerens HP15	CP001978	Y	mad	7	C	A	N	NT	Sonnenschein et al. (2011)
M. nauticus (aquaeolei) VT8	CP000514	Y	maq	5	C	A	Y	Fe oxidation	Singer et al. (2011), Lenneman et al. (2013)
M. subterrani JG233	LFBU01000000	Y	–	6	C	A	N	Fe oxidation	Bonis and Gralnick (2015)
M. nauticus ATCC49840^T	FO203363	Y	mhc	5	C	A	Y	NT	Mounier et al. (2018)
Marinobacter sp. BSs20148	CP003735	N	mbs	4	C	P	N	NT	Song et al. (2013)
M. salarius R9SW1^T	CP007152	N	msr	3	M	A	N	NT	Ng et al. (2014)
M. similis A3d10T	CP007151	N	msx	4	M	A	N	NT	Ivanova et al. (2014)
M. psychrophilus 20041^T	CP011494	N	mpq	3	C	M	N	NT	Zhang et al. (2008)
Marinobacter sp. LQ44	CP014754	N	mlq	6	C	A	N	NT	Zhou et al. (2020)
M. salinus Hb8^T	CP017715	N	msq	7	C	A	N	NT	Rani et al. (2017a)
Marinobacter sp. Arc7-DN-1	CP031848	N	mara	6	C	A	N	genetic potential	Meng et al. (2019)
Marinobacter sp. JH2	CP037934 CP037935	N	marj	6	C	A	N	NT	Hollensteiner et al. (2019)

[a]Y = yes, N = no, NT = not tested.

[b]C = complete pathway is annotated in the genome, M = most (>66%) of the pathway is annotated, P = partial pathway (33%–66%) is annotated, A = absent.

[c]2-C-methyl-D-erythritol 4-phosphate pathway.

[d]Mevalonate pathway.

experiments, where the current produced by *S. oneidensis* drops rapidly when fresh medium is used in the reactor (Kotloski & Gralnick, 2013). Such a large response to general environmental change may not be desirable in a situation where current production needs to be tuned to specific stimuli—such as in a sensor designed to change current production in response to a pollutant. In such a case, a chassis with engineered EET that does not produce soluble shuttles may be preferred even if the overall current is lower, due to the stability of having only direct EET as a current-producing mechanism. For this type of application, *Marinobacter* may be a good choice; as mentioned above, some species have a demonstrated ability to interact with the electrode in a reproducible manner, but produce low amounts of current, leaving plenty of room for an engineered increase in response to a specific stimulus, as would be needed for an engineered sensor with an electronic output. Recent work provides an example of this type of system in *M. atlanticus*: by engineering the electron transfer proteins from *S. oneidensis* into *M. atlanticus* under inducible promoters, a significant boost in current was induced by specific small molecules (Bird et al., 2022).

BIOFILM FORMATION

Like many microbial traits, the desirability of biofilm formation depends on the application. In many situations (such as in medical implants and tubing), biofilms are a nuisance to be avoided. In applications involving direct electron transfer to a solid surface, however, biofilms are vital: without physical contact, direct electron transfer cannot occur. Attempts have been made to engineer biofilm-forming capability in organisms that do it poorly—or poorly on certain surfaces—with varying levels of success (Lienemann et al., 2018; Suo et al., 2020). In *S. oneidensis*, which forms thin biofilms, increasing biofilm formation increased current production (Silva et al., 2020). Like the other traits described, biofilm formation can be native the chassis chosen or potentially engineered into a chassis.

Members of the *Marinobacter* genus are known to form biofilms at water-hydrocarbon interfaces (Ennouri et al., 2017; Klein et al., 2008) and on solid surfaces, including electrodes. Biofilm formation has been characterized in *M. atlanticus* CP1 (Phillips et al., 2020), which forms biofilms on glass as well as on carbon and indium tin oxide electrode materials, and even on gold (Yates et al., 2021), where the biofilms were shown to generate current in a nanolitre scale flow cell. Interestingly, the formation of biofilms under low nutrient conditions appears to be linked in part to the concentration of calcium in the medium; in the initial characterization of this species, artificial seawater medium with lowered calcium concentrations lead to higher planktonic growth, while the calcium levels found in seawater favoured biofilm formation (Bird et al., 2018). While this effect has not yet been tested in other *Marinobacter* species, it could prove useful in better controlling when biofilms are formed in an engineered application.

NATIVE METABOLIC CAPABILITIES

When considering applications such as living surface coatings and bioproduction (further discussed in the applications section below) the baseline metabolic capabilities of the potential chassis are an important consideration. *Marinobacter* species have several potentially useful metabolic pathways and enzymes that can be leveraged as a starting point for engineering production of a specific product. In fact, much of the early research in *Marinobacter* was centred on heterologous expression of enzymes such as wax ester and fatty acid synthases, as well as lipases and other catabolic enzymes (Table 2, and references therein). Now that better genetic tools exist, *Marinobacter*'s metabolic capabilities can be viewed through the lens of a chassis, rather than as a source of genetic parts.

Marinobacter species are particularly known for accumulating wax esters (a type of lipid) within their cells when faced with excess carbon and limited nitrogen

TABLE 2 Heterologous expression of *Marinobacter* enzymes. The halotolerance of *Marinobacter* enzymes has made them promising components in industrial applications.

Enzyme	Source	Heterologous host	References
Polyhydroxybutyrate depolymerase	*M. algicola*	*E. coli*	Martinez-Tobon et al. (2020)
Fatty acyl reductase (FAR/FAldhR)	*M. nauticus* VT8	*S. cerevisiae*	Wenning et al. (2019)
Wax synthase MhWS2	*M. nauticus* ATCC 49840	*S. cerevisiae*	Miklaszewska et al. (2018)
Fatty acyl-CoA/ACP reductase	*M. nauticus* VT8	*Synechocystis* *Rhodococcus opacus*	Kaczmarzyk et al. (2018), Lanfranconi and Alvarez (2017)
Fatty aldehyde dehydrogenase (FAldDH)	*M. nauticus* VT8	*E. coli*	Bertram et al. (2017)
Esterase LipBL	*M. lipolyticus*	*E. coli*	Perez et al. (2011)

(Alvarez, 2016). Wax esters are used in a range of industrial applications and can be further exploited for use as biofuels (Hwangbo & Chu, 2020; Tomko & Dunlop, 2015), lubricants (Domergue & Miklaszewska, 2022), pharmaceuticals (Gad et al., 2021) and cosmetics (Keng et al., 2009; Khan & Rathod, 2015). Bio-based fuels and lubricants are of increasing interest as a replacement for petrochemically derived compounds due to their lower toxicity, enhanced biodegradability and the wide range of environmental conditions within which they can operate (such as high salinity, low pH and high temperature) (Martin et al., 2021). Wax ester production in bacteria has typically focused on *Acinetobacter* species as model organisms (Fixter et al., 1986; Ishige et al., 2002; Luo et al., 2020). However, gene knockouts in *M. nauticus* VT8 (Márquez et al., 2005; Tindall, 2020) demonstrated the redundancy of certain enzymes within the wax ester synthesis pathway (Lenneman et al., 2013). This study also showed that the enzymes in *M. nauticus* have higher specific activities than the single enzymes associated with the pathway in *Acinetobacter*. In a more recent study, single gene knockouts of wax ester genes in *M. atlanticus* CP1 resulted in higher wax ester content than in wild-type strains (Bird et al., 2018), demonstrating that production of these molecules can be tuned and optimized.

In 2018, the crystal structure of a wax ester synthase, the enzyme responsible for the final step in either wax ester or triglyceride synthesis, was solved in *M. nauticus* VT8 (Mancipe et al., 2022; Petronikolou & Nair, 2018). This enzyme can accept a broad range of substrates including alcohols, diglycerides, and fatty acyl-CoAs for wax ester synthesis, and the various active sites within this enzyme were analysed in order to assess its utility as a biotechnological agent for producing high-value lipids and biofuels (Mancipe et al., 2022). As summarized in Table 2, six species of *Marinobacter* have demonstrated wax ester production (Barney et al., 2015; Holtzapple & Schmidt-Dannert, 2007; Lenneman et al., 2013; Lijewski et al., 2021; Nakano et al., 2012; Rontani et al., 2003; Wahlen et al., 2009), and *M. adhaerens* HP15 has genes for wax ester production, though it has not been tested.

While wax esters have received much attention as a broadly useful class of molecules, lipids with biosurfactant properties are also produced by *Marinobacter*. Biosurfactants have a wide range of uses in fields such as agriculture, pharmaceuticals and cosmetics and come in a variety of types, including glycolipids and phospholipids (Kumar et al., 2021). Rhamnolipids, a type of glycolipid, have been investigated in several *Marinobacter* species (Haque et al., 2020; Tripathi et al., 2019; Twigg et al., 2019). Additional research in *Marinobacter* has identified additional glycolipids (Dikit et al., 2019; Zenati et al., 2018), which may prove useful as biosurfactants.

In addition to lipid synthesis, *Marinobacter* species may be useful for production of other high-value chemicals. Most *Marinobacter* species that are present in the KEGG database (Kanehisa et al., 2020) possess the methylerythritol phosphate (MEP) pathway to generate geranyl diphosphate, one of the main branch points of terpene biosynthesis, allowing for a relatively easy entry point for engineering production of a wide variety of this valuable class of commodity and specialty chemicals. In two strains, this appears to be complemented by most of the genes necessary for the mevalonate (MVA) pathway (Table 1), which provides a second pathway to the branch point. In *E. coli* and *Synechocystis* PCC 6803, adding a heterologous MVA pathway to the endogenous MEP pathway bypassed native regulation of precursor pathways, which resulted in higher terpenoid product titres (Bentley et al., 2014; Martin et al., 2003).

Marinobacter species have a large number of acetyl-CoA acetyltransferase genes that catalyse the formation of acetoacetate from two acetyl-CoA molecules. This class of enzymes is involved in fatty acid synthesis (FadA) and polyhydroxybutanoate synthesis (PhbA) (Kanehisa et al., 2020). This innate ability to upgrade two-carbon molecules to longer chains may predispose *Marinobacter* species for a variety of anabolic biosynthetic reactions without using larger feedstock molecules such as the typical hexose-derived starting material. *Marinobacter* species are predicted to maintain a large pool of acyl-CoAs, including malonyl-CoA, which makes them good chassis organisms for reactions requiring these precursors. These pools can be further increased by deleting downstream pathways that consume them. This approach was used to create a strain that efficiently produced phloroglucinol from succinate in *M. atlanticus* CP1 (Meyer, Saaem, et al., 2019). *Marinobacter* species have also been considered as a potential production chassis for ectoine (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid), since ectoine biosynthesis is widespread in the genus (Lopes et al., 2020; Pastor et al., 2010). Ectoine is used by bacteria as an osmoprotectant and is a high value chemical in the skin care industry with cosmetic and sun protection applications (Liu et al., 2021).

Production of hydrophobic products can lead to toxicity in the production strain, but use of a strain that is already resistant would relieve this inhibition. Some *Marinobacter* species are known for their ability to attach to alkane droplets and for their resistance to alkane toxicity. Expression of hydrocarbon resistance genes, such as *yceI* from *M. nauticus* VT8 has been shown to improve resistance to pinene, a terpenoid precursor to biosynthetic jet fuel, when expressed in *E. coli* (Tomko & Dunlop, 2015). *Marinobacter* species may therefore be good candidates for producing hydrophobic compounds due to this natural resistance.

When considering potential biosynthesis systems, the required feedstock for the chassis is an important

consideration. Many *Marinobacter* species can degrade hydrocarbons and are associated with oils spills in the ocean (Bonin et al., 2015). *M. hydrocarbonoclasticus* and other *Marinobacter* strains have been shown to aerobically degrade a wide variety of hydrocarbons, including liquid and solid aliphatic and aromatic hydrocarbons, which serve as both a carbon and an energy source (Handley & Lloyd, 2013). Degradation of hydrocarbons generates a large acetyl-CoA pool. All *Marinobacter* strains examined in a recent study possessed the genes for the glyoxylate shunt (Cooper et al., 2022), which uses the acetyl-coA produced from hydrocarbon degradation to generate an extra malate molecule for each turn of the tricarboxylic acid (TCA) cycle. The generated malate could then fuel biosynthesis (Kornberg, 1966), making these species dual use for bioremediation and bioproduction. *Marinobacter* species are also equipped to degrade other insoluble biopolymers that make up potential feedstocks, like food waste, using enzymes including amylase (Kumar & Khare, 2012), protease (Masilamani & Natarajan, 2015), and cellulase (Shanmughapriya et al., 2010). Converting waste to products is one of the foundational principles of the circular bioeconomy. Halophiles like *Marinobacter* species may be beneficial for some more niche applications as well, such as treating seafood processing waste or brines from pickling and other fermented food processes, where they are found naturally (Anh et al., 2021; Chun et al., 2021; Sawada et al., 2021). The non-halotolerant bacteria traditionally used for wastewater treatment are not suitable for these applications, requiring dilution to bring salt concentrations down to tolerable levels.

The ability of *Marinobacter* species to synthesize a variety of compounds as well as to degrade hydrocarbons and thrive in high-salinity environments provides additional benefits, such as enabling point-of-need operation in extreme or remote locations, utilizing waste streams as carbon and energy sources and performing bioremediation within the environment. Additionally, the combination of electroactivity with hydrocarbon degradation (a combination some *Marinobacter* possess) has recently been considered as useful in oil-contaminated wastewater treatment (Chaudhary et al., 2022; D'Ugo et al., 2021).

APPLICATIONS

Given the traits described above, in what applications might an electroactive strain of *Marinobacter* species be a good fit? As shown in Figure 1, a few possibilities include sensors, point-of-need manufacturing, self-healing coatings and combinations of these functions. The possibility of using electrical signals as an output in a biological sensor has been considered in several reviews (Bird et al., 2021; Golitsch et al., 2013; Hassan et al., 2021). Such a system would provide the advantage of simplicity over visual outputs such as fluorescence, as well as the potential for greater sensitivity and specificity: microorganisms are often able to detect very low levels of specific molecules in their environment, making them potentially superior to traditional sensing technologies. Additionally, traditional sensors deployed in the environment are often negatively impacted by biofouling from endogenous organisms that form a biofilm on the sensor surface. Having a pre-existing biofilm colonized on the surface could serve a dual purpose as both the sensing layer and as a blocking film that prevents colonization by other organisms that may degrade sensor performance. *Marinobacter* could be well suited to this application because it naturally forms biofilms and can interact with electrodes but produces little current, leaving room for signal development against a low background.

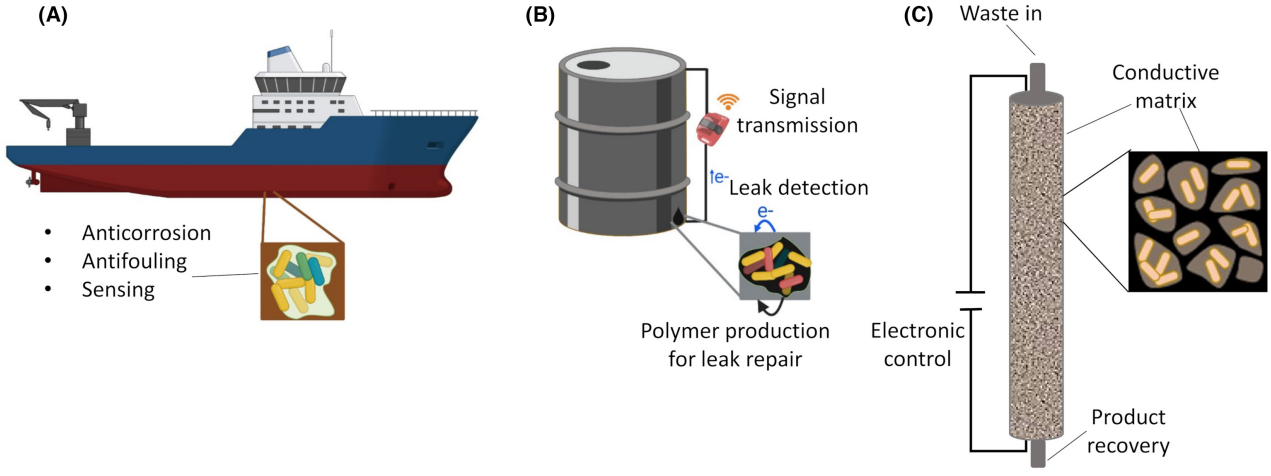

FIGURE 1 Possible applications of engineered electroactive Marinobacter. (A) As a living coating on marine surfaces to prevent corrosion and fouling and communicate electronically with the ship. (B) As living leak sensing system on storage units with repair capabilities. (C) As a low volume point of need manufacturing system converting waste to valuable product.

Biomanufacturing has also received considerable interest in recent years. Traditional biomanufacturing requires large amounts of potable water and aseptic conditions to generate products at titres that are economically viable. Additionally, traditional biomanufacturing processes utilize sugar-based feedstocks, which could otherwise be used by the food industry, creating a competitive dynamic between food and chemical industries. Research and development into processes based on non-traditional microbial species for biomanufacturing would circumvent many of these issues. Using a halophilic organism, such as *Marinobacter*, as a biomanufacturing strain would enable non-potable water to be used as the fermentation matrix (Yue et al., 2014). As manufacturing-scale batch fermentation processes using yeast or bacteria consume 30,000–250,000 L of water per batch (Meyer et al., 2017), developing biomanufacturing processes that use non-potable water could enable biomanufacturing in regions that are stressed for sources of potable water. Furthermore, using a halophilic bioproduction strain would preclude the growth of many organisms that could potentially contaminate a fermentation run, alleviating the need for equipment and processes to operate fermenters under stringent aseptic conditions. The wide range of substrates that *Marinobacter* is able to consume is also of interest for biomanufacturing. The ability to degrade non-traditional feedstocks to generate useful products would enable the capture and transformation of carbon derived from traditional waste streams into valuable materials. Incorporation of novel functions via synthetic biology only further enhances the viability of *Marinobacter* as a biomanufacturing strain. For example, controlling EET via synthetic biology could enable electrofermentation (Gong et al., 2020).

Electrofermentation is an underexplored microbial electrochemical process that allows for unbalanced fermentation reactions to occur by using an electrode that can act as a source or sink of electrons, depending on the product of interest (Moscoviz et al., 2016). In electrofermentation systems, unlike other microbial electrochemical technologies, current production is not the goal, and the electrode is not the main electron source or sink in the system. Instead, the goal is to shift the end-products of the fermentation toward a more desired product (Rabaey & Rozendal, 2010; Virdis et al., 2022). An example of the utility of electrofermentation can be found from the conversion of glycerol to ethanol by *Shewanella oneidensis*, a metabolic process made possible by transferring two electrons to an electrode (Flynn et al., 2010). We envision that using an EET-enabled *Marinobacter* species in a BES could be used to alter product profiles or modulate cell activity in a biomanufacturing context. In this use case, a *Marinobacter* strain that produces higher current densities would be desirable; this could be achieved either by screening the strains known to have multiheme cytochromes and/or metal-reducing capabilities (Table 1) for high electroactivity, or by engineering a strain with lower current production to be more electroactive, as described above. This is a field ripe with opportunities for further exploration.

Marinobacter species' ability to form biofilms, combined with their production of useful lipid molecules, paves the way for research and development into novel bioproduction processes based on immobilized whole-cell catalysis, such as packed bed bioreactors (Glaven et al., 2021). Similar to catalysis using immobilized enzymes (DiCosimo et al., 2013), fermentation by immobilized whole cells has the potential to improve production efficiency by enabling continuous fermentations, which is difficult for traditional batch fermentations due to loss of bacterial cells during continuous operation. Bioproduction processes based on immobilized whole cells have been explored for cells that are trapped in a synthetic matrix (Casali et al., 2012; Kumar & Chandrasekaran, 2003; Muffler et al., 2014) and produce highly soluble products but are only just beginning to be explored for cells that naturally form immobilized layers and produce longer chain hydrophobic products. The use of a biofilm also allows decoupling of growth from product formation, allowing a larger proportion of input substrate to go toward the product rather than additional biomass. In an ideal scenario, no growth would occur after the initial colonization, and the cells that are present would act solely as catalysts to convert raw materials into the desired product. Several strategies are used for obtaining products from solid-state fermentation processes which could be adapted here, including solvent or salt extraction (as suggested in [Glaven et al., 2021]), or export of soluble products (Fernández-Lahore et al., 1998). It may also be possible in the future, using synthetic biology, to genetically program cells to release intracellular compounds (Liu et al., 2011) as well as to boost concentrations of the desired products.

The ability of *Marinobacter* to form biofilms on electrodes also raises the intriguing possibility of utilizing conductive materials to construct the packed bed. As mentioned above, electrofermentation is a potentially useful mechanism for directing and increasing yields of useful molecules. The use of conductive materials as the support matrix in a bioreactor could provide another mechanism to control the metabolic processes occurring in the biofilm, either to control bioproduction through electrical signals or to trigger periodic cell release and lysis for collection of the products.

Finally, self-healing surface coatings have considerable potential, especially when combined with the two applications described above. A living biofilm on a ship or vehicle could reduce biofouling and fight corrosion, as has been shown for biomineralization by *Pseudoalteromonas lipolytica* (Liu et al., 2018). Living films could also be engineered to sense contaminants

and signals in the air or water. Such a film, if combined with EET capabilities, could communicate with the vehicle's operators through electrical signals transmitted through the vehicle's surface. Biosynthesis capabilities could also be useful: for example, an electroactive biofilm on the surface of a fuel tank could detect a small leak, send an electronic signal to alert the operators and convert some of the leaking fuel into a polymer that would heal the leak and prevent further damage. In this example, a *Marinobacter* species could have two of the four requirements for the chassis: it forms natural biofilms and can degrade hydrocarbons, while the signalling and polymer synthesis aspects would have to be engineered.

Among the many species of *Marinobacter*, there is also the question of which one is best suited for development as a chassis. Table 1 lists the most promising and best-studied *Marinobacter* strains. All have annotated genomes and can be explored using the KEGG database. Depending on the production pathways required, various strains might be chosen; for example, *M. atlanticus* and *M. adhaerens* have abundant acetyl-coA acetyltransferase genes, making them particularly useful for the synthesis of fatty acid derivatives including wax esters. Thus far, five strains in the table have proven amenable to genetic manipulation; however, it is likely that other strains and species are as well, as they have not yet been tested. Electrochemical activity is another trait that has not been characterized in most species: *M. atlanticus* is the best studied in this regard and is a good candidate for applications that require biofilm formation on electrodes with low-background current. If greater electron flow is required, strains such as Arc7-DN-1, which have multiheme cytochromes (Baker et al., 2022; Meng et al., 2019), warrant further exploration.

CONCLUSIONS

Synthetic biology opens the door to new application spaces, especially when combined with bioelectrochemical systems. Possibilities include living sensors, electrobioremediation, electrobiomanufacturing and living coatings for protection and self-repair of surfaces. Successful and streamlined development of these technologies will require proper selection of the chassis. *Marinobacter* species have a number of characteristics that make them potentially useful for certain applications: (1) they are halophilic, making them good candidates for marine and briny environments. (2) They can degrade hydrocarbons, making them a good fit for polluted substrates. (3) At least some species can colonize and interact with electrodes yet produce low amounts of current. This makes them primed for engineered current production with a low background. (4) Multiple species have at least a basic genetic system developed (Table 1). (5) They form biofilms, making them candidates for biomanufacturing in packed bed reactors and living surface coatings. (6) They have pathways for producing multiple useful lipid types, as well as resistance to hydrophobic compounds, making them a good candidate for bioproduction. Despite these advantages, there are difficulties in using *Marinobacter* species as chassis: engineering in many species is still slow, and there remain many gaps in our knowledge of the species' metabolisms, especially regarding electrochemical activity. As these issues are remedied, *Marinobacter* will become useful chassis within the parameters outlined above.

AUTHOR CONTRIBUTIONS

Lina J Bird: Conceptualization (lead); writing – original draft (equal); writing – review and editing (equal). **Rebecca Mickol:** Writing – original draft (equal); writing – review and editing (equal). **Brian Eddie:** Writing – original draft (equal); writing – review and editing (equal). **Meghna Thakur:** Writing – original draft (equal); writing – review and editing (equal). **Matthew D Yates:** Writing – original draft (equal); writing – review and editing (equal). **Sarah Glaven:** Conceptualization (equal); funding acquisition (lead); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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