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EPS bound flavins driven mediated electron transfer in thermophilic *Geobacillus* sp.

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ABSTRACT

Through extracellular electron transfer (EET), bacteria are capable of transforming different insoluble materials of geochemical interest into energy-rich molecules for their growth. For this process, bacteria have been depending directly or indirectly on molecules synthesized within the cells or by various synthetics as mediators. Herein, we studied the in-situ change in electrochemistry and supporting components for EET in the extracellular polysaccharide (EPS) producing biofilm of thermophilic *Geobacillus* sp. The CV and DPV resultsrevealed that the intact biofilm of bacteria was not able to generate any potential at 25 °C /- ≤50 °C. However, at 55 °C (optimal condition), the potential occurred drastically after the EPS production by bacteria. HPLC and MALDI-TOF results revealed that the presence of Flavins, which can able adsorbed to the electrodes from the cell surface. Moreover, the temperature-dependent EPS production and originally conceived ability of flavins to act as electron shuttles suggest that not much complexity in bacteria with minerals. Additionally, the electrochemical potential was severely affected upon removal of EPS/flavin moiety from the intact biofilm, revealed the necessity of EPS bound flavins in transferring the electrons across its thick cell walls. This paradigm shift to electrogenic nature of *Geobacillus* sp. biofilm will become evident in the adaptation of other microbes during mineral respiration in extreme environments.

1. Introduction

Flavins, an active redox compounds, have been reported to participate in extracellular electron transfer (EET) by bacteria (Marsili et al., 2008; von Canstein et al., 2008; Masuda et al., 2010; Kotloski and Gralnick, 2013; Engel et al., 2019). Although, flavins are produced endogenously by bacteria (Yatsyshyn et al., 2009), and are located at a specific locus in its genome (Light et al., 2018). These are required to cross and interact with the transmembrane barriers for energy derivations (Covington et al., 2010; Babanova et al., 2017). Despite, behaves distinctly in the electrochemical environment, the flavins precursors are essential for biogeochemistry (Shi et al., 2013), minerals (von Canstein et al., 2008), and electrode respiration (Yang et al., 2012). Till date, many reports available exclusive for *Shewanella* spp. (Marsili et al., 2008; von Canstein et al., 2008; Kotloski and Gralnick, 2013), and *Geobacter* spp. (Reguera et al., 2005; Okamoto et al., 2014), which is inherited the EET research. Although, many other bacteria have been characterized for EET activity so far (Pham et al., 2008; Yang et al., 2012; Dopson et al., 2016), however, the arguments unlikely differed on the gram (+) profile of the bacteria. These bacteria are widespread and are likely to execute in a different EET mechanism. Until recently, there are several studies are documented the EET mechanism in these type of bacteria (Marshall and May, 2009; Wrighton et al., 2011; Wu et al., 2014; Tokunou et al., 2016; Pankratova et al., 2019). Furthermore, their presence in the electrochemical system was extensively documented previously (Logan, 2009; Marshall and May, 2009; Dopson

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et al., 2016). More recently, riboflavin mediated extracellular metal reduction was demonstrated *clostridium*-dominated microbial community (Fuller et al., 2014). Such a self-mediating ET process was shown for an anodic microbial consortium, which is widely distrubuted in *Bacillus*, *Clostridium*, *Enterococccus*, *Eubacterium*, *Lactobacillus*, and *Lactococcus* species (Rabaey et al., 2004; Wu et al., 2014; Pankratova et al., 2018, 2019).

Unlike mesophiles, some of the gram-positive thermophiles surviving at high temperature makes it probable that they use alternative mechanism(s) for EET. Currently, MHC pathway from *Thermincola potens* is a widely accepted model for thermophilic EET (Carlson et al., 2012; Lusk, 2019). However, this mechanism does not explain the direct long-range EET in *T. ferriacetica*, because, it does not transfer of a electrons through the extracellular matrix (Parameswaran et al., 2013; Lusk et al., 2015a, b; Lusk et al., 2016). In contrast, some microbial energy and activity are deeply compromised with its environmental conditions. Also, under extreme conditions, the Gibbs free energy is always dependent on temperature, pressure, concentrations/activity; and pH of the electrolyte compared to normal conditions (Dopson et al., 2016). Hence, with only some resources for extreme conditions, it is difficult to establish a model system for these bacteria. Furthermore, a thick surface polysaccharide (EPS) around cells makes a structural difference which hinders the transfer of redox components to insoluble electron acceptors to the outer environment. It is known that the refractory polymers of the EPSs mainly cause redox behavior in the biofilm important for biogeochemical cyclic and organic pollutant (Flemming et al., 2007; Li et al., 2016). Recently, it was shown that the EETs in both gram profile bacteria and a yeast renders negligible as a core mechanism depends on EPSs activity (Xiao et al., 2017).

This study on a Gram-positive thermophilic *Geobacillus* sp. was investigated for their EET mechanism. The electrochemical behavior of the bacterium was found similar to previously reported bacteria. Moreover, the potential was generated mainly because of the endogenous flavins moiety that is bound to the EPS of the bacteria. Besides, the temperature-driven flavin stability has been found significant demerit for its electroactivity. This model of EET mechanism in thermophilic or extremophilic bacteria will open up the mechanistic understanding in the near future.

2. Experimental section

2.1. Bacterial electrochemistry

Thermophilic gram-positive *Geobacillus* sp. Iso5 was used in the current studies was isolated previously from thermal springs of southern India (Mahadevan and Neelagund, 2012). Prior electrochemical analysis, the seed and subsequent culturing were performed at 55 °C for 24 h in yeast extract free (Masuda et al., 2010), and all vitamins eliminated PBTA medium [contained (grams per liter): peptone (0.2%), beef extract (0.5 g), tryptone (0.1 g), and NaCl (2%)]. To ensure, no flavin interference from the medium, the uncultivated bacterial free medium was filtered through 0.22 µm membrane filters with subsequently analyzed by HPLC as previously described (Wu et al., 2014).

Molecular grade standards of riboflavin, FMN, and FAD were purchased from Sigma (Sigma Inc., USA). For *Geobacillus*, a modified three electrodes mini-three electrodes glass cell fitted with temperature control (up to 55 °C) system was connected to CHI 660D (*sc.*10 mV s^{-1}) cyclic voltammetry (CH Instruments, Texas, USA). Silica polished glassy carbon electrode, a platinum wire, and a saturated Ag/AgCl (3 mol L^{-1}KCl) were used as the working (WE), counter and reference electrodes (RE), respectively. All the potentials presented below are vs. the saturated Ag/AgCl in PBS 10 mmol L^{-1}, pH 8 at 55 °C under N_2-CO_2 (80:20, vol/vol) atmosphere. For DPV study, in the oxidation reactions, the staircase was 0.004 V, the initial potential was E_i -0.60 V, and the final potential was E_f-0.20 V (Wu et al., 2014). Bacterial cells and supernatant were saved after centrifugation at 5000 × *g* for 20 min at 4 °C; later collected cells were then washed thrice with the electrolyte. The cell suspension (~ 5 µl) was drop-casted with one µl of 1% Nafion on the GC electrode, and potential was recorded under dark. The supernatant on the other hand, replaces as electrolyte and electrode potential was recorded as above. For control, PBS dissolved standards were then filtered through 0.22 µm membrane filters.

2.2. EPS extraction and removal

EPS from cells were isolated by EDTA method, as previously described with slight modification (Liu and Fang, 2002; Cao et al., 2011; Li et al., 2016). In precise, the cells suspensions obtained after centrifugation at 5000 x g for 5 min were washed thrice with saline followed by resuspension in an equal volume of 2% Na_2-EDTA prepared in saline solution (pH 8.0). The resuspended mixture was heated to 55 °C in a water bath for 15 min and kept undisturbed for twenty-four hours at 4 °C. The supernatant obtained after centrifugation for 20 min at 3000 × g at 4 °C was filtered through a 0.22 µm membrane filter. Cells pellet were separately collected after centrifugation to study morphology and electrochemistry.

Furthermore, filtered EPS was dialyzed by a cellulose membrane (MW:1kDa) using the same 2% Na_2-EDTA buffer at 4 °C. The dialysate was further centrifuged for 20 min at 3000 × g at 4 °C to collect the supernatant. EPS fractions from the supernatant were then lyophilized and stored at −20 °C for use. Cell surface and microbial cell integrity analysis before and after EPS removal was imaged using Phase contrast microscope (Olympus, Japan) and scanning electron microscope (SIRION200, FEI Co., Netherlands).

2.3. Analytical methods

Proteins were concentrated from EPS by ammonium sulfate method (Bradford, 1976; Mahadevan and Neelagund, 2012). All the optical measurements were recorded in UV 1200 spectrophotometer (MAPADA, China). Broadford kit (Sangam Biotechnologies, China) was used for protein assay. The previously prescribed method was followed for HPLC measurements (Agilent, USA) (Wu et al., 2014). For UPLC-QTOF/MS analysis, an ACQUITY BEH C_{18} (1.7 µm, 50 mm) reverse phase column (Waters, MA) and fluorescence detector (Waters) was used with an excitation wavelength of 440 nm and an emission wavelength of 525 nm. The column wash was performed with 200 µL of strong washing (water/acetonitrile, 1:1) followed by 600 µL weak wash (water: acetonitrile, 95:5). The linear gradient separation was performed with ethanol:water (50:50) mobile phase at the flow rate of 1 ml/min at 40 °C with the injections of 2 µL samples and 200 µL of strong wash (water:acetonitrile, 1:1) followed by 600 µL weak wash (water/ acetonitrile, 95:5). Eluted compounds were analyzed by MS using a Thermo Electron LCQ Ion Trap spectrometer (Thermo Scientific, USA) in a positive ion mode. Alternatively, the molecular weight of pure (FAD, FMN, Riboflavin) and sample flavins was determined in Ultraflex MALDI-TOF/MS (Bruker, Germany) equipped with a nitrogen laser (337 nm). A series of 1–10 nM diluted samples were mixed in 1:1 matrix (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile /water with 0.1% TFA acid). The one µl mixture was placed on a MALDI target plate, and the spectrum was recorded in the linear positive ion mode. The spectral data were processed by Bruker Daltonics FLEX analysis software (version 2.0).

3. Results and discussion

Compared to *Shewanella* spp. (Marsili et al., 2008; von Canstein et al., 2008; Kotloski and Gralnick, 2013; Wu et al., 2014), *Geobacter* (Reguera et al., 2005; Rotaru et al., 2014; Fernandes et al., 2017), and other gram-positive EET models (Pham et al., 2008; Wrighton et al., 2011; Wu et al., 2014), only a limited resources available for thermophiles (Marshall and May, 2009; Lusk, 2019). Despite, several debacles

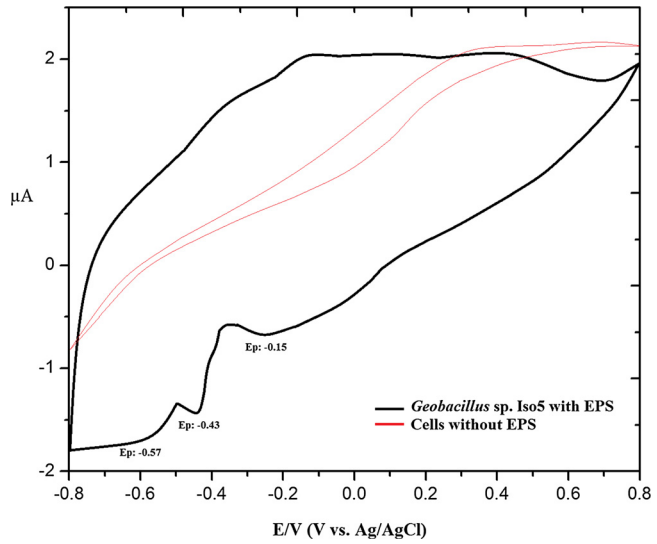

Fig. 1. Cyclic voltammetry of *Geobacillus* sp. Iso5 (Black solid line) during EPS production and after removal of EPS (red line). The scan was recorded at 55 °C in 10 mM PBS, pH 8.0 as a control. The results were verified thrice to the objectives under the same condition.

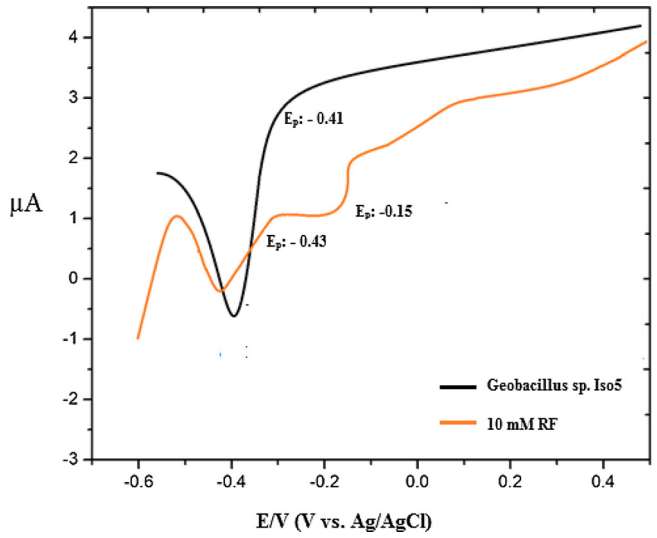

Fig. 2. Differential Pulse Voltammetry (DPV) of *Geobacillus* sp. Iso5 (Black solid line) during EPS production and standard riboflavin (red line). The scan was recorded at 55 °C in 10 mM PBS, pH 8.0 as a control. Results were verified thrice to the objectives under the same condition.

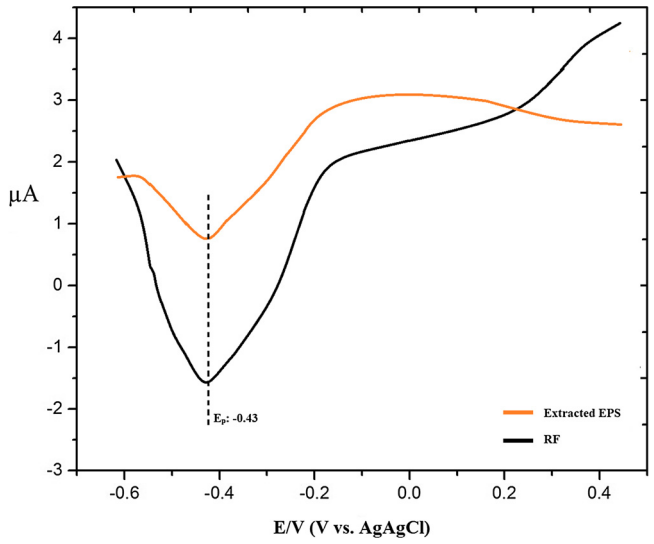

Fig. 4. DPV of extracted EPS from *Geobacillus* sp. Iso5 (red line) was compared with the standard RF (Black solid line). The potential was exactly at the position was observed at 55 °C in 10 mM PBS, pH 8.0 as a control. Results are verified in triplicates.

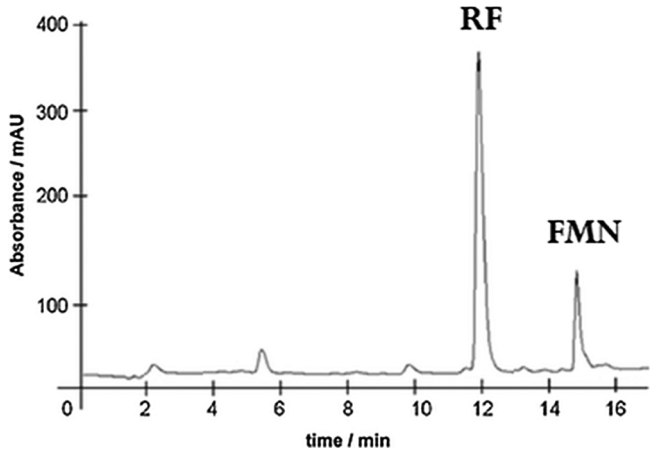

Fig. 5. HPLC elution pattern of EPS fraction showing two peaks corresponds to the riboflavin and FMN at 12 and 15 min, respectively.

over the use of thermophiles in MFCs, many thermophilic microbial groups have been mostly neglected. Not all known thermophiles are electrogens, but, some interest in EET mechanism is currently under research (Wrighton et al., 2011; Carlson et al., 2012; Shi et al., 2016). In this communication, we determined the mechanism of electrical

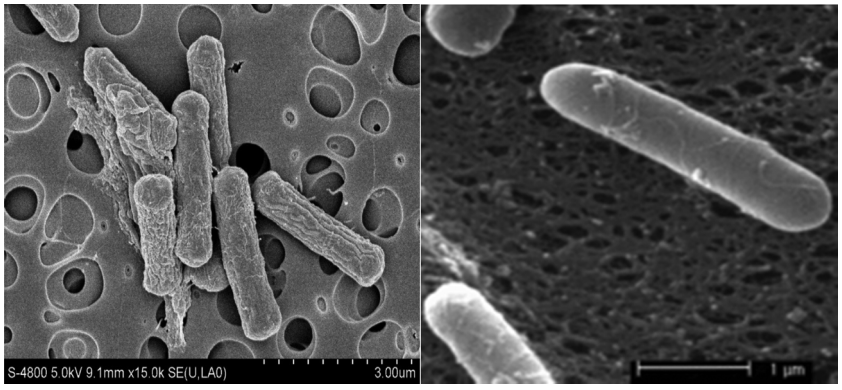

Fig. 3. Scanning electron micrograph of *Geobacillus* sp. Iso5 cells: (a) with EPS layer; (b) after EPS removal with the modified method.

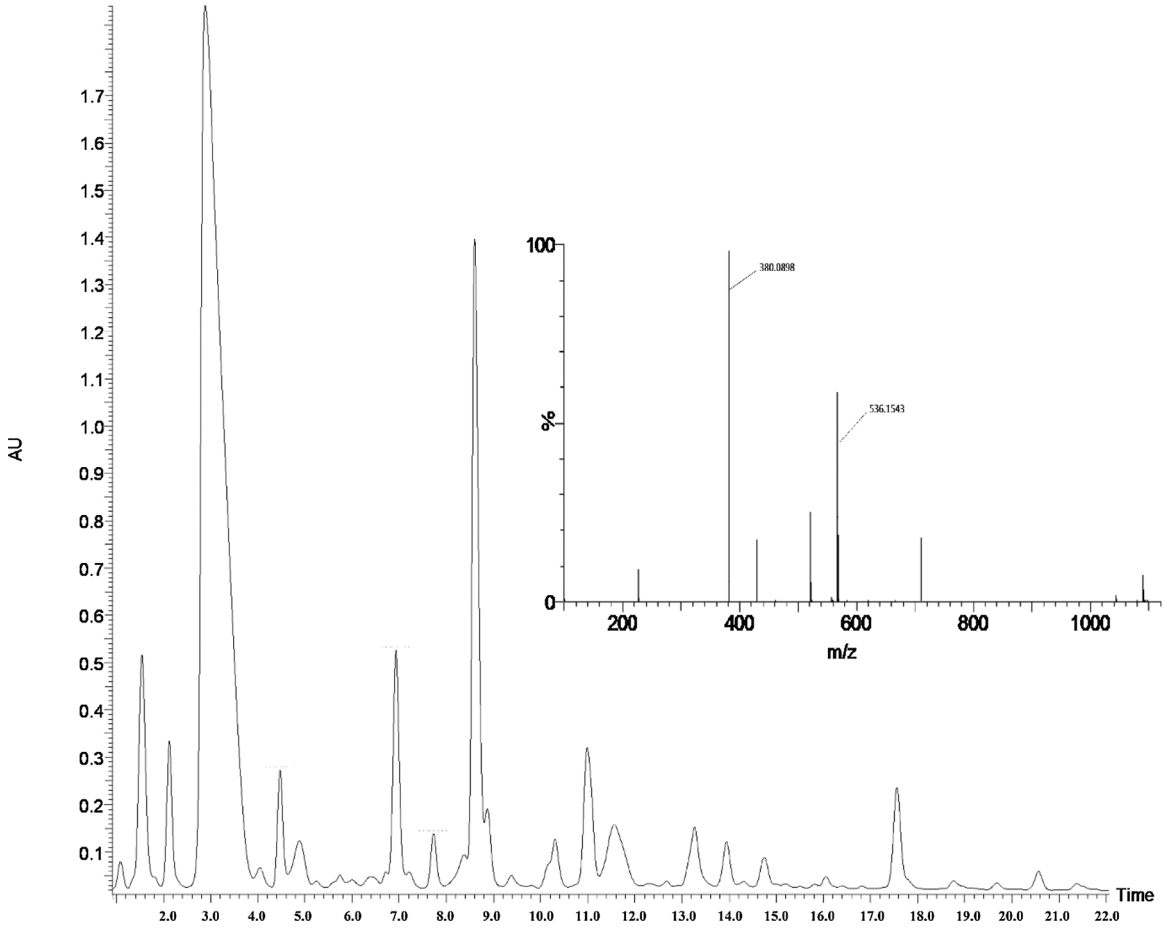

Fig. 6. UPLC spectrum of the EPS extracts from *Geoabcillus* sp. Iso5 showing elution peak for RF at 9 min and FMN at 18 min, respectively. Insert represents the *m/z* value of the elutents from UPLC combined, proving the presence of RF and FMN with its respective molecular mass. The parameters used for analysis: Methanol: water (50:50) mobile phase at the flow rate of 1 ml/min at 40 °C with the injections of 2 µL samples. Prior, the analysis was 200 µL of strong wash (water/ acetonitrile,1:1) followed by 600 µL weak wash (water/ acetonitrile,95:5).

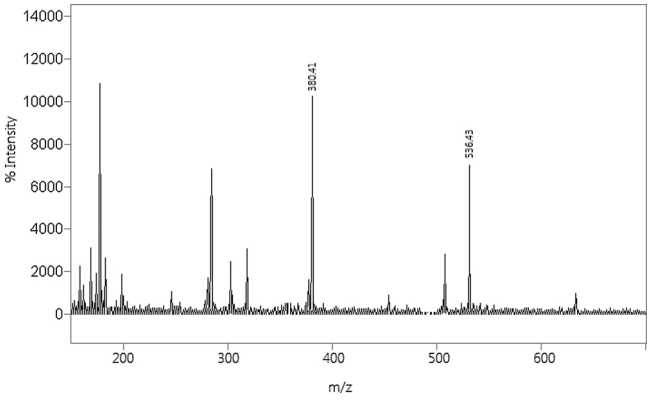

Fig. 7. Ionization of elutents by matrix-assisted laser desorption/ionization (MALDI) spectra for combine elutents showing *m/z* 380 and 536 for RF and FMN, respectively.

properties and factors that influence the EET in thermophilic gram-positive *Geobacillus* sp. biofilm. Provided the redox peaks (Fig. 1) which gave the first impression of electrochemical activity by thermophilic gram-positive *Geobacillus* sp. intact biofilm. However, Riboflavin yielded a much weak redox signal at -0.43 V, as compared to *Bacillus* sp. WS-—XY1 and yeast *P. stipitis* (Wu et al., 2014). These weak signals are of the process degradation rate may be high for flavins during CV analysis at 55 °C and also due to the Gibbs free energy changes under extreme conditions (Dopson et al., 2016). A small shift in potential for pure riboflavin (-0.41 ± 0.01) was seen, however, maybe because of the slightly alkaline pH medium and structural changes at a higher temperature. There are two other prominent redox peaks at -0.15 V and -0.57 V, which attributed the presence of *b* and *c*- cytochrome, respectively (Carmona-Martinez et al., 2011; Xiao et al., 2017) was omitted to flavin counterpart. DPV signals, on the other hand, a firmly visible peak at -0.43 V similar to WS-XY1 (Wu et al., 2014), and at -0.15 (omitted) which signifies the weak diffusion of riboflavin around the surfaces of the cells (Fig. 2)

In *Shewanella* spp. (Marsili et al., 2008; von Canstein et al., 2008) and *Bacillus* sp. (Wu et al., 2014), flavins (primarily, riboflavin) activity was found in supernatants. Genetically, not all bacteria were capable of using intercellular flavins for the respiration outside the cells (Yatsyshyn et al., 2009). However, under favored condition, it is not always true, which was similar in the case of *Geobacillus* sp. and standards (except at RT) at 55 °C (data not shown). This might be due to thick cell wall encased in a glycoprotein S-layer, and dense EPS hinders the movement of flavins out of the cell (Ehrlich, 2008; Pham et al., 2008; Wrighton et al., 2011). Furthermore, redox signals were completely distorted when surface polymeric substances (EPS) was exenterated from cells (Fig. 3). DPV also confirmed that the absence of these redox peaks, hypothesize the flavins in EPS is a significant factor for the electrochemical activity of *Geobacillus* sp. Moreover, it was further verified by exposing the extracted EPS to the electrode surface for CVs, yielding a small unshifted redox peak at -0.43 V which was similar to WS XY-1 (Xiao et al., 2017), and undepleted cells (Fig. 4).

The EPS synthesis vs. growth confirms that the mechanistic model of EET was monitored at a regular interval of time (data not shown). These results suggested that the cells of *Geobacillus* sp. alone cannot be able to generate any potential at initial growth without EPS synthesis. However, its potential was shortly restored at stationary growth and continued to be present late twenty-four hours from initial declination phase. A similar method of optical measurements for redox moiety in EPS was also confirmed decoloration of the azo dye Direct Blue 53 (Pearce et al., 2006; von Canstein et al., 2008). These results showed the presence of diffusible redox compounds in the EPS, but, the dye decoloration was slightly slower than *Shewanella* sp. previously reported (von Canstein et al., 2008), because of the lower Flavin content in the extracted sample. A quick faint two yellow eluate form HPLC revealing redox-active flavin separation at 12 min and 15 min for riboflavin and FMN, respectively (Fig. 5). Similar elution pattern was also observed to the pure flavins. In comparison, HPLC fraction from previously reported *Shewanella* strain was found to beat 28 min and 33 min for FMN and riboflavin, respectively at 35% methanol (von Canstein et al., 2008). Besides, two identical redox mediators were also confirmed by UPLC-MS, giving identical molecular weights (m/z) of 536.4 for the 9-minute fraction corresponding to FMN and of 380 for the 18-minute fraction corresponding to riboflavin (Fig. 6). Furthermore, results of MALDI TOF/MS showed the presence of low-intensity prominent peak for FMN at m/z 536.4 kDa and relatively high for riboflavin at 380.4 kDa (Fig. 7). These results indicated that the diffusible riboflavin might dominant in the electrogenic activity of *Geobacillus* sp. which is uncovered previously in thermophiles.

4. Conclusion

Though many of the thermophiles were largely uncovered and their metabolic processes in nature are unresolved. However, energy-yielding redox reactions by these microorganisms with a multitude of electron donors and acceptors arequite common in nature. Herein, a gram-positive thermophilic bacterium *Geobacillus* sp. Iso5 was exposed to the electrochemical system with its mesophilic *Bacillus* counterpart. The results indicated that the common analogy for EET mechanism as mesophiles were adopted even at the elevated temperate. It was determined that EPS was an important factor for respiration extracellularly in *Geobacillus* sp., which is uncommon in other bacteria. However, further observation of EPS depleted cells resulted in barely any potential even with the same condition applied. These factors of EPS, it was believed that some of the redox moiety probably involved in generating the potential. Furthermore, detailed analysis using UPLC and HPLC was shown in favor of flavins, which is ubiquitous proteins of redox property, which was known; mediate the EET in another bacterium. Hence, from this research, we believed that for EET, a common respiratory analogy exists in these bacteria irrespective of its habitats.

Declaration of Competing Interest

The authors ensure no conflict of interest exist

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References

Babanova, S., Matanovic, I., Cornejo, J., Bretschger, O., Nealson, K., Atanassov, P., 2017. Outer membrane cytochromes/flavin interactions in *Shewanella* spp.—a molecular perspective. Biointerphases 12 (2), 021004.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72 (1–2), 248–254.

Cao, B., Shi, L., Brown, R.N., Xiong, Y., Fredrickson, J.K., Romine, M.F., Marshall, M.J., Lipton, M.S., Beyenal, H., 2011. Extracellular polymeric substances from *Shewanella* sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics. Environ. Microbiol. 13 (4), 1018–1031.

Carlson, H.K., Iavarone, A.T., Gorur, A., Yeo, B.S., Tran, R., Melnyk, R.A., Mathies, R.A., Auer, M., Coates, J.D., 2012. Surface multiheme c-type cytochromes from *Thermincola potens* and implications for respiratory metal reduction by Gram-positive bacteria. Proc. Natl. Acad. Sci. U.S.A. 109 (5), 1702–1707.

Carmona-Martinez, A.A., Harnisch, F., Fitzgerald, L.A., Biffinger, J.C., Ringeisen, B.R., Schröder, U., 2011. Cyclic voltammetric analysis of the electron transfer of *Shewanella oneidensis* MR-1 and nanofilament and cytochrome knock-out mutants. Bioelectrochemistry 81 (2), 74–80.

Covington, E.D., Gelbmann, C.B., Kotloski, N.J., Gralnick, J.A., 2010. An essential role for UshA in processing of extracellular flavin electron shuttles by *Shewanella oneidensis*. Mol. Microbiol. 78 (2), 519–532.

Dopson, M., Ni, G., Sleutels, T.H.J.A., 2016. Possibilities for extremophilic microorganisms in microbial electrochemical systems. FEMS Microbiol. Rev. 40 (2), 164–181.

Ehrlich, H.L., 2008. Are gram-positive bacteria capable of electron transfer across their cell wall without an externally available electron shuttle? Geobiology 6 (3), 220–224.

Engel, M., Bayer, H., Holtmann, D., Tippkötter, N., Ulber, R., 2019. Flavin secretion of *Clostridium acetobutylicum* in a bioelectrochemical system - is an iron limitation involved? Bioelectrochemistry 129, 242–250.

Fernandes, A.P., Nunes, T.C., Paquete, C.M., Salgueiro, C.A., 2017. Interaction studies between periplasmic cytochromes provide insights into extracellular electron transfer pathways of *Geobacter sulfurreducens*. Biochem. J. 474 (5), 797–808.

Flemming, H.C., Neu, T.R., Wozniak, D.J., 2007. The EPS matrix: the “house of biofilm cells”. J. Bacteriol. 189 (22), 7945–7947.

Fuller, S.J., McMillan, D.G.G., Renz, M.B., Schmidt, M., Burke, I.T., Stewart, D.I., 2014. Extracellular electron transport-mediated Fe(III) reduction by a community of alkaliphilic bacteria that use flavins as electron shuttles. Appl. Environ. Microbiol. 80 (1), 128–137.

Kotloski, N.J., Gralnick, J.A., 2013. Flavin electron shuttles dominate extracellular electron transfer by *Shewanella oneidensis*. mBio 4 (1).

Li, S.W., Sheng, G.P., Cheng, Y.Y., Yu, H.Q., 2016. Redox properties of extracellular polymeric substances (EPS) from electroactive bacteria. Sci. Rep. 6, 39098.

Light, S.H., Su, L., Rivera-Lugo, R., Cornejo, J.A., Louie, A., Iavarone, A.T., Ajo-Franklin, C.M., Portnoy, D.A., 2018. A flavin-based extracellular electron transfer mechanism in diverse Gram-positive bacteria. Nature 562 (7725), 140–144.

Liu, H., Fang, H.H.P., 2002. Extraction of extracellular polymeric substances (EPS) of sludges. J Biotech 95 (3), 249–256.

Logan, B.E., 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nat. Rev. Micro 7 (5), 375–381.

Lusk, B.G., 2019. Thermophiles; or, the Modern Prometheus: the importance of extreme microorganisms for understanding and applying extracellular electron transfer. Front. Microbiol. 10 (818).

Lusk, B.G., Badalamenti, J.P., Parameswaran, P., Bond, D.R., Torres, C.I., 2015a. Draft genome sequence of the gram-positive thermophilic iron reducer *Thermincola ferriacetica* strain Z-0001T. Genome Announc. 3 (5), e01072–15.

Lusk, B.G., Parameswaran, P., Popat, S.C., Rittmann, B.E., Torres, C.I., 2016. The effect of pH and buffer concentration on anode biofilms of *Thermincola ferriacetica*. Bioelectrochemistry 112, 47–52.

Lusk, B.G., Khan, Q.F., Parameswaran, P., Hameed, A., Ali, N., Rittmann, B.E., Torres, C.I., 2015b. Characterization of Electrical current-generation capabilities from thermophilic bacterium *Thermoanaerobacter pseudethanolicus* using xylose, glucose, cellobiose, or acetate with fixed anode potentials. Environ. Sci. Technol. 49 (24), 14725–14731.

Mahadevan, G.D., Neelagund, E.S., 2012. Molecular characterization of industrially viable hyperthermostable α-amylase from thermoalklophilic *Geobacillus* sp. Iso5 isolated from geothermal spring. J. Pure Appl. Microbiol. 6 (4), 1759–1773.

Marshall, C.W., May, H.D., 2009. Electrochemical evidence of direct electrode reduction by a thermophilic Gram-positive bacterium, *Thermincola ferriacetica*. Synth. Lect. Energy Environ. Technol. Sci. Soc. 2 (6), 699–705.

Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A., Bond, D.R., 2008. Shewanella secretes flavins that mediate extracellular electron transfer. Proc. Natl. Acad. Sci. U.S.A. 105 (10), 3968–3973.

Masuda, M., Freguia, S., Tsujimura, S., Kano, K., 2010. Flavins contained in yeast extract are exploited for anodic electron transfer by *Lactococcus lactis*. Bioelectrochemistry 78 (2), 173–175.

Okamoto, A., Saito, K., Inoue, K., Nealson, K.H., Hashimoto, K., Nakamura, R., 2014. Uptake of self-secreted flavins as bound cofactors for extracellular electron transfer in *Geobacter species*. Synth. Lect. Energy Environ. Technol. Sci. Soc. 7 (4), 1357–1361.

Pankratova, G., Hederstedt, L., Gorton, L., 2019. Extracellular electron transfer features of Gram-positive bacteria. Anal. Chim. Acta 1076, 32–47.

Pankratova, G., Leech, D., Gorton, L., 2018. Hederstedt L. Extracellular electron transfer by the gram-positive bacterium *Enterococcus faecalis*. Biochemistry 57 (30), 4597–4603.

Parameswaran, P., Bry, T., Popat, S.C., Lusk, B.G., Rittmann, B.E., 2013. Torres CI. Kinetic, Electrochemical, and microscopic characterization of the thermophilic, anode-respiring bacterium *Thermincola ferriacetica*. Environ. Sci. Technol. 47 (9), 4934–4940.

Pearce, C.I., Christie, R., Boothman, C., von Canstein, H., Guthrie, J.T., Lloyd, J.R., 2006. Reactive azo dye reduction by *Shewanella* strain J18 143. Biotechnol. Bioeng. 95 (4), 692–703.

Pham, T.H., Boon, N., Aelterman, P., Clauwaert, P., De Schamphelaire, L., Vanhaecke, L., De Maeyer, K., Höfte, M., Verstraete, W., Rabaey, K., 2008. Metabolites produced by *Pseudomonas* sp. Enable a Gram-positive bacterium to achieve extracellular electron

transfer. Appl. Microbiol. Biotechnol. 77 (5), 1119–1129.
Rabaey, K., Boon, N., Siciliano, S.D., Verhaege, M., Verstraete, W., 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl. Environ. Microbiol. 70 (9), 5373.
Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., Lovley, D.R., 2005. Extracellular electron transfer via microbial nanowires. Nature 435 (7045), 1098–1101.
Rotaru, A.E., Shrestha, P.M., Liu, F., Markovaite, B., Chen, S., Nevin, K.P., Lovley, D.R., 2014. Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. Appl. Environ. Microbiol. 80 (15), 4599–4605.
Shi, L., Dong, H., Liu, J., Yu HQ, F.J.K., 2016. Extracellular electron transfer mechanisms between microorganisms and minerals. Nature. Rev. Microbiol. 14 (10), 651–662.
Wang, Z., Shi, Z., Zachara, J.M., Shi, L., Fredrickson, J.K., 2013. Reductive dissolution of goethite and hematite by reduced flavins. Geochim Cosmochim Ac 121, 139–154.
Tokunou, Y., Hashimoto, K., Okamoto, A., 2016. Acceleration of extracellular electron transfer by alternative redox-active molecules to riboflavin for outer-membrane cytochrome c of *Shewanella oneidensis* MR-1. J Phys Chem C 120 (29), 16168–16173.
von Canstein, H., Ogawa, J., Shimizu, S., Lloyd, J.R., 2008. Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. Appl. Environ. Microbiol. 74 (3), 615–623.
Wrighton, K.C., Thrash, J.C., Melnyk, R.A., Bigi, J.P., Byrne-Bailey, K.G., Remis, J.P., Schichnes, D., Auer, M., Chang, C.J., Coates, J.D., 2011. Evidence for direct electron transfer by a gram-positive bacterium isolated from a microbial fuel cell. Appl. Environ. Microbiol. 77 (21), 7633–7639.
Wu, S., Xiao, Y., Wang, L., Zheng, Y., Chang, K., Zheng, Z., Yang, Z., Varcoe, J.R., Zhao, F., 2014. Extracellular electron transfer mediated by flavins in gram-positive *Bacillus* sp. WS-XY1 and *Yeast Pichia stipitis*. Electrochim. Acta 146, 564–567.
Dai, Y., Xiao, Y., Zhang, E., Zhang, J., Yang, Z., Christensen, H.E.M., Ulstrup, J., Zhao, F., 2017. Extracellular polymeric substances are transient media for microbial extracellular electron transfer. Sci. Adv. 3 (7), e1700623.
Yang, Y., Xu, M., Guo, J., Sun, G., 2012. Bacterial extracellular electron transfer in bioelectrochemical systems. Process Biochem. 47 (12), 1707–1714.
Yatsyshyn, V.Y., Fedorovych, D.V., Sibirny, A.A., 2009. The microbial synthesis of flavin nucleotides: a review. Appl. Biochem. Microbiol. 45 (2), 115.