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Citation: AIP Conference Proceedings **1784**, 020010 (2016); doi: 10.1063/1.4966720
View online: https://doi.org/10.1063/1.4966720
View Table of Contents: http://aip.scitation.org/toc/apc/1784/1
Published by the American Institute of Physics

Antimicrobial Activity and Determination of Bioactive Components from Marine *Alcaligenes faecalis* Extract Against a Sulfate-Reducing Bacteria

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Abstract. Biogenic souring and microbial-influenced corrosion is a common scenario in petroleum reservoir .The serious threat normally comes from sulfate-reducing bacteria (SRB). *Alcaligenes faecalis* was tested in this study for the ability to inhibit the growth of SRB. Ethyl acetate extraction of *A. faecalis* grown in marine broth was carried out to produce crude ethyl acetate of *A. faecalis* (CEAF). CEAF was diluted at concentrations 0.2–12.8 mg/mL and was tested for anti-microbial activity by microdilution susceptibility tests in 96-wells plate. CEAF was then analyzed by Gas Chromatography Mass Spectrometry (GC-MS). The microdilution susceptibility tests showed that the crude have anti-microbial activities on SRB. CEAF showed immediate killing effect against SRB in liquid medium which suggest the presence of active chemical compounds with antimicrobial activity. The GC-MS analysis showed the presence of 20 different chemical compounds in CEAF, The major components in CEAF can be related to antimicrobial, antifungal, antioxidant, pesticide, metabolism, toxicity, anticancer and corrosion inhibition activities. In conclusion, crude ethyl acetate extract of *A. faecalis* has the ability to inhibit SRB growth.

INTRODUCTION

The electrochemical reaction that exist between the environment and material such as metal such as tank pipeline can alter the property of the material and cause corrosion [1]. Sulphate reducing bacteria (SRB) are the main bacteria group that involved in harmful processes of metal biocorrosion. Using biocides to control growth of sulfate-reducing bacteria (SRB) can be impeded by antimicrobial resistance which often occurs; biofilms with biocides [2]. Toxicity, persistence of biocides and residual concentration in industrial effluents is detrimental to the environmental. The petroleum industries are looking for environmental friendly treatments of anticorrosion as alternatives to replace the synthetic biocides. Natural bacteria products are used widely as compound in developing antibacterial agents against bacterial infection [3]. Organic compounds products from marine microorganisms against terrestrial microorganisms are extensively used for treatment in many diseases [4]. The present study highlights the activity of compounds from *Alcaligenes faecalis* that was isolated from the Malaysian marine and also found in the soil, water, and wastewater treatment systems and has the ability to inhibit biofilm formation [5]. The genus *Alcaligenes* is known among bacteria to have antagonistic activity [6] and inhibit the growth of many bacteria [7]. However, no research on the inhibitory effect of *A. faecalis* against *Desulfovibrio sp*. has been reported. There are few studies demonstrating antibiosis effect of isolated strains from marine bacteria [8]. Moreover, the antibacterial effect of *A. faecalis* is not yet been elucidated in Malaysian ecological zones, especially against SRB.

The 2016 UKM FST Postgraduate Colloquium
AIP Conf. Proc. 1784, 020010-1–020010-5; doi: 10.1063/1.4966720
Published by AIP Publishing. 978-0-7354-1446-4/$30.00

MATERIALS AND METHODS

Microorganisms

Marine bacterial strain *A. faecalis* was obtained from [5]. SRB was isolated from local crude oil from Malaysia according to [9].

Preparation of Bacterial Extracts:

A liquid-liquid extraction was carried out using ethyl acetate (EA) for extraction of antimicrobial compounds. An inoculum of *A. faecalis* was cultivated in marine broth (MB, Difco, NJ, USA) in rotary agitation at 150 rpm at 30°C for 96 h. After incubation, the culture was centrifuged at 6000 rpm for 10 min at 4°C to obtain clear supernatant. The supernatant was recovered, sterilized by filtration and ethyl acetate was added at the ratio of 1 part of EA for every 2 parts of supernatant. Vacuum evaporation at 40°C of the extracts was done to obtain dry extract.

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

The microdilution susceptibility tests and growth were performed according to [10]. Dry crude extract was diluted in sterile medium of VMNI and dilution was prepared in 96-well microtiter plates at 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg/ml. SRB growth was observed the blackish coloration of the medium following incubation at 30°C for 7 days due to iron sulfide precipitation in VMNI medium. Minimum inhibitory concentration (MIC) was determined by measuring the optical density (OD) at 630 nm wavelength of SRB culture. The test was done in triplicate.

Screening of Bacterial Metabolite by GC/MS:

CEAE was analyzed by gas chromatography and mass spectrophotometer (GC/MS) for detection of the main compounds. Ten µl of CEAE was directly injected into the injection port of gas chromatograph (Agilent Technologies 7890A GC system) directly coupled with a mass spectrometer system (MS) (Agilent Technologies 5975C inert MSD with Triple-Axis Detector).The GC was operated on an Agilent DB-5MS UI GC column (30 m × 0.25 mm, id. with 0.25µm film thickness of 5%-phenyl-methylpolysiloxane) and helium was used as the carrier gas. The temperature program was started with an initial temperature at 50°C and held for 2 minutes at this temperature, then 6 °C/min to 280 °C for 10 min a flow rate of 1 mL/min and run time 50.333 min. The MSD Chemstation was used to determine all the peaks in raw GC chromatogram. Library search was done for all the peaks using the National Institute of Standards and Technology NIST/EPA/NIH version 2.0 All results were combined in a single peak table.

Statistical Analysis

Microsoft Excel was used to calculate mean and mean standard deviation. Statistical comparisons of the results were performed by one-way ANOVA using SPSS ver.20 with significant differences is set at $P<0.05$. All the results were calculated from the mean of three replicate samples for each data point.

RESULTS AND DISCUSSIONS

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC):

The MIC of CEAF was established at 3.2 mg/ ml according to the spectrophotometric assay result (Fig. 1). MIC, sub-MIC and supra-MIC levels were considered as 3.2 mg /ml, 1.6 mg /ml and 6.4 mg / ml respectively. The MBC of CEAF was determined as the same value as the MIC, as no cell growth was recovered from any of the three

replicate wells. The OD reading of untreated SRB is around 1.687 ± 0.03242 nm ($p<0.001$). According to the OD reading results any wells that give OD reading less than this range can be considered to have effect with the CEAF. Therefore, CEAFE showed a bactericidal effect against the SRB. The time kill test at MIC level showed an immediate bactericidal effect. This result indicates that *A. faecalis* acts by substance (s) secreted in the medium and which are soluble in ethyl acetate, considering the large number of different groups of chemical compounds present in CEAF (Table 1). It is likely that the antibacterial activity is not attributed by one specific mechanism but to several targets in the cell.

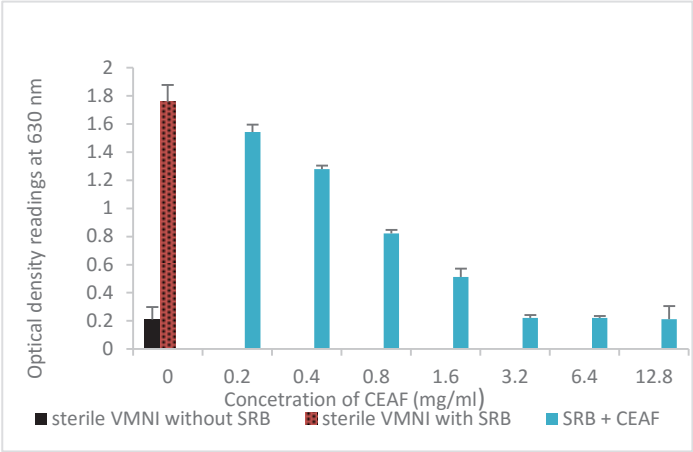

FIGURE 1. MIC determination of CEAF by optical density (OD) reading of SRB cell cultures treated with different concentrations.

GC-MS profile analysis

The GC-MS profile analysis of CEAF showed the presence of 58 major different chemical compounds (Fig. 2), the major chemical compounds were identified with similarity index ≥90%. The chosen criteria was selected from other studies where they have been used to differentiate within constituent's compounds according biological activities showed the presence of 20 different chemical compounds in CEAE but there might be some other compounds in the extract which could not be detected by this particular technique. In addition, the presence of many other compounds that have small peaks or similarity index ≤ 90% in the spectrum suggests that the extract contains several other unidentified components. The major components in CEAF were related to antimicrobial, antifungal, antioxidant, pesticide, metabolism toxicity, anticancer and inhibit corrosion activities (Table 1). The most abundant in CEAF were antimicrobials and according to the peak area, the content in CEAF was 49.334 %. (Table 1) and most were fatty acids. In a previous study, similar profile of fatty acids and some aromatic compounds were isolated from *Apus bamboo* using GC-MS for identification [11]. According to [12] free fatty acids have antibacterial properties. Free fatty acids not only disrupt the electron transport chain and oxidative phosphorylation but also interfere with cellular energy production, enzyme activity and nutrient uptake, generate toxic peroxidation, direct lysis of bacterial cells and prevent initial bacterial adhesion and subsequent biofilm formation. Many organisms use those natural compounds to defend against parasitic or pathogenic bacteria. Esters also had antibacterial activity because they could be metabolized to yield fatty acids by lipase enzyme in the body.

CONCLUSIONS

Our findings showed that the secondary metabolite produced from marine *A. faecalis* has bioactive compounds. These compounds have been identified, characterized and tested as an antimicrobial activity against sulfate-reducing bacteria (SRB). This investigation provides promising results that show the inhibitory effects of 3.2 mg/ml of CEAF on the SRB growth and thus lead to the control of the corrosion influenced by SRB. According to our results, we propose that CEAF has antimicrobial active compound with anticorrosion effect which can be a future option in

controlling SRB planktonic, sessile growth and biocorrosion mitigation of pipeline, tanks and other petroleum industrial related equipment.

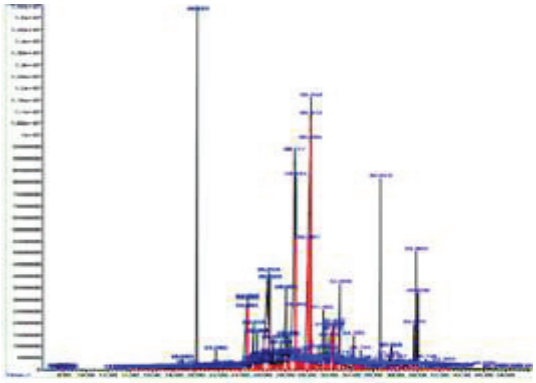

FIGURE 2: GC-MS chromatogram of CEAE.

TABLE 1. GC-MS analysis of CEAF and their reported biological activities.

S. No	R.T	Compound Name	M.W	Formula	P%	Activity
1	20.148	Phenol, 2,4-bis(1,1-dimethylethyl)-	282	$C_{18}H_{34}O_2$	1.58	Antimicrobial, ntifungal activity,UV stabilizer and an antioxidant for hydroarbon-based products [13].
2	23.745	Hexacosane	226.45	$C_{16}H_{34}$	0.4	Antioxidant,Cancer preventive,Cosmetic, Hypercholesterolemic, Nematicide[14].
3	25.214	Tetradecanoic acid	228.38	$C_{14}H_{28}O_2$	1.6929	Antibacterial, ntioxidant, Antitumor,Cancer preven tive, Immunostimulant, Chemo prev- entive, Pesticide and Lipoxygenaseinhibitor [15].
4	25.511	5-Octadecene, (E)-	252	$C_{16}H_{32}$	0.4797	Pesticide and antibiotic. [16]
5	28.817	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	12.52	Phthalate (pesticides and insect repellents. met-abolism, toxicity, antioxide. [17]
6	28.816	n-Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	12.518	Antimicrobial antioxidant [18]
7	29.536	Heptadecanoic acid	270	$C_{17}H_{34}O_2$	0.26	Anticancer, cholesterol lowering effect, nticoa-gulant, Increase stamina and mprove strength and reaction time for athletes. [19]
8	31.309	cis-13-Octadecenoic	282	$C_{18}H_{34}O_2$	1.58	Cytotoxic activity. [20]
9	31.403	cis-Vaccenic acid	282.46	$C_{18}H_{34}O_2$	1.6541	Antimicrobial [21]
10	31.662	Octadecanoic acid	284	$C_{18}H_{36}O_2$	1.000	Antibacterial [22]
11	30.261	Palmitoleic acid	254.41	$C_{16}H_{30}O_2$	9.31	Antimicrobial [23]
12	34.797	1-Nonadecene	266	$C_{19}H_{38}$	0.242	Antimicrobial [24]
13	32.968	9-Hexadecenoic acid	298.54	$C_{20}H_{42}O$	2.85	Stronger sexual characters [24]
14	36.620	Bis(2-ethylhexyl) phthalate	390.56	$C_{24}H_{38}O_4$	2.9448	Anti-fungal activity[25]
15	36.621	Diisooctyl phthalate	390.56	$C_{24}H_{38}O_4$	2.94	Antimicrobial ntifouling[26]
16	39.869	Decanedioic acid, bis(2-ethylhexyl)E	426.67	$C_{26}H_{50}O_4$	1.7648	Nematicidal , anticancer, antioxidant and antimicrobial [24]
17	40.027	Squalene	410	$C_{30}H_5$	1.0507	Good therapeutic agent for the treatment of sleep disorders and pain [25]
18	42.393	Octacosyltrifluoroace tate	506.76	$C_{30}H_{57}F_3O_2$	0.17	Pesticide and antibiotic [16]
19	42.393	1-Heptacosanol	396.74	$C_{27}H_{56}O$	0.81	Antimicrobial and antioxidant [26]
20	42.399	Octacosanol	410	$C_{28}H5_8O$	0.81	Antimicrobialand anticorrosion activities [27]

R.T = Retention Time .M.W=Molecular Weight .P% = Peak Area%

ACKNOWLEDGMENT

This work was supported Science Fund Research Grant from MOSTI (04-01-02-SF0754).

REFERENCES

1. H. H. Fang, L.-C. Xu and K.-Y. Chan, Water Res. **36** (19), 4709-4716 (2002).
2. P. S. Stewart and J. W. Costerton, The Lancet **358** (9276), 135-138 (2001).
3. H. Bredholdt, O. A. Galatenko, K. Engelhardt, E. Fjærvik, L. P. Terekhova and S. B. Zotchev, Environ. Microb**.** **9** (11), 2756-2764 (2007).
4. M. S. Butler, Nat. Prod. Rep. **25** (3), 475-516 (2008).
5. Z. Lutfi, G. Usup and A. Ahmad, presented at the THE 2014 UKM FST Postgraduate Colloquium: Proceedings of the Universiti Kebangsaan Malaysia, Faculty of Science and Technology 2014 Postgraduate Colloquium, 2014 (unpublished).
6. B. Austin, J. App. Bact. **67** (5), 461-470 (1989).
7. Z. Li, Y. Hu, Y. Huang and Y. Huang, Microbiol. **76** (4), 494-499 (2007).
8. A. K. Rai, N. Bhaskar, P. M. Halami, K. Indirani, P. Suresh and N. Mahendrakar, Appl. Microbiol. and Biotech. **83** (4), 757-766 (2009).
9. F. K. Sahrani, Z. Ibrahim, A. Yahya and M. Aziz, Sains Malays. **37** (4), 365-371 (2008).
10. P. Das, S. Mukherjee and R. Sen, J. Appl. Microbiol. **104** (6), 1675-1684 (2008).
11. W. Sujarwo, I. B. K. Arinasa and I. N. Peneng, Bul. Littro **21**, 129-137 (2010).
12. A. P. Desbois and V. J. Smith, Appl. Microbiol. Biotechnol. **85** (6), 1629-1642 (2010).
13. M. Govindappa, S. Prathap, V. Vinay and R. Channabasava, Int. J. Bio. Pharm. Res. **5** (11), 861-869 (2014).
14. J. Devi and A. K. Muthu, Int. J. Pharm. Pharm. Sci. **6**(2), 755-759 (2014).
15. G. Rajeswari, M. Murugan and V. Mohan, J. Pharm. Biomed. Sci **29**, 818-824 (2013).
16. A. Shettima, Y. Karumi, O. Sodipo, H. Usman and M. Tijjani, J. Appl Pharm Sci 3, 146-150. (2013).
17. F. Arcadi, C. Costa, C. Imperatore, A. Marchese, A. Rapisarda, M. Salemi, G. Trimarchi and G. Costa, Food Chem. Toxicol. **36** (11), 963-970 (1998).
18. T. Marrufo, F. Nazzaro, E. Mancini, F. Fratianni, R. Coppola, L. De Martino, A. B. Agostinho and V. De Feo, Molecules **18** (9), 10989-11000 (2013).
19. A. Dalli, G. Saha and U. Chakraborty, Ind. J. Exp. Biol. **45** (3), 285 (2007).
20. A. Manilal, S. Sujith, G. S. Kiran, J. Selvin and C. Shakir, Global J Pharmacol **3** (2), 90-94 (2009).
21. F. Mujeeb, P. Bajpai and N. Pathak, Biomed Res. Int. **2014**, 497606. (2014).
22. S. Keawsa-Ard, B. Liawruangrath, S. Liawruangrath, A. Teerawutgulrag and S. G. Pyne, Nat. Prod. Commun. 7(7):955-958. (2012).
23. A. B. Hsouna, M. Trigui, R. B. Mansour, R. M. Jarraya, M. Damak and S. Jaoua, Int. J. Food Microb. **148** (1), 66-72 (2011).
24. B. V. Raman, S. La, M. P. Saradhi, B. N. Rao, A. Khrisna, M. Sudhakar and T. Radhakrishnan, Asian J. Pharm. Clin. Res **5** (2), 99-106 (2012).
25. B. F. Cravatt, R. A. Lerner and D. L. Boger, J. Am. Chem. Soc, **118**(3), 580-590 (1996).
26. I. S. Melo, S. N. Santos, L. H. Rosa, M. M. Parma, L. J. Silva, S. C. Queiroz and V. H. Pellizari, Extremophiles **18** (1), 15-23 (2014).
27. N. Sheoran, A. V. Nadakkakath, V. Munjal, A. Kundu, K. Subaharan, V. Venugopal, S. Rajamma, S. J. Eapen and A. Kumar, Microbiol. Res. **173**, 66-78 (2015).