Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 24, No. (4) : 2022 : 707-712

ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2022.v24i04.015

BIOSURFACTANT PRODUCTION BY ENDOPHYTIC ISOLATES FROM RHIZOMES OF *ZINGIBER ZERUMBET*

HARSHA K., SHALIMA M.V. AND ASWATI R. NAIR*

Department of Biochemistry and Molecular Biology, Central University of Kerala (CUK), Kasaragod 671 320, Kerala,

(Received 2 May, 2022; Accepted 25 July, 2022)

Key words: *Biosurfactant,Bacillus spp, Zingiber zerumbet, Emulsification Index*

Abstract– Biosurfactants (BS) or green surfactants are amphiphilic compounds that enhance the bioavailability of hydrophobic organic compounds and also exhibit anti-phytopathogenic properties. BS-producing isolates thus find applications in soil remediation and development of biocontrol agents. Endophytes that confer several benefits to host plant constitute a good resource for prospecting BS-producing isolates. The present study screened the *Z. zerumbet* endophytes identified in earlier studies for BS production by hydrocarbon overlay agar method, CTAB- Methylene blue agar assay and determining Emulsification Index (EI24). EI24 was observed as the most desirable parameter to determine BS production compared to hydrocarbon overlay agar and CTAB- methylene blue agar plate assay. Isolate *ZzADK*4 (*Bacillus* spp) showed highest emulsification activity (58%) determined as EI% with kerosene. UV-vis spectral scans identified the endophytes as producing LP class of BS with absorbance maxima spanning 207- 216 nm. LP class of BS producing endophytic isolates identified in the present study constitute potential solutions with applications in soil remediation and the development of biocontrol agents (BCAs).

INTRODUCTION

Microbial surface-active compounds (biosurfactants) are amphiphilic compounds that reduce the surface and interfacial tension thereby finding wide applications as emulsifiers, wetting agents and detergents (Marchut-Mikolajczyk *et al*., 2021). Biosurfactants (BS) or green surfactants have emerged as an alternative to synthetic surfactants due to low toxicity and high biodegradability (El-Sheshtawy *et al*., 2015). Contrarily the petroleum-based synthetic surfactants like carboxylates, sulfonates and sulfate acid esters (Moldes *et al*., 2021) that find use as adjuvants in crop protective formulations (Deleu and Paquot, 2004) are toxic and difficult to degrade making them potent organic pollutants with adverse environmental effects (Petrovic and Barcelo, 2004; Sachdev and Cameotra, 2013). These reasons attribute to the emergence of BS as promising cost effective and eco-friendly remediation technology to deal with environmental pollutants (Guerra *et al*., 2018; Marchut Mikolajczyk *et al*., 2018). Several microbial species are known to produce BS with unique physico-chemical and biochemical properties and often exhibiting considerable stability over wide temperature and pH ranges (Jiménez-Peñalver *et al*., 2018; Patil *et al*., 2014). The diverse classes of BS produced by microbes include lipopeptides, glycolipids, phospholipids and polymeric macromolecules (Jiménez-Peñalver *et al*., 2018; Kaur *et al*., 2019).

Amongst the vast diversity of microbes thriving in the biosphere, endophytic microbes with ubiquitous distribution constitute a prospective resource to search for BS producers. One primary reason is their existence in symbiotic association with plants thereby conferring several benefits to the host including nutrient acquisition, resistance to biotic and abiotic factors, phytohormone production and metal chelation (Gaiero *et al*., 2013; Abbamondi *et al*., 2016; Santoyo *et al*., 2016). Endophytes besides promoting plant growth and resistance/ tolerance also display the ability to survive in the presence of xenobiotics (Rosenblueth and Martinez-Romero, 2006). Degradation and/or detoxification of organic pollutant particularly hydrocarbons by endophytes signifies their application in soil bioremediation (Afzal *et al*., 2014; Arslan *et al*., 2017; Zahoor *et al*.,

2017; Fenibo *et al*., 2019). BS production by endophytes has been implicated with hydrocarbon degradation considering their ability to emulsify and/or increase solubility of hydrocarbons thereby facilitating microbial degradation (Pawlik *et al*., 2017; Marchut-Mikolajczyk *et al*., 2021). Majority of the endophytic BS producers have been identified as *Pseudomonas* spp and have been reported to produce cyclic lipopeptides (LPs) and rhamnolipid (glycolipid) class of BS (D'aes *et al*., 2010). Bioprospecting for endophytic BS producers have been undertaken in few plants like *Phragmites australis* (Wu *et al*., 2018), *Scirpus triqueter* (Zhang *et al*., 2014), *Chelidonium majus* (Marchut-Mikolajczyk *et al*., 2018), *Lotus corniculatus* and *Oenothera biennis* (Pawlik *et al*., 2017). Such BS producing isolates constitute potential candidates that can replace the harsh surfactants presently used in pesticide formulations (Lima *et al*., 2011). Furthermore, in view of the various biological roles ascribed to BS that includes antimicrobial, facilitating assimilation of poorly water soluble nutrient, nutrient reserve and biofilm development (Twigg *et al*., 2021), the BS producing isolates hold potential for development of biocontrol agents (BCAs).

Zingiber zerumbet (L) Smith, a ginger congener, is known to exhibit durable resistance to biotic stress factors (Kavitha *et al*., 2007; Aswati and Thomas, 2012). Earlier studies have isolated and characterized bacterial and fungal endophytes from this taxon. BS producing isolates constitute promising BCAs considering their ability to form biofilm that helps to (i) increase nutrient availability; (ii) helps plants to grow in contaminated areas and (iii) antimicrobial activity of BS thereby preventing phytopathogenic ingress from soil (Sachdev and Cameotra, 2013). Present study was undertaken to identify potential BS producers amongst the *Z. zerumbet* endophytes.

MATERIALS AND METHODS

Endophytic isolates

Isolates *ZzMNGD*1 (*Bacillus megaterium*), *ZzMGRL*1 (*Kosakonia* spp), *ZzKSD*1 (*Bacillus* spp), *ZzKSD*7 (*Enterobacter tabaci*), *ZzKSD*8 (*Klebsiella aerogenes*), *ZzADK*3 (*Pseudomonas fuscovaginae*) and *ZzADK*4 (*Pantoea ananatis*) characterized earlier in our laboratory were used for the present experiments. The isolates were cultured in Luria-Bertani (LB) broth (Tryptone-0.05g, Yeast extract-0.025g NaCl-0.05g) at 37 °C for 16 hours at 150 rpm.

Screening of BS production

Hydrocarbon overlay agar method

BS production was screened by hydrocarbon overlay agar method (Kokare *et al.,* 2007). Hydrocarbon overlay agar method involved spotting the isolate on LB agar plate smeared with crude oil (1 ml). Formation of zone of clearance around the colony after incubation at 37 °C for 24 h indicates a positive result.

CTAB- Methylene blue agar assay

For CTAB- methylene blue agar plate method (Walter *et al.,* 2010), filtered cell-free supernatant (150 µl) of isolates was added to wells (6.5 mm diameter) punched in CTAB agar plates [CTAB (0.06 g); Methylene blue (0.002 g) and agar (7.2 g) of 400 mL; pH 7.0]. After incubation at room temperature for 24 – 48 hours, formation of dark blue halo around the colonies confirms formation of anionic BS (Walter *et al*., 2010).

Emulsification Index (EI %)EI (%) was estimated (Cooper and Goldenberg, 1987) by adding 48 hour grown culture supernatant (2 ml) to 2 mL of petrol, coconut oil, kerosene, sun flower oil and 2 mL supernatant (Cooper and Goldenberg 1987). The mixture was vortexed for 5 minutes and allowed to stand for 24 hrs. EI (%) was calculated as: EI (%) = (Height of emulsion / Total height of solution) x 100.

Characterization of BS

Spectroscopic analysis of BS

BS was extracted by acid precipitation of endophytic cultures, *ZzKSD*1, *ZzKSD*8, *ZzMNGD*1, *ZzMGRL*1, *ZzADK*3 and *ZzADK*4. For the same, isolate cultures grown in Luria Bertani (LB) broth (100 ml) for 3 days at 180 rpm was centrifuged at 8000 rpm for 15 min at 4 °C. The cell- free medium was acidified to pH 2.0 with concentrated HCl and left to precipitate overnight at 4 °C. Precipitates were collected by centrifugation at 8000 rpm for 15 min at 4 °C, washed with absolute methanol (5 ml) and dried overnight at 42 °C. The dried precipitate was suspended in methanol (200 µl) and presence of BS was spectroscopically analyzed (Perkin Elmer) by spectral scanning from 200 - 400 nm.

Thin Layer Chromatography

Preliminary characterization of BS was done by TLC. TLC was carried out on pre-coated silica gel 60 F254. The extracted BS sample was spotted on the

TLC plates and separated using chloroform: methanol: water (65:25:4) as the mobile phase. Plates were developed using iodine for visualization of lipids and identification of BS. Retention factor was calculated by the formula: Rf = Distance moved by the component/ Distance moved by the solvent front.

RESULTS AND DISCUSSION

Screening isolates for BS production

Endophytes characterized in earlier studies from *Z. zerumbet* rhizomes were screened in present study to identify BS producers. Hydrocarbon overlay agar method identified only isolate *ZzMNGD*1 (*Bacillus megaterium*) to exhibit hydrocarbon degradation potential (Fig. 1) whereas CTAB- methylene blue agar plate assay did not identify any of the selected isolates as positive indicating absence of anionic BS production. CTAB- methylene blue assay presents limitations as only anionic BS can form insoluble pair of CTAB with methylene blue seen as halo zone in plates (daSilva *et al*., 2021) besides the CTAB reagent, cetrimide being broad-spectrum antimicrobial thereby leading to false-negative results (Twigg *et al*., 2021). However, the endophytic isolates showed emulsification activity in experiments determining emulsification index (EI24) that is known as a simpler and effective method as it measures ratio between emulsified volume and total volume of sample (daSilva *et al*., 2021). Measurement of EI24 using petroleum, kerosene, coconut oil and sunflower oil yielded good emulsification indices for isolates, *ZzKSD*1 (*Bacillus* spp), *ZzKSD*8 (*Klebsiella aerogenes*) and *ZzADK*4 (*Pantoea ananatis*) (Table 1). Amongst the isolates screened, highest emulsification activity (58%) was observed for *ZzADK*4 with kerosene

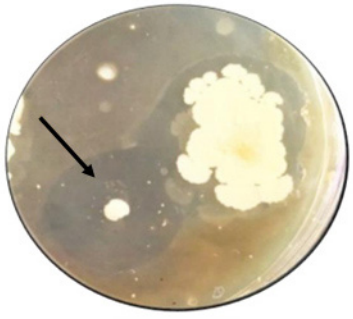

Fig. 1. Hydrocarbon overlay agar test showing the halo zone as observed for the endophytic isolate, *ZzMNGD*1.

Table 1. Emulsification index (EI%) as determined for endophytic isolates used in present study with petrol, kerosene, coconut oil and sunflower oil. Hyphen indicates non-detection of EI.

Endophytic isolates	Emulsification index (EI%)			
	Petrol	*Kerosene*	*Coconut oil*	*Sunflower oil*
*ZzKSD*1	32	54	26	22
ZzKSD7	-	8	20	15
*ZzKSD*8	8	11	48	9
*ZzADK*3	-	-	42	15
*ZzADK*4	8	58	48	30
*ZzMNGD*1	-	-	28	15
*ZzMGRL*1	-	5	32	16

(58%) wherein the emulsification height was determined as 18 mm and total solution height as 31 mm (Fig. 2). Earlier studies have also reported negative results in complementary assays like hydrocarbon overlay agar and CTAB-methylene blue agar assay while detecting BS production by EI determination. These studies have subsequently concluded EI as the most desirable parameter to determine BS production (Adebajo *et al*., 2020) such as for BS producing isolates, *B. amyloliquefaciens* and *B. subtilis* (Sharma *et al*., 2018) and in *Bacillus* spp. isolated from hydrocarbon contaminated soils from automobile workshop (Santhini *et al*., 2014).

Characterization of BS

Absorption spectra for BS production was determined by spectral scan between 200- 400 nm. The spectral range chosen corresponds to the characteristic absorbance spectra of anionic BS (Magthalin *et al*., 2016). Absorbance maxima was detected at 207 nm for *ZzMNGD*1, 211 nm for *ZzADK*4, 212 nm for *ZzKSD*1, 215 nm for *ZzMGRL*1, 216 nm for *ZzADK*3 and 212 nm for *ZzKSD*8. The spectral peaks correspond to the characteristic absorbance maxima of peptide bonds in lipopeptide (LP) BS (Meena *et al*., 2021). Production of LP class of BS by the endophytic isolates was confirmed by TLC analysis based on the Rf values. The Rf values of spots detected by *ZzKSD*1 (Rf: 0.5 cm), *ZzKSD*8 (Rf: 0.6 cm), *ZzMNGD*1 (Rf: 0.7 cm) and *ZzMGRL*11 (Rf: 0.4 cm) corresponded to those reported for iturin (0.55- 0.72 cm) (Joy *et al*., 2017) and surfactin (Rf: 0.3 cm) class of LP (Ramyabharathi *et al*., 2018). The identified BS producing endophytic isolates constitute potential endophytes with applications in agriculture. BS which are also termed as "green surfactants" constitute viable alternatives to the

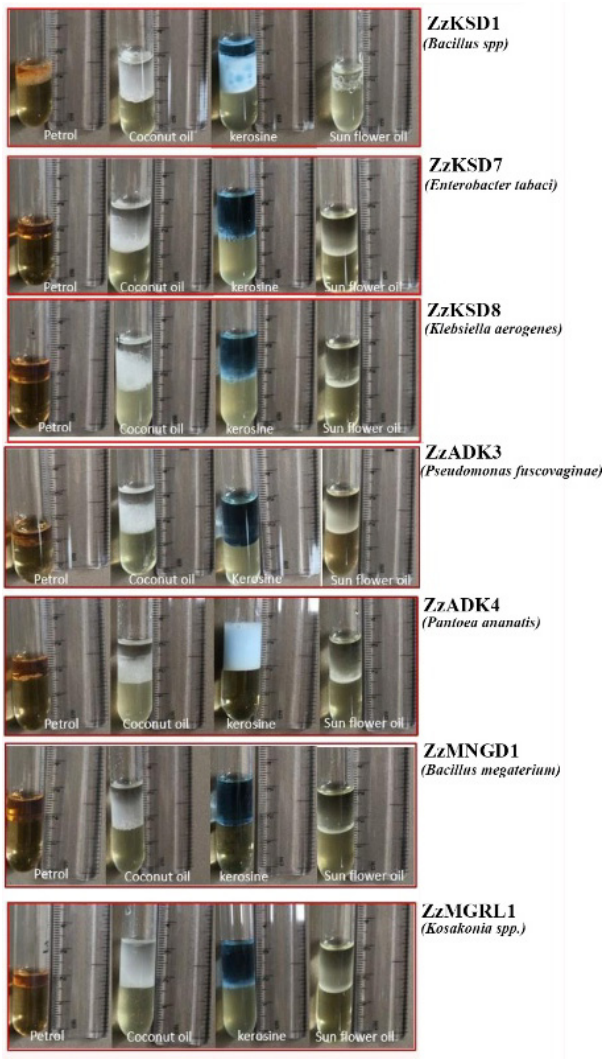

Fig. 2. Emulsification index (E24) observed for BS producing endophytic isolates with different oil samples.

toxic synthetic surfactants currently used widely as adjuvants in pesticides (Sachdev and Cameotra, 2013). Being biodegradable, less toxic and high stability, there is growing research interest to prospect BS producing endophytic isolates from various plant sources (Marchut-Mikolajczyk *et al*., 2018; Sachdev and Cameotra, 2013) for applications as biocontrol agents (Banat *et al*., 2000; Adetunji and Olaniran, 2021). BS are known to enhance degradation of chemical pesticides that accumulates in agriculture soil (Zhang *et al*., 2011; Singh *et al*., 2009; Wattanaphon *et al*., 2008). Indiscriminate use of pesticides and fertilizers is a problem seriously confronting ginger fields after every harvesting season. In this perspective, the BS producing isolates identified in present study can provide solutions for augmenting quality of agriculture soil after harvest by remediating the soil of synthetic agrochemicals applied during the ginger growing season. Development of such sustainable solutions are necessary in the context of excessive soil degradation in terms of depletion of organic matter and nutrients witnessed after ginger harvesting every year due to heavy reliance on synthetic agrochemicals (Mahadevan and Gonemaituba, 2013). Besides soil remediation, the LP class of BS also exhibit antimicrobial and zoospore lytic effects due to their emulsification activity (Kruijt *et al*., 2009; Sachdev and Cameotra, 2013). Considering these aspects, the BS producing isolates constitute potential candidates for BCA development and soil remediation.

ACKNOWLEDGEMENT

Authors acknowledge the facilities extended at Central University of Kerala (CUK) for conduct of the experiments.

REFERENCES

Abbamondi, G.R., Tommonaro, G., Weyens, N., Thijs, S., Sillen, W., Gkorezis, P., Iodice, C., Rangel, W.de.M., Nicolaus, B. and Vangronsveld, J. 2016. Plant growth-promoting effects of rhizospheric and endophytic bacteria associated with different tomato cultivars and new tomato hybrids. *Chem. Biol. Technol. Agric*. 3: 1.

Adebajo, S.O., Akintokunb, P.O., Ojoa, A.E., Akintokuna, A.K. and Badmosa, O.S. 2020. Recovery of biosurfactant using different extraction solvent by rhizospheric bacteria isolated from rice-husk and poultry waste biochar-amended soil. *Egyptian J. of Basic and Appl. Sci*. 7(1): 252–266.

Adetunji, A.I. and Olaniran, A.O. 2021. Production and potential biotechnological applications of microbial surfactants: An overview. *Saudi J. of Biol. Sci.* 28(1): 669-679.

Afzal, M., Khan, Q.M. and Sessitsch, A. 2014. Endophytic bacteria: prospects and applications for the phytoremediation of organic pollutants. *Chemosphere* 117 : 232–242.

Arslan, M., Imran, A., Khan, Q.M. and Afzal, M. 2017. Plant-bacteria partnerships for the remediation of persistent organic pollutants. *Environ Sci Pollut Res Int*. 24(5): 4322-4336.

Aswati R.N. and Thomas, G. 2012. Functional genetic diversity at nucleotide binding site (NBS) loci: Comparisons among soft rot resistant and susceptible *Zingiber* taxa. *Biochemical Systematics and Ecol*. 44: 196-201.

Banat, I., Makkar, R. and Cameotra, S. 2000. Potential

commercial applications of microbial surfactants. *Appl. Microbiol. Biotechnol*. 53: 495–508.

Cooper, D.G. and Goldenberg, B.G. 1987. Surface-active agents from two *Bacillus* species. *Appl. and Envt. Microbiol*. 53(2): 224-229.

D'aes, J., De Maeyer, K., Pauwelyn, E. and Höfte, M. 2010. Biosurfactants in plant-*Pseudomonas* interactions and their importance to biocontrol. *Environ. Microbiol. Rep*. 2: 359–372.

da Silva, A.F., Banat, I.M., Giachini, A.J. and Robl, D. 2021. Fungal biosurfactants, from nature to biotechnological product: bioprospection, production and potential applications. *Bioprocess Biosyst Eng*. 44: 2003–2034.

Deleu, M. and Paquot, M. 2004. From renewable vegetables resources to microorganisms: new trends in surfactants. *Comptes Rendus Chimie* 7(6–7): 641-646.

El-Sheshtawy, H.S., Aiad, I., Osman, M.E., Abo-ELnasr, A.A. and Kobisy, A.S. 2015. Production of biosurfactant from *Bacillus licheniformis* for microbial enhanced oil recovery and inhibition the growth of sulfate reducing bacteria. *Egyptian J. of Petroleum* 24(2): 155-162.

Fenibo, E.O., Ijoma, G.N., Selvarajan, R. and Chikere, C.B. 2019. Microbial surfactants: The next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. *Microorganisms*. 7(11): 581.

Gaiero, J.R., McCall, C.A., Thompson, K.A., Day, N.J., Best, A.S. and Dunfield, K.E. 2013. Inside the root microbiome: bacterial root endophytes and plant growth promotion. *Am. J. Bot*. 100(9): 1738-1750.

Guerra, A.B., Oliveira, J.S., Silva-Portela, R.C.B., Araújo, W., Carlos, A.C., Vasconcelos, A.T.R., Freitas, A.T., Domingos, Y.S., de Farias, M.F., Fernandes, G.J.T. and Agnez-Lima, L.F. 2018. Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation. *Environ Pollut*. 235 : 869-880.

Jiménez-Peñalver, P., Castillejos, M., Koh, A., Gross, R., Sánchez, A., Font, X. and Gea, T. 2018. Production and characterization of sophorolipids from stearic acid by solid-state fermentation, a cleaner alternative to chemical surfactants. *J Clean Prod*. 172: 2735–2747.

Joy, S., Rahman, P.K. and Sharma, S. 2017. Biosurfactant production and concomitant hydrocarbon degradation potentials of bacteria isolated from extreme and hydrocarbon contaminated environments. *Chemical Engg. J.* 317 : 232-241.

Kaur, G., Wang, H., To, M.H., Roelants, S.L.K.W., Soetaert, W. and Lin, C.S.K. 2019. Efficient sophorolipids production using food waste. *J Clean Prod*. 232: 1–11.

Kavitha, P.G., Nair, P., Aswati R.N., Jayachandran, B.K., Sabu, M. and Thomas, G. 2007. AFLP polymorphism and *Pythium* response in *Zingiber* species. In: *Recent Trends in Horticultural Biotechnology*. New India Publishing Agency, New Delhi Eds. Raghunath Kesavachandran et al, pp 497-503.

Kokare, C.R., Kadam, S.S., Mahadik, K.R. and Chopade, B.A. 2007. Studies on bioemulsifier production from marine *Streptomyces* sp. S1. *Indian J Biotechnol*. 6: 78–84.

Kruijt, M., Tran, H. and Raaijmakers, J.M. 2009. Functional, genetic and chemical characterization of biosurfactants produced by plant growth-promoting *Pseudomonas putida* 267. *J. Appl. Microbiol*. 107: 546–556.

Lima, T.M., Procópio, L.C., Brandão, F.D., Carvalho, A.M., Tótola, M.R. and Borges, A.C. 2011. Biodegradability of bacterial surfactants. *Biodegradation* 22: 585–592.

Magthalin, J.C., Varadharajan, A., Swarnalatha, S. and Sekaran, G. 2016. Utilization of chicken tallow for the production of cationic biosurfactant and thereof for decontamination of Cr(III) containing soil. *Procedia Environmental Sci*. 35: 895– 913.

Mahadevan, R. and Gonemaituba, W. 2013. A note on soil erosion and the loss in crop production: case study of Fiji's ginger farms. *J of Asia Pacific Economy* 18(3): 502-511.

Marchut-Mikolajczyk, O., Dro¿d¿yński, P., Pietrzyk, D. and Antczak, T. 2018. Biosurfactant production and hydrocarbon degradation activity of endophytic bacteria isolated from *Chelidonium majus* L. *Microb Cell Fact*. 17: 171.

Marchut-Miko³ajczyk, O., Dro¿d¿yński, P., Polewczyk, A., Smu³ek, W. and Antczak, T. 2021. Biosurfactant from endophytic *Bacillus pumilus* 2A: physico-chemical characterization, production and optimization and potential for plant growth promotion. *Microb Cell Fact*. 20: 40.

Meena, K.R., Dhiman, R., Singh, K., Kumar, S., Sharma, A., Kanwar, S.S., Mondal, R., Das, S., Franco, O.L. and Mandal, A.K. 2021. Purification and identification of a surfactin biosurfactant and engine oil degradation by *Bacillus velezensis* KLP2016. *Microb. Cell Factor*. 20: 1–12.

Moldes, A.B., Rodríguez-López, L., Rincón-Fontán, M., López-Prieto, A., Vecino, X. and Cruz, J.M. 2021. Synthetic and Bio-Derived surfactants versus microbial biosurfactants in the cosmetic industry: An overview. *Int J Mol Sci*. 22(5): 2371.

Patil, S., Pendse, A. and Aruna, K. 2014. Studies on optimization of biosurfactant production by *Pseudomonas aeruginosa* F23 isolated from oil contaminated soil sample. *Int J Curr Biotech*. 2(4): 20–30.

Pawlik, M., Cania, B., Thijs, S., Vangronsveld, J. and Piotrowska-Seget, Z. 2017. Hydrocarbon degradation potential and plant growth-promoting activity of culturable endophytic bacteria of *Lotus corniculatus* and *Oenothera biennis* from a long-term polluted site. *Environ. Sci. Pollut. Res. Int*. 24(24): 19640-19652.

Petrovic, M. and Barcelo, D. 2004. Analysis and fate of surfactants in sludge and sludge-amended soil. *Trends Analyt Chem* 23:10–11.

Ramyabharathi, S., Meena, K.S., Rajendran, L., Karthikeyan, G., Jonathan, E.I. and Raguchander, T. 2018. Biocontrol of wilt-nematode complex infecting

gerbera by *Bacillus subtilis* under protected cultivation. *Egypt J. Biol. Pest Control*. 28 : 21.

Rosenblueth, M. and Martínez-Romero, E. 2006. Bacterial endophytes and their interactions with hosts. *Mol. Plant Microbe Interact*. 19(8): 827-837.

Sachdev, D.P. and Cameotra, S.S. 2013. Biosurfactants in agriculture. *Appl. Microbiol. Biotechnol*. 97: 1005-1016.

Santhini, K. and Parthasarathi, R. 2014. Isolation and screening of biosurfactant producing micro-organisms from hydrocarbon contaminated soils from automobile workshop. *Intl. J. of Pharm. and Biol. Arch.* 5(2): 158-167.

Santoyo, G., Moreno-Hagelsieb, G., Orozco-Mosqueda, Md.C. and Glick, B.R. 2016. Plant growth-promoting bacterial endophytes. *Microbiological Res*. 183: 92-99.

Sharma, R., Singh, J. and Verma. N. 2018. Production, characterization and environmental applications of biosurfactants from *Bacillus amyloliquefaciens* and *Bacillus subtilis*. *Biocatalysis and Agricultural Biotechnol*. 16: 132-139.

Singh, P.B., Sharma, S., Saini, H.S. and Chadha, B.S. 2009. Biosurfactant production by *Pseudomonas* sp. and its role in aqueous phase partitioning and biodegradation of chlorpyrifos. *Lett. Appl. Microbiol*. 49: 378-383.

Twigg, M.S., Baccile, N., Banat, I.M., Déziel, E., Marchant, R., Roelants, S. and Van Bogaert, I.N.A. 2021. Microbial biosurfactant research: time to improve the rigour in the reporting of synthesis, functional characterization and process development. *Microb Biotechnol*. 14(1) : 147-170.

Walter, V., Syldatk, C. and Hausmann, R. 2010. Screening concepts for the isolation of biosurfactant producing microorganisms. *Biosurfactants*. 1-13.

Wattanaphon, H.T., Kerdsin, A., Thammacharoen, C., Sangvanich, P. and Vangnai, A.S. 2008. A biosurfactant from *Burkholderia cenocepacia* BSP3 and its enhancement of pesticide solubilization. *J. Appl. Microbiol*. 105 : 416-423.

Wu, T., Xu, J., Xie, W., Yao, Z., Yang, H., Sun, C. and Li, X. 2018. *Pseudomonas aeruginosa* L10: A hydrocarbon-degrading, biosurfactant-producing, and plant-growth-promoting endophytic bacterium isolated from a Reed (*Phragmites australis*). *Front Microbiol*. 9: 1087.

Zahoor, M., Irshad, M., Rahman, H., Qasim, M., Afridi, S.G., Qadir, M. and Hussain, A. 2017. Alleviation of heavy metal toxicity and phytostimulation of *Brassica campestris* L. by endophytic *Mucor* sp. MHR-7. *Ecotoxicol Environ Safety.* 142 : 139-149.

Zhang, C., Wang, S. and Yan, Y. 2011. Isomerization and biodegradation of beta-cypermethrin by *Pseudomonas aeruginosa* CH7 with biosurfactant production. *Bioresour. Technol*. 102 : 7139–7146.

Zhang, X., Liu, X., Wang, Q., Chen, X., Li, H., Wei, J. and Xu, G. 2014. Diesel degradation potential of endophytic bacteria isolated from *Scirpus triqueter*. *Intl. Biodeter Biodegrad*. 87 : 99-105.