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HPLC Profiling of Viscosin Producing *Fluorescent pseudomonads* Isolated From Sugarbeet -Maize Ecosystem Enhances Plant Growth Promotion

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A B S T R A C T

Antimicrobial biosurfactant producing Fluorescent Pseudomonads biovar (Pf-V1) was isolated from Maize/Sugar beet rhizosphere in sandy loam soil and tested for the presence of viscosinby HPLC analysis. The strain performed its antifungal activity against major fungal disease of maize with zoosporicidal activity against downy mildew pathogen of maize. The strain is grouped under V1 (Associated with the production of Viscosin). The strain improves growth & systemic protection of maize crop against foliar & soil-borne pathogens and surprisingly drought tolerance. Genetic improvement of Pf strains with increased Viscosinproduction, biosurfactant properties & wide applicability to other crops will be aimed as separate research theme.

Introduction

Biosurfactants are surfaceactive compounds physiologically secreted by microorganisms for utilization of hydrocarbons (Salek & Euston, 2019). Microbial surfactants exhibit superior properties compared to chemically manufactured alternatives, of which the most important is outstanding surface activity, stability in a wide range of pH and temperature, biodegradability, low toxicity and extraordinary emulsifying and demulsifying activity (Fardami *et al.,* 2022). Numerous genera have been described for biosurfactant production, with *Bacillus spp*., *Acinetobacter spp*., *Rhodococcus spp*., *Halomonas spp*. and *Candida spp*. being the best-known biosurfactant producers, as well as *Pseudomonas*, described as a dominant genus involved in biosurfactant synthesis.

Pseudomonas is a cosmopolitan microorganism found in miscellaneous habitats including soil, water, organic matter, plants and animals (Shekhar *et al.,* 2015). Due to its metabolic complexity, Pseudomonas is capable of

synthesizing both glycolipid and lipopeptide biosurfactants (Geudens & Martins, 2018). (Biniarz *et al.,* 2017) Biniarz and coworkers highlighted that the complex group of Pseudomonas lipopeptides, originally represented by viscosin, amphisin, tolaasin and syringomycin, has been recently expanded by several newly identified compounds (Mazzola *et al.,* 2009). In fact, the extensively studied group of viscosins has so far included 17 characterized compounds, represented by viscosin, viscosinamide, WLIP (white line-inducing principle) pseudodesmin, massetolide and pseudophomins (Chauhan *et al.,* 2023). (Cesa-Luna *et al.,* 2023) Cesa – Luna reported that the antibiotic was secreted in *P. viscosa* culture and therefore named viscosin. Later, (Neu *et al.,* 1990) Neu and his workers determined the surface-active properties of viscosin. Given the dearth of knowledge regarding the lipopeptides' production by the Pseudomonas genus, this paper aims at describing, for the first time, the capability of a Pseudomonas strain to produce the lipopeptide biosurfactant viscosin

Materials and Methods

Isolation of surfactant producing *Pseudomonas spp*. strains

Soil samples were collected from loamy sand, where maize crop was intercropped with sugarbeet and kept at 5°C until use. The samples were weighed for 50 g in polythene vials with the bulk density 1.1 g cm-3. Maize seeds were sown in vials (3 seeds/vial) and kept in 15°C under 16 h light and 8 h dark cycle. The seedlings were uprooted along with adhering soils and transferred to 10 ml sterile 0.9% Nacl. The sample was vortexed for 1 min and sonicated for 0.5 min and plated in solid media. High density population of *Pseudomonas spp*. was obtained in two different media: (i) On King‘s B medium fluorescent Pseudomonas spp. were detected by exposing the agar plates with UV light (254 nm) and the fluorescent colonies were randomly picked. (ii) Gould‘s S1 medium, containing 10 g sucrose, 10 ml of glycerol, 5 g of casamino acids, 1 g of $NaHCO_3$, 1 g of $MgSO_4.7H_2O$, 2.3 g of K_2HPO_5, 1.2 g of sodium lauryl sulphate and 15 g of agar per liter was autoclaved, and then 5 ml of 100 mg of trimethoprim, 8.5 ml of methanol, and 16.5 ml of Milli-Q water was added to the medium.

The colonies appearing in Gould´S1 selective medium were eligible for random picking. Isolates from the two media were further streaked onto Gould‘ S1 agar and checked for fluorescence before culturing in 3 ml of Luria Bertani medium per liter containing 10 g of tryptone, 5 g of yeast extract, 10 g of Nacl, and 1 g of glucose pH 7.2 for subsequent preservation at -80°C.

Swarming and biofilm assays

Bacterial cells grown for 24 h on GS1 (Gould‘s S1) medium agar plates were dissolved in sterile distilled water to a final density of 109 CFU ml-1 (OD600 = 1), pelleted by centrifugation and washed once with sterile distilled water. Swarming assays were performed on soft agar plates (KB medium with 0.6% (W/V) agar, five microlitres of the cell suspension were placed in the centre of a soft agar plate. The ability of the bacterial colony to spread was evaluated after 24, 48 and 72 h of incubation at 25°C. The biofilm assays were performed in flat-bottom non-detachable 96 wells plates (Nunc.Immuno TM Micro Well TM, SIGMA-ALDRICH, USA) according to the methods described by (10)O‘Toole and (11) Bruine. The 96 wells were filled with 180 µl of Gould‘s S1 medium and 20 µl bacterial suspension (1×109 cells ml-1) and 20 µl bacterial suspension (1×109 cells ml-1) and incubated for 24 h at 25°C. Biofilms were stained with crystal violet and visualized at 600 nm (Artursson *et al.,* 2006). The biofilms were observed in side walls of the 96 well plates and the OD was measured at 600 nm.

Structural diversity of Pseudomonas spp. surfactants

The first characterization of the Pseudomonas spp. surfactants by high-pressure liquid chromatography (HPLC) analysis was performed after culturing of all isolates at 20ºC for 2 days in 25ml glass tubes with 3ml of King’s B broth. Samples were obtained by extraction for 1h with 5ml of ethyl acetate containing 1% formic acid. HPLC analysis of surfactant compounds was performed by using a Hypersil BDS C18 column (100 by 4.6mm; 3µM particle diameter) held at 40ºC, and UV detection (200-400 nM) was performed on a Hewlett-Packard model 1100 HPLC diode array detector.

The samples were analyzed in a gradient of 85% eluent B to 100% after 40min. Eluent flow rate was 1ml per min. Chromatograms were analyzed using the Hewlett-Packard Chemstation Software package. Surfactants were considered identical when retention times in HPLC

chromatograms varied by less than 0.1min. The retention times of one (Occasionally two) major surfactant peak were used to cluster the isolates, hereafter referred to as *Pseudomonas spp*. strain groups.

Peaks (retention time between 27 and 36 min) with the absorption spectra at approximately 200 nm (endpoint absorption) were identified as surfactant producing Pf strains and they were found to be antifungal against major diseases of maize. Three strains were selected based on their color reactions in Hiassorted Rapid Biochemical Identification-Test kit based on their sugar utilization and subsequently used for the extraction of Viscosin for testing their antifungal potential, antiserum production and formulation. Pf-V1 strain belongs to group 1 was colonized well in dual antibiotic selection pressure (Trimethoprim, Streptomycin) and tested against maize pathogens under field conditions

Effect of biosurfactants producers on maize seed germination and seedling vigor

The germination test was conducted based on the paper towel method using seeds treated with pure PGPR suspensions. Treated and control seeds were seeded onto paper towels rinsed in a sterilized distilled water. Ten maize seeds were positioned equidistantly on a paper towel and enclosed with another pre-soaked paper towel, rolled along with the polythene packaging to avoid drying of towels. The rolled paper towels were then incubated in an incubation chamber at 24 ± 1 °C.

After incubation, the paper towels were opened and the number of germinated seeds was recorded and signified as the percent. The seedling vigor index was calculated after 10 days of incubation. To evaluate the vigor index, the mean length of the root and shoot in each variant of inoculation were measured. The vigor index (VI) was calculated using the formula VI = (mean root length + mean shoot length) × germination percent. The experimentation was conducted with four replicates of hundred seeds each, and the entire experiment was repeated thrice.

Results and Discussion

Our present investigation shows antibiotic, biosurfactant producing fluorescent pseudomonads were isolated from sugar beet-maize intercropped in sandy loam soils at Maize Research Station, Vagarai, TNAU. The strains were distinguished based on their growth, CFU/g, fluorescence, and pigment production (Fig.1). The impact of viscosin producing flurorescent pseudomonads strain on the zoospores of downy mildew pathogen of maize was studied by direct microscopy and encysted zoospores were observed. When a total of 20 fluorescent *Pseudomonads* spp. tested for their frequencies of swarming, biofilm assays where highly variable. The initial screening step for surfactant-producing Pseudomonas spp. strains was based on the drop collapse assay (Table 1).

The particular strain was tested for its growth promoting activity by treating the maize seeds for their germination, and seedling vigour performance. HPLC analysis of purified compounds confirmed the presence of Viscosin (Based on the retention time between 27 & 36 min). By comparison, V group (Associated with the production of Viscosin) produced CLPs with MW value of approximately 1120-1125 with the retention time between 31-33min (Table 2, Fig 2 & 3). We found that 5 out of 20 surfactant- producing isolates form one single group V1, since one major surfactant peak was present in all the isolates. The V1 group surfactant viscosin, produced by 25% of the strains (Table 2).

We found an interesting result of CLP producing Fluorescent Pseudomonads inhabiting Maize/Sugar beet inter cropping in sandy loam soils. Fluorescent pseudomonads can be affiliated to group under viscosin producing biotypes/biovars. Purification and characterization of viscosin by HPLC analysis was carried out. Viscosin in general receive considerable attention as potent antimicrobial drugs. viscosin was found to impact plant root architecture parameters with enhanced growth (Table 3) dependent on plant genotype.

*5 µl droplets of bacterial cell suspensions (OD600 = 1) were tested in a drop–collapse assay on Parafilm; '+' indicates a drop collapse.

Zoospore motility was observed microscopically after addition of bacterial cell suspensions (OD600 = 1) to zoospores (104 zoospores ml)1) of Downy mildew sporangia in a 1 : 1 (v/v) ratio. '+' indicates cessation of zoospore motility.

Zoospore lysis was observed microscopically after bacterial cell suspensions (OD600 = 1) were mixed with zoospores (104 zoospores ml)1) of Downy mildew sporangia in a 1 : 1 (v/v) ratio. '+' indicates zoospore lysis.

**Biofilm formation of the bacterial strains was tested in 96-well plates filled with 150 ll liquid KB medium per well. Biofilms were stained with crystal violet after 48 h of incubation. "+" indicates blue colour.

Table.1 Biosurfactant analysis of Pf-strains

Sl.No.	Strains	Biofilm formation	Drop collapse Assay	Zoospore motility	Zoospore lysis
1	Pf-V1	+	+	+	+
2	Pf-V2	+	+	+	+
3	Pf-V3	+	+	+	+
4	Pf-V4	-	-	-	-
5	Pf-V5	-	-	-	-
6	Pf-V6	+	+	+	-
7	Pf-V7	-	-	-	-
8	Pf-V8	-	-	-	-
9	Pf-V9	-	-	-	-
10	Pf-V10	+	+	+	+

Table.2 Grouping of Viscosin strains

Viscosin group	Retention time	Molecular weight (App.)	*Pf*-Biotype
V1	32.097,32.007	1125	A
V2	31.814,31.741	1124	B
V3	31.892	1124	B
V4	31.821	1124	B

Table.3 Effect of surfactant producing *Pf* strains on growth parameters of maize

Sl.No	Strains	Shoot length (cm)*	Root length (cm)*	Root weight (g)*	shoot weight (g)*
1	Pf-V1	25.83^{a}	34.45^{a}	1.35^{a}	2.00^{a}
2	Pf-V2	19.00^{c}	26.67^{c}	1.30^{b}	1.50^{c}
3	Pf-V3	13.65^{d}	27.82^{c}	0.85^{c}	1.45^{c}
4	PF	20.57^{b}	32.30^{b}	1.30^{b}	1.85^{b}
5	Control	9.05^{e}	23.70^{d}	0.50^{d}	0.75^{d}

Figure.1 Surfactant producing *Pf* strains

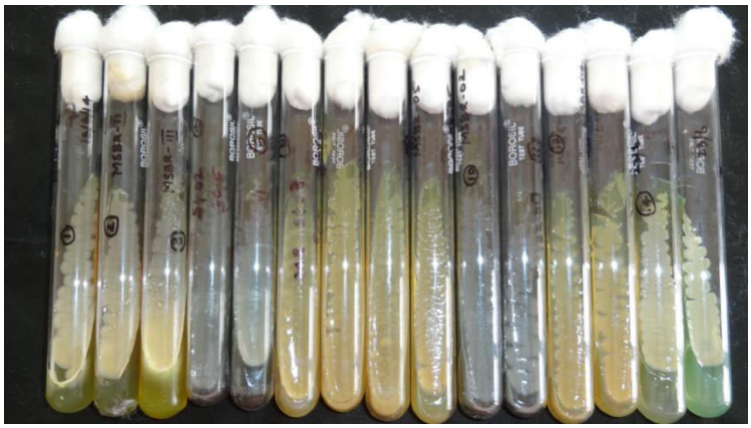


Figure.2 HPLC analysis of visosin from *Pf* surfactant strains

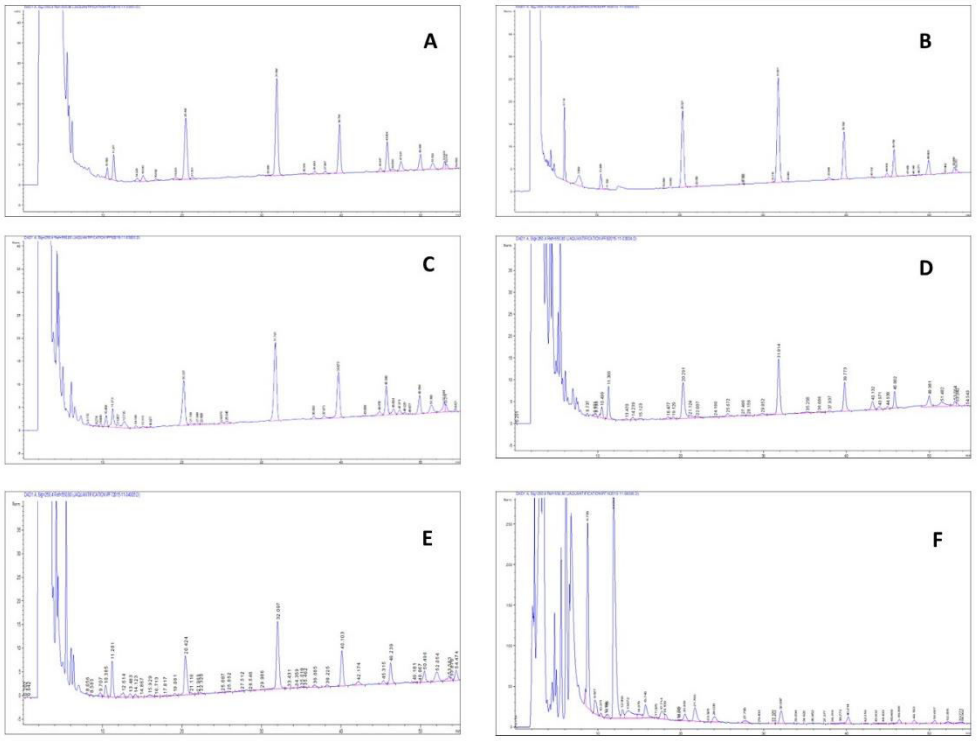
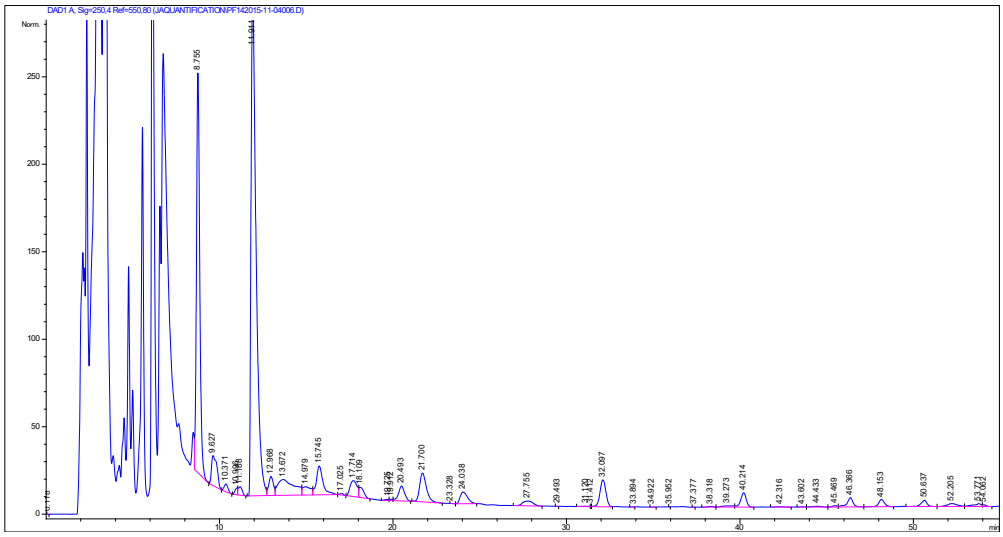


Figure.3 HPLC-Profiling of Pf-V1



Pseudomonas fluorescens can promote plant growth by producing hormones or inducing systemic resistance, but many naturally occurring strains also significantly improve plant growth by improving the bioavailability of nutrients and antagonizing pathogenic fungi and oomycetes (Raaijmakers *et al.,* 2010; Radhajeyalakshmi Raju *et al.,* 2016; Singh *et al.,* 2022). Antagonism can be conferred by the production of siderophores and of surfactants, such as viscosin and viscosinamide, as well as antimicrobial compounds, such as hydrogen cyanide, phenazines, pyrrolnitrin or 2,4-diacetylphloroglucinol (DAPG) (Haas and Défagon, 2005). Biosurfactants are involved in bacterial virulence, biofilm formation and defence against predators and pathogens in addition to having a role in multiple motility mechanisms.

Although biosurfactants include many types of molecule, the lipopeptides, which include an oligopeptide and a lipid tail, are a particularly important and well-studied family (Haas and Défagon, 2005). *Pseudomonas fluorescens* strain SBW25 is known to produce the potent antimicrobial lipopeptide viscosin (Laycock, 1991).

Moreover, we show that viscosin is important for plant root colonization and plays a further role in protecting plant roots from an oomycete pathogen. The biosurfactant viscosin has been characterized by several other research groups and has been implicated as important for motility (de Bruijn *et al.,* 2007), as a biocontrol agent that lyses oomycete zoospores, antiviral (Groupé *et al.,* 1951) for protozoan grazing defence and in altering soil water characteristics (Fechtner *et al.,* 2011). Here, we provide direct evidence that biosurfactant viscosin production is crucial in the expression of the sliding motility phenotype seen when flagella function is lost. Importantly, the observations from the two studies suggest that flagellum-based swarming is insufficient for surface movement over drier surfaces, and so the role of viscosin in promoting surface spreading in drier conditions is a good target for future research.

Cyclic lipopeptides (CLPs) with antibiotic, biosurfactant producing Fluorescent Pseudomonads were isolated and enumerated from maize rhizosphere. The impact of flurorescent Pseudomonads strain (CLP) on the zoospores of Downy mildew pathogen of maize was studied by direct microscopy and encysted zoospores were observed and field experiments were conducted for the performance of Pf-V1 strain against fungal pathogens of maize. Pf-V1 strains were found to be as effective as systemic fungicides in controlling leaf blight pathogens under field conditions in terms of systemic protection with growth promotion.

Author Contributions

Radhajeyalakshmi Raju: Investigation, formal analysis, writing—original draft. Sethuraman Kandhasamy: Validation, methodology, writing—reviewing. Sathyasheela Veluchamy:—Formal analysis, writing—review and editing. Selvakumar Thambiyannan: Investigation, writing—reviewing. Satheesh Kumar Natesan: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

References

Artursson, V., Finlay, R.D and Jansson, J. K. 2006. Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environmental Microbiology.8: 1-10. https://doi.org/10.1111/j.1462-2920.2005.00942

Biniarz, P., Łukaszewicz, M., Janek, T. 2017. Screening concepts, characterization and structural analysis of microbial-derived bioactive lipopeptides: a review. Crit Rev Biotechnol.37(3):393-410. https://doi.org/10.3109/07388551.2016.1163324.

Bruine de Bruin, W., Parker, A.M., Fischhoff, B. 2007. Individual differences in adult decision-making 221 competence. J. Person. Soc. Psychol. 92(5):938-956. http://dx.doi.org/10.1037/0022-3514.92.5.938

Cesa-Luna, C., Geudens, N., Girard, L., De Roo, V., Maklad, H.R. 2023. Martins J, De Mot, R. Charting the lipopeptidome of nonpathogenic Pseudomonas. MSYSTEMS.8(1). https://doi.org/10.1128/msystems.00988-22

Chauhan, V., Mazumdar, S., Pandey, A., Kanwar, S.S. 2023. Pseudomonas lipopeptide an excellent biomedical agent. Med Comm – Biomater Appl.; 2:27. https://doi.org/10.1002/mba2.CHAUHAN *et al.,* | 15 of 15 Page 9 of 11 The Plant Pathology Journal

de Bruijn, I., De Kock, M.J.D., Yang, M., De Waard, P., Van Beek, T.A and Raaijmakers, J.M. 2007. Genome based discovery, structure prediction and functional analysis of cyclic lipopeptide antibiotics in Pseudomonas species. Mol Microbio.63: 417–428. https://doi.org/10.1111/j.1365-2958.2006.05525.x

Fardami, A.Y., Kawo, A.H., Yahaya, S., Lawal, I., Abubakar, A.S., Maiyadi, K.A., 2022. Review on Biosurfactant Properties, Production and Producing Microorganisms. Journal of Biochemistry, Microbiology and Biotechnology.10 (1): 5–12. https://doi.org/10.54987/jobimb.v10i1.656

Fechtner, J., Koza, A., Sterpaio, P.D, Hapca, S.M and Spiers, A.J. 2011. Surfactants expressed by soil pseudomonads alter local soil-water distribution, suggesting a hydrological role for these compounds. FEMS Microbiol Ecol.78: 50–58. https://doi.org/10.1111/j.1574-6941.2011.01141.x

Geudens, N., Martins, J.C., 2018. Cyclic Lipodepsipeptides from Pseudomonas spp. - Biological Swiss Army Knives. Front Microbiol.Aug;14(9) :1867. https://doi.org/10.3389/fmicb.2018.01867.

Groupé, V., Pugh, L., Weiss, D., Kochi M. 1951. Observations on antiviral activity of viscosin. Proc Soc Exp Biol Med.1951;78:354–358 https://doi.org/10.3181/00379727-78-19071

Haas, D and Défagon, G. 2005. Biological control of soilborne pathogens by fluorescent 236 pseudomonads. Nat Rev Microbiol.3: 307–319 https://doi.org/10.1038/nrmicro1129

Laycock, M.V., Hildebrand, P.D., Thibault, P., Walter, J.A and Wright, J.L.1991. Viscosin, a potent peptidolipid biosurfactant and phytopathogenic mediator produced by a pectolytic strain of *Pseudomonas fluorescens*. J Agric Food Chem.39: 483–489. Page 10 of 11 The Plant Pathology Journal Only

Mazzola, M., de Bruijn, I., Cohen, M.F and Raaijmakers, J.M. 2009. Protozoan-induced regulation of cyclic lipopeptide biosynthesis is an effective predation defense mechanism for *Pseudomonas fluorescens*. Appl.Environ.Microbiol.75: 6804–6811. https://doi.org/10.1128/AEM.01272-09

Neu, T.R., Härtner, T & Poralla, K.1990. Surface active properties of viscosin: a peptidolipid antibiotic. Appl. Microbiol. Biotechnol.32; 518–520. https://doi.org/10.1007/BF00173720

O'Toole, G.A., Pratt, L.A., Watnick, P.I., Newman, D.K., Weaver, V.B. & Kolter.R. 1999. Genetic approaches to the study of biofilms. Methods

Enzymol.310:91-109. https://doi.org/10.1016/s0076-6879(99)10008-9

Raaijmakers, J.M., De Bruijn I., Nybroe O and Ongena, M. 2010. Natural functions of lipopeptides from Bacillus and Pseudomonas: more than surfactants and antibiotics. FEMS Microbiol Rev.34: 1037–1062 https://doi.org/10.1111/j.1574-6976.2010.00221.x

Radhajeyalakshmi Raju., Sethuraman Kandhasamy., Ganesan Kalipatty Nalliappan., Kumari Vinodhana Natarajan., Karthikeyan Gandhi and Bharathi Chandrasekaran. 2016. "Cyclic depsipeptide producing fluorescent pseudomonads exerts antifungal activity against fungal pathogens of maize (*Zea mays*). African Journal of Microbiology Research.10(42): 1767-1774. http://dx.doi.org/10.5897/AJMR2016.8265

Salek, K. and Euston, S.R. 2019. Sustainable microbial biosurfactants and bioemulsifiers for commercial exploitation. Process Biochemistry.85:143-155. https://doi.org/10.1016/j.procbio.2019.06.027

Shekhar, S., Sundaramanickam, A., Balasubramanian, T. 2015. Biosurfactant Producing Microbes and their Potential Applications: A Review. Critical Reviews in Environmental Science and Technology.45 (14): 1522 – 1554. https://doi.org/10.1080/10643389.2014.955631

Singh, P., Singh, R.K., Zhou, Y., Wang, J., Jiang, Y., Shen, N. 2022. Unlocking the strength of plant growth promoting Pseudomonas in improving crop productivity in normal and challenging environments: a review. Journal of Plant Interactions.17:220-238. https://doi.org/10.1080/17429145.2022.2029963

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