ANTIMICROBIAL EFFICACY OF BIOACTIVE COMPOUNDS OF RARE ENDOPHYTIC ACTINOBACTERIA, *Actinoalloteichus* *cyanogriseus* SIR5 (MK793584)

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ABSTRACT

To address the problem of antibiotic resistance in pathogens, our research aimed for endophytic actinobacteria, producers of a diverse array of significant bioactive metabolites. Endophytic actinobacteria SIR5 was isolated from roots of *Sphaeranthus indicus* Linn. and was identified to be *Actinoalloteichus cyanogriseus* via 16S rRNA sequencing. With the accession number MK793584, the gene sequence was deposited to NCBI. In the current study, a rare actinobacteria *Actinoalloteichus cyanogriseus*, has been reported as an endophyte for the first time. Both Microbial Type Culture Collection (MTCC) and Clinical Cultures (CC) were used to investigate the antimicrobial property of the bioactive chemicals synthesized by *A. cyanogriseus* SIR5. A significant zone of inhibitions was recorded against clinical cultures: *B. cereus* (12.16±0.16 mm), *Candida albicans* (12.83±0.44 mm), *E. coli* (15.33±0.33 mm), *S. epidermidis* (11.50±0.28 mm) and MTCC pathogens: *B. cereus* (11.16±0.16 mm), *B. subtilis* (13.33±0.16 mm), *P. aeruginosa* (13.33±0.33 mm), *S. epidermidis* (12.33±0.33 mm). The production of bioactive compound was enhanced by optimization using one factor at a time (OFAT), which was achieved with modified ISP-4 medium (starch - 1% w/v, NH₄NO₃ - 1% w/v, CaCO₃ - 2 g/l, K₂HPO₄ - 1 g/l, MgSO₄ - 1 g/l, NaCl - 1g/l, trace solution - 1 ml/l) with inoculum size - 13%, incubation period - 16 days, pH - 8.0 and temperature - 28°C.

Keywords: *Actinoalloteichus cyanogriseus*, Bioactive Compounds, Antimicrobial Activity, Endophytic Actinobacteria

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INTRODUCTION

The increased multidrug resistance (MDR) in pathogens as a result of anthropogenic activities in addition to natural processes (through hereditary changes, efflux pump, β lactamases, etc.) is alarming for public health and modern medicine.^{1,2} The situation has resulted in reduced effectiveness of approved antibiotics and thus efforts are being made to find efficient and broad-spectrum antibiotics from actinobacteria which are potential producers of diverse metabolites. Since currently available antibiotics are mainly derived from soil actinobacteria, research on endophytic actinobacteria is underway to replace repetitive discovery of known antibiotics. Endophytic actinobacteria are more likely to be involved in the metabolic pathway of the host plant and thus chances of production of some potential novel bioactive metabolites in addition to chemically similar ones are more.³ Taxane (taxol), an anticancer compound produced by the plants *Taxus brevifolia* and *Taxus baccata*, has also been obtained from its endophyte *Micromonospora* sp. and *Kitasatospora* sp. respectively,⁴ possibly evidencing the involvement of both in their metabolism. Thus to ascertain more efficient compounds, recent research has focused on rare endophytic actinobacteria, an underexplored group of microorganisms.

In this study, a rare actinobacteria, *Actinoalloteichus cyanogriseus* strain SIR5 was procured from the root tissue of medicinal weed *Sphaeranthus indicus* from Raipur, Chhattisgarh and its antimicrobial activity was observed against MTCC and Clinical pathogens. Previously, *A. cyanogriseus* has been isolated from the soils of China and the strain was authenticated to be of family *Pseudonocardiaceae*, based on phylogenetic analysis.⁵ Bioactive alkaloids, caerulomycins and cyanogramide, obtained from marine *A. cyanogriseus*, exhibited significant antibacterial, antifungal, anticancer and antiamebic activity.⁶⁻⁸

Chhattisgarh has tremendous diversity and abundance of ethnomedicinal plants, but only a few endophytic actinobacteria have been reported so far.⁹ Therefore, the present research focused on endophytic actinobacteria from this underexplored province.

EXPERIMENTAL

Materials and Methods

Isolation of Endophytic Actinobacteria

Blooming *S. indicus* plants were collected from the vicinity of Pt. Ravishankar Shukla University, Raipur, Chhattisgarh. The root tissues were processed aseptically within 24h of collection following Chandrakar & Gupta, 2015.¹⁰ The surface-sterilized root tissues were then inoculated either directly on the growth medium or were macerated and spread on ISP-1, starch casein nitrate agar (SCN agar) and yeast extract malt extract agar media. The growth of other endophytic fungus and fast-growing bacteria was inhibited by the addition of cycloheximide/ nystatin (50 µg/ml) and nalidixic acid (50 µg/ml), respectively to the culture media. The incubation period provided was 21 days at 28°C.

Morphological Characterization

The morphological characteristics such as growth pattern, colony nature, pigmentation, aerial and substrate mycelium of *A. cyanogriseus* were observed on seven different culture media; ISP-3, ISP-4, ISP-5, ISP-6, ISP-7, glucose soybean meal agar (GSMA) and SCN agar.^{11,12} The spore chain morphology was analyzed under the light microscope (Leica DM 1000) and scanning electron microscope (SEM).

16S rRNA Gene Sequencing and Phylogenetic Analysis

Extraction of the genomic DNA and PCR amplification of the strain SIR5 was done using ZR Bacterial DNA Mini Prep kit (Make Zymo Research) and universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (TACGGYTACCTTGTTACGACTT), respectively. QIAquick Gel Extraction Kit (Qiagen) was utilized for purification of the amplicon and Sanger DNA sequencing method was applied for obtaining the purified product. The analysis of obtained gene sequence was done by Finch TV software version 1.4. The constructed sequences of 16S rRNA gene were compared via BLAST tool in EzBiocloud portal (<http://www.ezbiocloud.net/>). The neighbor-joining approach was used to create a phylogenetic tree for strain SIR5 by MEGA6 software.

Test Pathogens

MTCC pathogens: *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96), *Staphylococcus epidermidis* (MTCC-435), *Escherichia coli* (MTCC-1687), *Klebsiella pneumoniae* (MTCC-3384), *Proteus vulgaris* (MTCC-744), *Pseudomonas aeruginosa* (MTCC-741), *Aspergillus niger* (MTCC-872) and *Candida albicans* (MTCC-183) were acquired from Microbial Type Culture Collection and Gene Bank, IMTECH Chandigarh. Clinical cultures (CC) of *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa*, *A. niger* and *C. albicans* were obtained from Jawaharlal Nehru Medical College, Raipur, Chhattisgarh, India.

Antimicrobial Activity

Primary Screening

A. cyanogriseus SIR5 was screened for its antimicrobial potential against the test pathogens by the cross-streak method.¹³ Growth inhibition was noted as complete inhibition (CI), declined growth (DG) and uninhibited growth (UG).

Secondary Screening

The secondary screening was performed against test pathogens, using the disc diffusion method.¹⁴ Briefly, *A. cyanogriseus* was inoculated and incubated in 50 ml of SCN broth for 14 days at 28°C and 150 rpm. The sterile disc was then loaded with crude filtrate, placed on the plates inoculated with test cultures and left for incubation at 37°C for 24 h. The antimicrobial activity was measured in terms of the diameters of halos generated. Standard antibiotic discs viz., tetracycline (10 µg) and chloramphenicol (10 µg) were used as control.

Optimization of Culture Conditions Using One Factor at a Time (OFAT) Method

Various physical, chemical and nutritional parameters were optimized by the OFAT method by varying a particular parameter and keeping the other factors constant. The most appropriate nutrition medium was selected from different culture media namely; Czapek-Dox broth (CZB), glucose soybean meal broth (GSMB), glycerol asparagine broth (GAB), ISP-4 broth, nutrient broth (NB), oat meal broth (OMB), SCN broth, soybean meal broth (SMB), tyrosine yeast extract broth (TYEB) and yeast extract malt extract broth (YEMEB). Strain SIR5 was inoculated and incubated in 100 ml of each culture medium at 28°C for 14 days at 150 rpm and then the antimicrobial activity of each of the culture filtrates was investigated. Various physical parameters affecting the production of antimicrobial compound were analyzed by taking different ranges of particular parameter such as inoculum size (1, 3, 5, 7, 9, 11, 13, 15, 17, 19%), incubation period (1- 20 days), pH (5.0, 6.0, 7.2, 8.0, 9.0) and temperature (26, 28, 30, 32, 34, 36°C). Different carbon sources (fructose, glucose, glycerol, lactose, maltose, mannitol, sorbitol, starch, sucrose, xylose) were used at 1% w/v in the medium for studying their effect on the production of the antimicrobial compound. Similarly, to observe the effect of nitrogen source, ammonium nitrate, ammonium sulfate, beef extract, malt extract, peptone, potassium nitrate, sodium nitrate, soybean meal, urea and yeast extract were dissolved in the production media at 1% w/v. For each optimization set up, *A. cyanogriseus* SIR5 was inoculated and incubated in 50 ml of SCN broth and the bioactive compound production was observed in terms of its antimicrobial efficacy. Besides, dry cell weight (g/100ml) of the culture biomass was also measured for each optimization set up.

RESULTS AND DISCUSSION

Isolation of Endophytic Actinobacteria

A gray colored colony of *A. cyanogriseus* SIR5 was isolated from the root tissue of *S. indicus* on SCN agar medium. The successful isolation of the endophytic actinobacteria was possible due to the surface sterilization process, which eliminated the epiphytic micro-organisms. To our knowledge, this study is the first to report *A. cyanogriseus* SIR5 as an endophyte. Previously, *A. cyanogriseus* has been reported from the soil⁵ and marine sediments⁸ of China. Several other diverse genera of rare actinobacteria have been reported as endophytes like; *Actinoallomurus*, *Amnibacterium*, *Amycolatopsis*, *Kineococcus*, *Micromonospora*, *Nonomuraea*, *Oerskovia*, *Promicromonospora* and *Rhodococcus*.¹⁵⁻¹⁹ Mostly, the isolated free-living actinobacteria belong to the genus *Streptomyces*, while reports on rare actinobacteria are poor. Many studies indicate the likeliness of plant internal tissues to harbor rare actinobacteria.²⁰

Morphological Characterization

The culture characteristics such as colony nature, growth pattern, pigmentation, aerial and substrate mycelium are one of the classical approaches for the identification of actinobacteria. In this study, various cultural characteristics have been observed (Table-1) in seven different culture media. The fast growth of the strain SIR5 was observed in ISP-4, GSMA and SCN agar media, whereas moderate growth was noticed in ISP-3, ISP-5, ISP-6 and ISP-7 media. Strain SIR5 was found to release soluble and colored pigments in all the used culture media, except in ISP-5. The color and development pattern of aerial and substrate mycelia also varied with culture media. On visualization under the light microscope (Leica DM 1000), the mycelium was found to be branched and fragmented with rod-shaped spores (Fig.-1B). SEM analysis revealed long, wavy spore chain morphology and rod-shaped spores of average size 1.23 x 0.46 µm (Fig.-1C and D). Marine-derived *Actinoalloteichus* species²¹ also revealed similar observations. However, contrasting culture characteristics have been recorded for the strain *A. cyanogriseus* IFO 14455^T isolated from the soil of Yunnan province of China.⁵ The differences in cultural characteristics of type strains might be due to ecological and physiological variations.

Molecular Characterization

16S rRNA gene sequencing of strain SIR5 revealed 100% similarity with *Actinoalloteichus cyanogriseus* DSM 43889 (AUBJ01000042) and thus ascertained the strain to be of the family Pseudonocardaceae. The phylogenetic tree revealed 99.56% and 96.59% similarity of *A. cyanogriseus* SIR5 to *Actinoalloteichus spitiensis* RMV-1378 (AGVX02000100)²² and *Actinoalloteichus hymeniacidonis*

HPA177, respectively while *Streptomyces carpaticus* NBRC 15390 (AB184641) was put in the out-group. Further, an accession number MK793584 was received on submission of the gene sequence of strain SIR5 to the NCBI gene bank.

Table 1: Culture Characteristics of *Actinoalloteichus cyanogriseus* SIR5

Medium	Growth and Colony Nature	Aerial mycelium	Substrate mycelium	Pigmentation
GSMA	Fast & powdery	Whitish gray	Black	Bluish black
SCN agar	Fast & powdery	Gray	Black	Bluish black
ISP-3	Moderate & powdery	Grayish white	Black	Bluish black
ISP-4	Fast & smooth	White	Black	Bluish black
ISP-5	Moderate & powdery	Off White	Gray	No soluble pigment
ISP-6	Moderate & smooth	White	Pink	Light pink
ISP-7	Moderate & powdery	Cream	Off white	Creamish white

Fig.-1: Morphology of *Actinoalloteichus cyanogriseus* SIR5; (A) in ISP-4 Medium, (B) Light Microscopic (Leica DM 1000) image at x1000, (C-D) SEM Micrograph at x5000 Magnification.

Fig.-2: Neighbor-joining Phylogenetic Tree of *Actinoalloteichus cyanogriseus* SIR5 based on 16S rRNA Gene Sequence. Values on the Nodes indicate Bootstrap Values, expressed in percentages of 1000 replication.

Antimicrobial Activity

Primary Screening

The antimicrobial activity of strain SIR5 was assessed primarily against 20 pathogens from MTCC and CC, including eight Gram-positive and eight Gram-negative bacteria and four fungal cultures. The results

(Table-2) showed that all the bacteria except *P. aeruginosa* (CC) were inhibited and significant antimicrobial activity was recorded against *B. cereus*, *S. aureus*, *S. epidermidis*, *B. subtilis* and *E. coli* (Fig.-3). However, all the fungal pathogens except *C. albicans* (CC) were found to be resistant. This result supports the hypothesis of the existence of antimicrobial potential in *A. cyanogriseus* SIR5. Several investigations on the bioactive potential of endophytic actinobacteria indicate that they may be a reservoir of many important pharmaceutical compounds.²³⁻²⁵

Table-2: Primary Screening of Antimicrobial Activity of *Actinoalloteichus cyanogriseus* SIR5

Pathogens		An	Bc	Bs	Ca	Ec	Kp	Pa	Pv	Sa	Se
Antimicrobial Activity	MTCC	UG	DG	DG	UG	CI	DG	DG	DG	DG	DG
	Clinical	UG	CI	CI	DG	CI	DG	UG	DG	DG	CI

An - *A. niger*, Bc - *B. cereus*, Bs - *B. subtilis*, Ca - *C. albicans*, Ec - *E. coli*, Kp - *K. pneumoniae*, Pa - *P. aeruginosa*, Pv - *P. vulgaris*, Sa - *S. aureus*, Se - *S. epidermidis*, DG - declined growth, UG - uninhibited growth, CI - complete inhibition

Fig.-3: Antimicrobial Activity of *Actinoalloteichus cyanogriseus* SIR5 against the pathogens; (A-B) Test Plate, (C) Control

Secondary Screening

The crude filtrate of *A. cyanogriseus* SIR5 was tested for antimicrobial activity against the pathogens inhibited in primary screening. The zone of inhibition (ZOI) was recorded (Fig.-4) as 15.33 ± 0.33 , 13.33 ± 0.16 , 13.33 ± 0.33 , 12.33 ± 0.33 , 12.83 ± 0.44 , 12.16 ± 0.16 , 11.50 ± 0.28 and 11.16 ± 0.16 mm against *E. coli* (CC), *B. subtilis* (MTCC), *P. aeruginosa* (MTCC), *S. epidermidis* (MTCC), *Candida albicans* (CC), *B. cereus* (CC), *S. epidermidis* (CC) and *B. cereus* (MTCC), respectively. However, the filtrate produced less effect against *S. aureus* (CC), *P. vulgaris* (CC) and *K. pneumoniae* (MTCC) with ZOI recorded as 9.16 ± 0.16 , 9.83 ± 0.33 and 9.33 ± 0.33 mm, respectively. The overall findings suggested that Gram-negative bacteria were more resistant than Gram-positive bacteria, except *E. coli* (CC) and *P. aeruginosa* (MTCC). The endophytic actinobacteria isolated from medicinal herbs also exhibited similar antimicrobial activity.¹⁰ The susceptibility to antimicrobial compounds may be explained in terms of the cell wall complexity. Vulnerability of Gram-positive bacteria to antimicrobial compounds is attributed to the existence of a single peptidoglycan layer in their cell wall, whereas the existence of lipopolysaccharide (LPS) layer over the peptidoglycan layer in Gram-negative bacteria makes them less susceptible.²⁶

Optimization of Culture Conditions

In this study, *A. cyanogriseus* SIR5 was grown with variations in nutritional, physical and chemical parameters and the antimicrobial activity, in terms of bioactive compound production efficacy, was recorded against the pathogens. The maximum inhibition zone against the pathogens indicated higher antimicrobial compound production. Minimum and maximum production was observed in OMB and ISP-4 medium, respectively (Fig.-5A). Optimization of inoculum size (Fig.-5B) revealed a gradual increase in metabolite production from 1-13% of inoculum size and thereafter decline was observed. The dry cell biomass of the strain also increased accordingly. The production of antimicrobial compound started from the 6th day of incubation and maximum yield was noticed on the 16th day (Fig.-5C). The optimum pH of the medium was found to be 8.0 by OFAT (Fig.-5D), while at pH 5, 6 and 9, strain SIR5 was unable to produce the antimicrobial compound. Optimum temperature was determined by growing the strain at

various temperatures, such as 26, 28, 30, 32, 34 and 36°C. Maximum and minimum production of the compound was noticed at 28°C and 34°C, respectively (Fig.-5E).

Fig.-4: Secondary Screening of Antimicrobial Activity of *Actinoalloteichus cyanogriseus* SIR5

Fig.-5: Effect of Nutritional, Physical and Chemical Parameters on Antimicrobial Activity of *Actinoalloteichus cyanogriseus* SIR5 against *E. coli* (CC) and *B. subtilis* (MTCC); A - Culture media, B - Inoculum size, C - Incubation period, D - pH, E - Temperature, F - Carbon sources, G - Nitrogen sources.

Medium containing starch and ammonium nitrate as carbon and nitrogen source respectively were found to be best for compound production (Fig.-5F and G) while xylose and peptone containing medium showed minimum production. When cultured in optimized conditions, the antimicrobial compound production enhanced significantly which was visible by increased ZOI from 14.66 ± 0.16 to 30.33 ± 0.16 mm against *E. coli* (CC) and 12.66 ± 0.33 to 26.33 ± 0.33 mm against *B. cereus* (MTCC). These results are in agreement with various other studies, which suggest that after optimization, the efficiency of bioactive compounds increases significantly and the optimum fermentation conditions for metabolite production may vary with strains, species, and genus of actinobacteria.²⁷⁻²⁹

CONCLUSION

Actinobacteria have contributed significantly to the discovery of efficient antibiotics to combat multidrug-resistant bacteria. The current study suggested that special attention to rare endophytic actinobacteria may uncover many diverse and broad-spectrum antibiotics, since they are an underexplored group. Our findings showed that *A. cyanogriseus* SIR5 has potent antimicrobial activity against a wide range of pathogenic bacteria and also ensures the existence of some rare endophytic actinobacteria in Chhattisgarh as it has huge therapeutic floral diversity.

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REFERENCES

- G. L. Conn, V. Bavro, C. Davies, *Frontiers in Molecular Biosciences*, **6**, 71(2019), <https://doi.org/10.3389/fmolb.2019.00071>
- World Health Organization, High Level of Antibiotic Resistance Found Worldwide, New Data Shows, World Health Organization, Geneva, Switzerland (2018).
- L. M. Castronovo, A. Vassallo, A. Mengoni, E. Miceli, P. Bogani, F. Firenzuoli, R. Fani, V. Maggini, *Pathogens*, **10**, 106(2021), <https://doi.org/10.3390/pathogens10020106>
- M. Caruso, A. L. Colombo, L. Fedeli, A. Pavesi, S. Quaroni, M. Saracchi, G. Ventrella, *Annals of Microbiology*, **50**, 3(2000).
- T. Tamura, L. Zhiheng, Z. Yamei, K. Hatano, *International Journal of Systematic and Evolutionary Microbiology*, **50**, 1435(2000), <https://doi.org/10.1099/00207713-50-4-1435>

6. P. Fu, S. Wang, K. Hong, X. Li, P. Liu, Y. Wang, W. Zhu, *Journal of Natural Products*, **74**, 1751(2011), <https://doi.org/10.1021/np200258h>
7. P. Fu, F. Kong, X. Li, Y. Wang, W. Zhu, *Organic Letters*, **16**, 3708(2014), <https://doi.org/10.1021/ol501523d>
8. Y. Zhu, P. Fu, Q. Lin, G. Zhang, H. Zhang, S. Li, C. Zhang, *Organic Letters*, **14**, 2666(2012), <https://doi.org/10.1021/ol300589r>
9. S. Chandrakar, A. K. Gupta, *Probiotics and Antimicrobial proteins*, **11**, 1055(2018), <https://doi.org/10.1007/s12602-018-9451-6>
10. S. Chandrakar, A. K. Gupta, *Proceedings of the National Academy of Sciences. India Section B*, **87**, 905(2017), <https://doi.org/10.1007/s40011-015-0668-9>
11. Q. Li, X. Chen, Y. Jiang, C. Jiang, Morphological identification of actinobacteria, *Actinobacteria-Basics and Biotechnological Applications*, *InTech Open*, Rijeka, Croatia, 59(2016) <https://doi.org/10.5772/61461>
12. E. B. Shirling, D. Gottlieb, *International Journal of Systematic and Evolutionary Microbiology*, **16**, 313(1966).
13. B. Pandey, P. Ghimire, V. P. Agrawal, *Journal of Biological Sciences*, **23**, 44(2004).
14. M. Balouri, M. Sadiki, S. K. Ibnsouda, *Journal of Pharmaceutical Analysis*, **6**, 71(2016), <https://doi.org/10.1016/j.jpha.2015.11.005>
15. C. A. Bascom-Slack, C. Ma, E. Moore, B. Babbs, K. Fenn, J. S. Greene, B. D. Hann, J. Keehner, E. G. Kelley-Swift, V. Kembaiyan, S. J. Lee, P. Li, D.Y. Light, E. H. Lin, M. A. Schorn, D. Vekhter, L. A. Boulanger, W. M. Hess, P.N. Vargas, G. A. Strobel, S. A. Strobel, *Microbial Ecology*, **58**, 374(2009), <https://doi.org/10.1007/s00248-009-9494-z>
16. G. K. Bian, Z. Z. Feng, S. Qin, K. Xing, Z. Wang, C. L. Cao, C. H. Liu, C. C. Dai, J. H. Jiang, *Antonie van Leeuwenhoek*, **102**, 621(2012), <https://doi.org/10.1007/s10482-012-9757-4>
17. C. Li, H. Wang, P. Jin, W. Zheng, L. Chu, C. Liu, X. Wang, *Antonie van Leeuwenhoek*, **108**, 453(2015), <https://doi.org/10.1007/s10482-015-0498-z>
18. I. Kim, G. Chhetri, J. Kim, T. Seo, *Antonie van Leeuwenhoek*, **112**, 1731(2019), <https://doi.org/10.1007/s10482-019-01302-7>
19. K. Zhao, P. Penttinen, T. Guan, J. Xiao, Q. Chen, J. Xu, K. Lindstrom, L. Zhang, X. Zhang, G. A. Strobel, *Current Microbiology*, **62**, 182(2011), <https://doi.org/10.1007/s00284-010-9685-3>
20. A. S. Azman, I. Othman, S. S. Velu, K. G. Chan, L. H. Lee, *Frontiers in Microbiology*, **6**, 856(2015), <https://doi.org/10.3389/fmicb.2015.00856>
21. P. A. Jose, S. R. D. Jebakumar, *Indian Journal of Geo-Marine Sciences*, **44**, 428(2015).
22. A. K. Singla, *Thesis*, CSIR-IMTECH, Chandigarh/Jawaharlal Nehru University, New Delhi, India, (2004).
23. N. Bunbamrung, C. Intaraudom, A. Dramaee, C. Thawai, S. Tadtong, P. Auncharoen, P. Pittayakhajonwut, *Phytochemistry*, **172**, 112275(2020), <https://doi.org/10.1016/j.phytochem.2020.112275>
24. S. Qin, J. Li, H. H. Chen, G. Z. Zhao, W. Y. Zhu, C. L. Jiang, L. H. Xu, W. J. Li, *Applied and Environmental Microbiology*, **75**, 6176(2009), <https://doi.org/10.1128/AEM.01034-09>
25. S. Qin, K. Xing, J. H. Jiang, L. H. Xu, W. J. Li, *Applied Microbiology and Biotechnology*, **89**, 457(2011), <https://doi.org/10.1007/s00253-010-2923-6>
26. D. W. Green, *Expert Opinion on Therapeutic Targets*, **6**, 1(2002), <https://doi.org/10.1517/14728222.6.1.1>
27. G. Rajivgandhi, R. Vijayan, M. Kannan, M. Santhanakrishnan, N. Manoharan, *Bioactive Materials*, **1**, 140(2016), <https://doi.org/10.1016/j.bioactmat.2016.11.002>
28. R. Ranjan, V. Jadeja, *Journal of Pharmaceutical Analysis*, **7**, 343(2017), <https://doi.org/10.1016/j.jpha.2017.05.001>
29. R. Balachandar, N. Karmegam, R. Subbaiya, P. Boomi, D. Karthik, M. Saravanan, *Biocatalysis and Agricultural Biotechnology*, **20**, 101186(2019), <https://doi.org/10.1016/j.bcab.2019.101186>

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