16S rRNA-Based Metagenomic Analysis of Endophytic Actinomycetes Diversity from *Tinospora crispa* L. Miers

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Endophytic actinomycetes associated with medicinal plants is very important as source of various bioactive compounds. The fact that more than 99% of microbes that exist in nature may have the potency but still unexplored. Published data regarding diversity of endophytic actinomycetes from *Tinospora crispa* is mainly based on a culturable approach. This paper describes the first reported data regarding metagenomic analysis on the diversity of endophytic actinomycetes from *T. crispa* based on 16S rRNA gene using PCR-DGGE. There were some similarities amongst endophytic actinomycetes found in stems, roots, and leaves with soil actinomycetes community in the rhizosphere of *T. crispa*. There were a total of 21 bands found from the DGGE analysis which were interpreted using Phoretix 1D software. Diversity of actinomycetes in the stems, leaves, roots were represented by 17, 16, and 14 bands, respectively. Whereas only 10 bands represented diversity of actinomycetes in the soil rhizosphere. The 12 dominant and or different bands with 180 bp in size were molecularly sequenced. The A4 and A9 bands have 95% and 86% similarities with *Williamsia* and *Streptomyces*, respectively. These similarities were less than 97% thus may indicate novel actinomycetes. The other 10 sequenced bands have closed similarity ranging from 97-100% and they were closely related to the genus *Streptomyces*, *Microbacterium*, *Amycolatopsis*, *Actinomadura*, *Actinoplanes*, *Actinokineospora*, *Kibdelosporangium*, *Williamsia*, and *Kocuria*. These findings indicate that diversity of actinomycetes can be found associated with *T. crispa*.

**Key words:** 16S rRNA, DGGE, endophytic actinomycetes, metagenomic, *Tinospora crispa*

Endophytic actinomycetes from *T. crispa* are capable of producing secondary metabolites which can also function as an antidiabetic agent (Pujiyanto *et al*. 2012). This phenomenon is presumably the result of genetic exchange and evolution between endophytes and their host (Tan and Zou 2001). The biological function of *T. crispa* as an antidiabetic agent may be associated with the presence and diversity of actinomycetes in the host plant tissue. Actinomycetes are very important microbes as they have an ability to

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*Tinospora crispa* L. Miers is a medicinal plant traditionally utilized by Asian people as an antidiabetic agent (Puranik *et al*. 2010; Patela and Mishrab 2012).

Endophytic actinomycetes from *T. crispa* are capable of producing secondary metabolites which can also...
produce various metabolite compounds. Previous study done by Pujiyanto et al. (2012) showed that among several medicinal plants which were traditionally used as antidiabetics possessed endophytic actinomycetes which may function as source of antidiabetics. Amongst the 65 culturable endophytic actinomycetes isolated from those antidiabetic plants, 32 isolates were found from T. crispa.

Diversity of actinomycetes in T. crispa can be explored using culture dependent and or independent approaches. Isolation of actinomycetes using a culture-dependent method has faced several constraints such as cultivation conditions, techniques used, isolation media and cultivation time (Qin et al. 2012). These partly because many members of the actinomycetes genus are relatively slow-growing and some genera are often difficult to be cultured (Nimnoi et al. 2010). Moreover, it is only 0.1-10% of all microbes in nature can be cultured in the laboratory (Zeyaullah et al. 2009). Culturable microbes can grow rapidly in nutrient-rich media, aerobic conditions and at moderate temperatures (Thontowi 2009), however, more than 99% of microbes that exist in nature, which may have a very useful potential, are still not known nor utilized (Zeyaullah et al. 2009). The suitable method, i.e. a culture-independent, needs to be used to explore whole diversity of endophytic actinomycetes. For that purpose, a metagenomic approach, which does not require culture, is used for genome analysis of bacterial communities (Thontowi 2009).

The DGGE (Denaturing Gradient Gel Electrophoresis) technique is used to study microbial communities in situ. PCR-DGGE result can be used to observe metagenomic data, especially from the dominant endophytic actinomycete species that colonizes plant organs and the rhizosphere (Nimnoi et al. 2010). Information on endophytic actinomycete diversity from T. crispa based on 16S rRNA gene using a culture-independent technique has remained obscure. This research aimed to explore diversity of endophytic actinomycetes in T. crispa based on 16S rRNA gene using actinomycetes specific primers (Table 1). Step 1 amplification was conducted using Promega GoTaq Green Master Mix with the mixed composition consisted of 12.5 µL of master mix 27f dan 16Sact1114r 60 pmol primers at 0.25 µL each, 5 µL of DNA template and added with nuclease free water to a volume of 25 µL. PCR conditions used were pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 60 s, annealing at 55 °C for 45 s (a decrease at 0.5 °C per cycle was set for the first 20 cycles, the last decrease was at 55 °C), elongation at 72 °C for 120 s for 30 cycles and final elongation at 72 °C for 7 min (Zhang et al. 2013). Step 2 amplification also used Promega GoTaq Green Master Mix with the mixed composition of 25 µL of Promega GoTaq Green, P338f-GC dan P518r 100

**MATERIALS AND METHODS**

**Sample Extraction and Surface Sterilization.** T. crispa plants were obtained from Medicinal Plants Collection Garden, Biopharmaca Research Center, Institut Pertanian Bogor. The leaves, stems and roots of plants were cleaned and cut into pieces, then their surfaces were sterilized sequentially by soaking in 70% alcohol for 1 min, then in 1% sodium hypochlorite (NaOCl) for 5 min, followed by soaking in 70% alcohol for 1 min. In the final step, samples were rinsed 3 times with sterile distilled water. To prove that only endophytic actinomycetes genom were extracted, the last soaking water during surface sterilization process was spreaded on Humic Acid Vitamin B (HV) medium and checked for their grown colonies (Combs and Franco 2003) used as negative control test. Soil samples were taken from rhizosphere of T. crispa at a depth of approximately 1-5 cm from the soil surface.

**Genomic DNA Extraction from Endophytic Actinomycetes and Soil Actinomycetes.** Genomic DNA of endophytic actinomycetes in plant samples was extracted using Genomic DNA Mini Kit (Plant) Geneaid. Soil DNA was extracted using the Power Soil DNA Isolation Kit (Mobio Laboratories, Carlsbad, CA, USA). The used procedures followed the standard protocol recommended by the manufacturer. The extraction results were then quantitatively checked using Nano Drop 2000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

**PCR for DGGE.** Amplification was done using nested PCR technique which was conducted to obtain propagation of 16S rRNA gene using actinomycetes specific primers (Table 1). Step 1 amplification was conducted using Promega GoTaq Green Master Mix with the mixed composition consisted of 12.5 µL of master mix 27f dan 16Sact1114r 60 pmol primers at 0.25 µL each, 5 µL of DNA template and added with nuclease free water to a volume of 25 µL. PCR conditions used were pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 60 s, annealing at 65 °C for 45 s (a decrease at 0.5 °C per cycle was set for the first 20 cycles, the last decrease was at 55 °C), elongation at 72 °C for 120 s for 30 cycles and final elongation at 72 °C for 7 min (Zhang et al. 2013). Step 2 amplification also used Promega GoTaq Green Master Mix with the mixed composition of 25 µL of Promega GoTaq Green, P338f-GC dan P518r 100
pmol primers at 0.25 µL each, 1 µL of step 1 PCR products was used as a DNA template and added with nuclease free water to a volume of 50 µL. Amplification was conducted using T1-thermocycler (Biometra, Goettingen, Germany) with these following optimizations: pre-denaturation at 94 °C for 5 min, denaturation at 94 °C for 60 s, annealing at 55 °C for 45 s, elongation at 72 °C for 60 s for 30 cycles and final elongation at 72 °C for 5 min (Zhang et al. 2013).

The amplification result was then observed by migrating 5 µL of the amplicon on 1% agarose gel, 80 volts for 45 min. The migration result was then stained with 0.1% ethidium bromide for 15 min and then viewed under UV transiluminator and documented using Gel Doc. The remaining PCR products were stored at -20 ºC prior to be analysed using DGGE.

DGGE (Denaturing Gradient Gel Electrophoresis). The DGGE was conducted using the D Code Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). The amount of 30 µL template (25 µL of PCR products + 5 µL of Loading Dye) was migrated at 1 mm 8% polyacrylamide gel (acrylamide-bisacrylamide [37.5:1]) in a 7 L 1X TAE (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA) using urea as a denaturant. The denaturant gradient of 30% -70% (100% denaturant was made with 8.4 g of 7 M urea, 8 ml of formamide, 0.4 mL of 50x TAE, 4 mL of acrylamide-bisacrylamide and added with sterile distilled water to a volume of 20 mL). Migration was conducted at 60 °C and 150 volts for 5 h. Once the migration was completed, the gel was soaked and stained with SYBR Safe (Invitrogen-Molecular Probes, Carlsbad, CA, USA) for 1 h. Gel visualization was conducted using the G:BOX (Syngene, Frederick, MD, USA). The selected dominant and or different bands were cut and each band was put into a microtube containing 100 µL of ddH2O and stored for 24 hours at 4 °C. Acrylamide gel image results with G:BOX were analyzed using 1D Phoretix software to estimate the total band appeared. DGGE products was settled for 24 h, then the products were vortexed and amplified in PCR instrument using primers without GC Clamp with similar PCR conditions.

Sequencing of 16S rRNA Gene and Construction of Phylogenetic Tree. The PCR products were then sent to the sequencing service company (1stBASE Malaysia). Sequencing was performed using double stranded PCR-DGGE product. Sequencing results were compared to the data base from the NCBI website (http://www.ncbi.nlm.nih.gov) with the Basic Local Alignment Search Tool (BLAST). Nucleotide sequence alignment and phylogenetic tree construction were conducted using MEGA 5.2 software. Construction of a phylogenetic tree was conducted using the Neighbour Joining method.

RESULTS

PCR-DGGE Profile and Phylogenetic Tree of Actinomycetes Based on 16SrRNA Genes. The last soaking water which was placed in HV test medium showed no growth, proving that only genomic DNA of endophytic actinomycetes were extracted. The genomic DNA concentrations of 35.1 ng µL⁻¹, 37.2 ng µL⁻¹, and 30.9 ng µL⁻¹ were obtained from the roots, stems and leaves, respectively. The soil g enomic DNA concentration obtained was 3.7 ng µL⁻¹. The purity of DNA obtained from this extraction with the A260/A280 parameter ranged from 1.6 to 1.7 which slightly lower than the recommended value (1.8-2.0) (Sambrook and Russell 2001), indicating some
impurities. However, this impurity seemed to not interfere during the DNA replication process.

Amplification with nested PCR was conducted in 2 steps using actinomycetes specific primers i.e. 27f/16Sact1114r and P338fGC/P518r. Step 1 PCR was successfully amplifying the band with a length of ~1087 bp (Fig 1a), while step 2 PCR was at ~180 bp length (Fig 1b). In this study, 12 dominant and different bands were obtained from DGGE profile (Fig 2a). There were some similarities between endophytic actinomycetes community found in stems, roots, leaves and soil. Interpretation using Phoretix 1D software showed that diversity of actinomycetes community were represented by 17, 16, 14 and 10 bands in the stems and leaves, roots and soil, respectively. Total number of bands obtained with this software was 21 bands (Fig 2b). The 12 dominant sequenced bands revealed their genetic relationship based on their phylogenetic tree (Fig 2c).

Analysis of phylogenetic tree based on genetic distance matrix (p-distance) showed nucleotide sequence similarity in the stems and leaves, but there were several differences from the roots and soil (Fig 2d). Almost all endophytic actinomycetes who reside in leaves have also been found in the stems, while for roots and soil, their actinomycetes were more varied.

The alignment results of 12 partial sequences (~180 bp) of endophytic actinomycetes from *T. crispa* plant and soil actinomycetes in the rhizosphere was analyzed using the BLAST program (Table 2). The A2 and A10 sequences have 100% similarity with Actinokineospora auranticolor strain IFO 16518 and Amycolatopsis orientalis strain HCCB10007, respectively. A1, A3, A5, A6, A7, A8, A11 and A12 sequences have ≥ 97% similarity with *Streptomyces acidiscabies* strain ATCC 49003, *Williamsia marianensis* strain DSM 44944, *Kocuria halotolerans* strain YIM 90716, *Microbacterium ginsengisoli* strain Gsoil 259, *Kibdelosporangium philippinense* strain A 80407, *Actinomadura rifamycini* strain JCM 3309 and *Actinoplanes* sp., respectively. A relatively new sequence was also found based on the percentage of a maximum identity from the BLAST results showing the data base sequence similarity at 95% and 86% of the ~180bp (A4 and A9 sequences). These sequences were closely related with *Williamsia marianensis* strain DSM 44944 and *Streptomyces javensis* strain B22P3, respectively. The phylogenetic relatedness of endophytic actinomycetes 16S rRNA gene sequences from *T. crispa* and rhizosphere soil was compared with closely related strains sequenced from database (Fig 3).

**Abundance of Endophytic Actinomycetes in Plants and Soil Actinomycetes.** Based on the nucleotide partial sequenced at ~180 bp length, there were 12 species of endophytic and soil actinomycetes dominant in *T. crispa* plant. Among the 12 species obtained, one species found in the soil has a closely related with *Streptomyces acidiscabies* strain ATCC 49003 (1). Meanwhile, six endophytes and soil actinomycetes have a close relationship to *Actinokineospora auranticolor* strain IFO 16518 (2), *Kocuria halotolerans* strain YIM 90716 (5), *Microbacterium ginsengisoli* strain Gsoil 259 (6), *Kibdelosporangium philippinense* strain A 80407 (8), *Streptomyces javensis* strain B22P3 (9), and Acti-

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**Fig 1** (A) Step 1 amplification results of 16S rRNA gene of endophytic actinomycetes in roots, stems, leaves of *T. crispa* plant (A), (B), (D) and soil (T) (~1087bp) and (B) Step 2 with length of ~180bp.
Fig 2  (A) DGGE profile 16S rRNA gene of endophytic actinomycetes from *T. crispa* plant and soil actinomycetes; (B) Interpretation from Phoretix 1D software. Numbers next to bands indicate the cut/splitted bands for re-amplification. Wells from left to right: (T) soil, (D) leaves, (B) stems, and (A) roots; (C) Re-amplification DGGE bands; (D) p-distance analysis.

**Table 2** Sequence similarity of 16S rRNA gene of endophytic actinomycetes from medicinal plant *T. crispa* and soil actinomycetes with reference strain (GenBank)

<table>
<thead>
<tr>
<th>Comparison sequence from database</th>
<th>Band</th>
<th>Maximum identity</th>
<th>E-Value</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces acidiscabies</em> strain ATCC 49003</td>
<td>A1</td>
<td>97%</td>
<td>1e-81</td>
<td>NR116534.1</td>
</tr>
<tr>
<td><em>Streptomyces javensis</em> strain B22P3</td>
<td>A9</td>
<td>86%</td>
<td>6e-46</td>
<td>NR028958.1</td>
</tr>
<tr>
<td><em>Williamsia marianensis</em> strain DSM 44944</td>
<td>A3</td>
<td>97%</td>
<td>2e-80</td>
<td>NR043263.1</td>
</tr>
<tr>
<td><em>Williamsia marianensis</em> strain DSM 44944</td>
<td>A4</td>
<td>95%</td>
<td>1e-76</td>
<td>NR043263.1</td>
</tr>
<tr>
<td><em>Kocuria halotolerans</em> strain YIM 90716</td>
<td>A5</td>
<td>99%</td>
<td>8e-89</td>
<td>NR044025.1</td>
</tr>
<tr>
<td><em>Kocuria halotolerans</em> strain YIM 90716</td>
<td>A7</td>
<td>99%</td>
<td>1e-83</td>
<td>NR044025.1</td>
</tr>
<tr>
<td><em>Actinokineospora auranticolor</em> strain IFO 16518</td>
<td>A2</td>
<td>100%</td>
<td>2e-89</td>
<td>NR040873.1</td>
</tr>
<tr>
<td><em>Microbacterium ginsengisoli</em> strain Gsoil 259</td>
<td>A6</td>
<td>97%</td>
<td>1e-81</td>
<td>NR041516.1</td>
</tr>
<tr>
<td><em>Kibdelosporangium philippinense</em> strain A 80407</td>
<td>A8</td>
<td>98%</td>
<td>2e-85</td>
<td>NR025572.1</td>
</tr>
<tr>
<td><em>Amycolatopsis orientalis</em> strain HCCB10007</td>
<td>A10</td>
<td>100%</td>
<td>3e-89</td>
<td>NR103940.1</td>
</tr>
<tr>
<td><em>Actinomadura rifamycini</em> strain JCM 3309</td>
<td>A11</td>
<td>99%</td>
<td>3e-88</td>
<td>NR113155.1</td>
</tr>
<tr>
<td><em>Actinoplanes</em> sp. SE 50/110 strain SE 50/110</td>
<td>A12</td>
<td>98%</td>
<td>2e-84</td>
<td>NR074431.1</td>
</tr>
</tbody>
</table>
nomadura rifamycini strain JCM 3309 (11). There were 5 species which found only as endophytes which were closely related with Williamsia marianensis strain DSM 44944 (3,4), Amycolatopsis orientalis strain HCCB10007 (10), Kocuria halotolerans strain YIM 90716 (5), Actinoplanes sp. strain SE 50/110 (12) (Fig 4a).

There were several similar endophytic actinomycetes found in different plant organs (Figure 4b). Actinomycetes that have a close relationship with Williamsia marianensis strain DSM 44944 (3,4) and Actinoplanes sp. strain SE 50/110 (12) were found in roots and stems, while Kocuria halotolerans strain YIM 90716 (5) were found in roots and leaves. The actinomycetes which were found in roots, stems and leaves were closely related with Actinokineospora auranticolor strain IFO 16518 (2), Microbacterium ginsengisoli strain Gsoil 259 (6), Amycolatopsis orientalis strain HCCB10007 (10) and Actinomadura rifamycini strain JCM 3309 (11), Streptomyces javensis strain B22P3 (9), Kibdelosporangium philippinense strain A 80407 (8) and Kocuria halotolerans strain YIM 90716 (7).
DISCUSSION

As the first published report, this study describes metagenomic diversity of endophytic actinomycetes in roots, stems, leaves of *T. crispa* plant and actinomycetes from rhizosphere soil based on 16S rRNA-gene using PCR-DGGE technique. DGGE profiles shows each separate band which representing one of its own species community. The dominant species and the intensity of each band indicates their relative abundance (Nimnoi et al. 2010). Amplification with nested PCR and DGGE was also successfully detects actinomycetes community in the soil, plant rhizosphere of *Artemisia tridentata* and from roots of *Aquilaria crassna* (Heuer et al. 1997; Franco et al. 2009; Nimnoi et al. 2010). Genetic distance (p-distance) analysis of 16S rRNA gene sequences of the selected bands indicates similarity of the actinomycetes community between stems and leaves, but has several differences from roots and soil. This phenomenon is predicted due to the migration of actinomycetes from stems to the leaves, soil and roots. Differences in the nucleotide sequences based on the p-distance matrix indicates species diversity between each sample. The differences are thought to be influenced by differences in isolated parts of plant organs and the effect of actinomycetes found in phyllosphere.

Endophytic actinomycetes community was found to be more diverse than rhizosphere soil actinomycetes. Relatively low actinomycetes community in the soil was allegedly due to the high competitive niches in the soil (Hibbing et al. 2010). Endophytes are sheltered from environmental stresses and microbial competition by the host plant and they seem to be ubiquitous in plant tissue (Nimnoi et al. 2010). Endophytic actinomycetes population in the stems and leaves exhibit a more diverse than in the roots. While relatively low actinomycetes community in the roots was allegedly due to the migration of roots endophytic actinomycetes to other plant organs as a result of the old plants used in this research work.

Association between actinomycetes in the soil with endophytic actinomycetes occur as it is seen in Figure 4a. Several endophytic actinomycetes in *T. crispa* plant organs are also found in the soil rhizosphere. Actinomycetes which abundantly live in the rhizosphere soil can colonize and penetrate into plant roots (Sardi et al. 1992; Nimnoi et al. 2010). With a passive penetration, microbial endophytic can also move in the open space or cracks in the root tip (Nimnoi et al. 2010). Actinomycetes in roots migrate to other plant organs through the intercellular and vascular system (Feng et al. 2004). Similar results were also reported by Qin et al. (2012) that the abundance of endophytic actinomycetes on the stems and leaves of medicinal plant *Maytenus austroyunnanensis* showed greater diversity than the roots. These phenomenon may affects the production cycle of the active compounds in the leaves and stems of *T. crispa*, which often used for medicinal treatment. Microbial endophytics of medicinal plants have a role in the biochemical cycles and production of bioactive compounds in their host (Zhao et al. 2011).

The study shows that several similar endophytic actinomycetes are found in different plant organs (Fig 4b), which is presumably due to the migration of the endophytic actinomycetes between plant organs (Tian et al. 2007). The endophytes can come from the surrounding environments, such as rhizosphere and phyllosphere of plants which can enter the plant tissue through stomata, lenticel and physical injuries (broken trachoma) or area in which the lateral roots emerged (Susilowati 2010). Interaction between endophytic microbes and plants is regarded as symbiosis mutualism. Under this type of interaction, endophytic microbes obtains nutrients and protection from the host plant, but they produce compounds that can trigger plant growth and suppress pathogens, phosphate solvent, assimilation of nitrogen for plants (Rosenblueth and Romeo 2006) and able to produce enzyme inhibitor (Khamna et al. 2009; Pujiyanto et al. 2012).

Based on the partial sequences similarity and phylogenetic relatedness of 16S rRNA gene, there are two species which were closely related to the genus *Streptomyces*. *Streptomyces* is the dominant actinomycetes isolated from the soil rhizosphere (Khamna et al. 2009). The structure of microbial community in the soil is determined by environmental factors such as soil characteristics, type of plants and cultivation that can cause the changes in the structure of soil microbes (Zhao et al. 2012). Plant roots exudates stimulate the growth of actinomycetes in the rhizosphere and the synthesis of antimicrobial compounds. The diversity of endophytic actinomycetes species in roots and stems of potato samples which were analyzed with DGGE showed to have a high similarity with *Streptomyces* (Sessitsch et al. 2002). On the agar medium (a culture-dependent), *Streptomyces* are the more frequently found genus.
Microbacterium (Zhang was reported to have antibacterial and anti-fungal activity (Qin et al. 2009) and producing β-glucosidase (Park et al. 2008). Using a similar approach, endophytic Actinomadura was isolated from roots of Aquilaria crassna (Nimnoi et al. 2010). Actinomadura has been reported to produce Indole Acetics Acid (IAA) as a growth hormone and produce siderophore (Khamna et al. 2009) as well as antibacterial compounds (Qin et al. 2009). This work found a band which has sequence similarity with Kocuria. Kocuria halotolerans strain YIM 90716 is rarely found as endophytic actinomycetes. This genus is reported to have been found in seawater, marine sediment, water and desert soil (Kaur et al. 2011). It has the ability to produce IAA and siderophore (Khamna et al. 2009) as well as biosurfactant (Sarafin et al. 2014). For Williamsia which are found in this study as endophyte, this genus has also been found in roots of Eucalyptus microcarpa (Kaewkla & Franco 2012). It has an ability to produce RDX (Hexahydro-1,3,5-Trinitro-1,3,5-Triazine) bioremediation (Thompson et al. 2005). Kibdelosporangium is also a rare genus reported as an endophytic (Janso & Carter 2010). It has a capability in producing antiviral cycloviracins (Tomita et al. 1993) and kibdelomycin (Philips et al. 2011). As endophytic actinomycetes, Amycolatopsis and Actinoplanes were isolated from uncultivatedly rice paddy roots (Tian et al. 2007). Amycolatopsis was found to have antibacterial and anti-fungal activity (Qin et al. 2009), whereas Actinoplanes produced Teicoplanin, a glycopeptide antibiotic for the treatment of multiresistant gram-positive infection (Taurino et al. 2011) and α-glucosidase inhibitors (Zhang et al. 2003). Meanwhile the α-glucosidase inhibitors can also be produced by Streptomyces isolated from T. crispa (Pujiyanto et al. 2012). It is clearly described that actinomycetes able to produce various bioactive compounds thus have open the potency to discover new bioactive compounds.

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REFERENCES

Abdelmohsen UR, Cheng C, Viegellman C, Zhang T,


