INTRODUCTION

There has been a large body of literature describing potential uses of plant-associated bacteria as agents stimulating plant growth and managing soil and plant health1). Some of these bacteria colonize the root surfaces and the closely adhering soil surface, rhizosphere, but some others enter root interior, followed by establishing endophytic populations. Regardless of the survival sites, almost all microbes produce and secrete secondary metabolites such as lytic enzymes2,3), plant hormones, toxins, antibiotics, siderophores and other biologically active materials, as well-documented in reviews by Compant et al.1), Takahashi and –Omura4) and Kohmoto and Otani5). As is expected, these molecules very often interfere with growth of other organisms.

Since Matsukuma et al.6) and Okazaki et al.7) discovered that a variety of actinomycetes inhabit a wide range of plants as either symbions or parasites, endophytic actinomycetes have been studied extensively as potential sources of novel antibiotics and physiological activators8–10). Shimizu et al.11–13) developed a unique biocontrol method using an endophytic actinomycete: they isolated Streptomyces galbus strain R-5 that shows a broad spectrum of antimicrobial activity, from field-grown rhododendron and added it to flasks in which tissue-cultured seedlings of rhododendron were growing to successfully render them disease-resistant. Similarly, Nishimura and colleagues14,15) succeeded to produce disease-resistant tissue-cultured seedlings of mountain laurel by applying an endophytic strain AOK-30 of S. padanus to the flakes with growing mountain laurel seedlings. Furthermore, Hasegawa et al.16,17) showed increase of drought tolerance in the AOK-30-treated seedlings by enhancement of osmotic pressure in leaf cells, acceleration of callose and lignin accumulation in cell walls. Igarashi et al.18–20) discovered novel antibiotics and growth regulators of plants and animals from several species of actinomycetes of plant origin. These papers show that secondary bioactive metabolites of endophytic actinomycetes apparently affect physiological properties of host plants. In this paper, we report isolation and selection of a strain of endophytic actinomycete that is able to stimulate rooting of plants.

MATERIALS AND METHODS

Isolation of actinomycetes from field-grown rhododendron

Endophytic actinomycetes were isolated from young plants of rhododendron that were harvested from the Akatsu Garden Co. Ltd. (Tsu-City, Mie Prefecture, Japan), by following the method of Shimizu et al.13). Briefly, small pieces of leaves, stems and roots were rinsed in 0.1% Tween 20 for a few seconds and then in 1% sodium hypochlorite for 5 min and washed in sterilized distilled water for a few minutes. They were further surface-sterilized in 70% ethanol for 1 min and air-dried in a laminar flow chamber. Each piece was placed on IMA-2 agar medium11). Actinomycetous filaments growing from the respective samples were transferred onto freshly pre-

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pared IMA-2 medium with fine glass fibers and incubated at 30°C for several days. Colonies on the medium were independently transferred onto membrane filters (mixed cellulose ester, pore size = 0.2 μm, Advantec-Toyo, Japan), which were placed on the medium surface with sterilized needles and incubated at 30°C for 1 week. New colonies which grew on the medium surface several days after removing the membrane filters, were isolated. A total of 102 isolates was obtained from leaves, stems and roots, and numbered as isolates 1~102. To simplify the experiments, isolates Nos. 1~29 that grew poorly on IMA-2 medium were not used for the subsequent experiments. A total of 73 isolates (Nos. MBR 30~102) were suspended respectively in 20% glycerin solution and then maintained at −80°C until use.

Selection of the isolates that promote root growth of cucumber seedlings

The 73 stocked isolates were transferred into A-3M liquid medium and cultured on a shaker at 30°C for 3 days. One ml of each culture was transferred into freshly-prepared A-3M liquid medium and subjected in a further 6-day shaking culture. To extract metabolites of each isolate, the same volume of acetone as that of the medium was added into these culture tubes and incubated on a shaker at 30°C for 24 hr. The supernatant was obtained from the medium-acetone mixture by centrifugation (3,000rpm) at 4°C for 10 min. Acetone was evaporated at 35°C with a rotary evaporator and the resultant extract was used for the subsequent test. The acetone extract of fresh A-3M medium was prepared as similarly as above for control.

Cucumber seeds (cv. Kaga-fushinari) were surface-sterilized with 1% sodium hypochlorite for 3 min followed by washing with sterilized deionized water three times. They were incubated on sterilized wet filter papers in Petri dishes for 28°C in dark to allow them to germinate.

Three germings were placed on a sterilized filter paper infiltrated with 0.5 ml of 1/2 diluted extract in an autoclaved glass tube (10 cm long and 2.5 cm diameter). The glass tubes were sealed with sterilized aluminum sheets. Two glass tubes were prepared for each isolate. In controls the filter papers were infiltrated with 1 ml of sterilized deionized water or 1/2 diluted extract of A-3M medium. All tubes were incubated at 28°C in dark for 7 days. Length of a main root of each seedling was measured with a digital scale and a total dry weight of roots of three seedlings was determined for each isolate.

As described later, 7 out of 73 test isolates that promoted growth of main roots (longer than 80 mm) were further used to test their promoting effects using 1/10, 1/100 and 1/1000 diluted extract as similarly as above.

Because isolate MBR-52 showed the most conspicuous promoting effect as explained later, adventitious roots from seedlings were counted until 3 days after initiation of treatment with 1/1000 diluted extract of MBR-52

Colonization of MBR-52 on tissue-cultured seedlings of rhododendron

Colonization of this isolate on tissue-cultured seedlings of rhododendron (cultivars: Sakigake and Wedding bouqet) was examined by the modified method of Shimizu et al.12). Tissue-cultured seedlings of rhododendron were grown as described in detail in previous paper12). Briefly, 500 μl of the stock suspension of MBR-52 was inoculated to 100 ml of GY broth and incubated on a shaker at 30°C overnight. Two ml of the mycelial suspension (3~4 × 10⁶ cfu/ml) was spread on the surface of the multiplication medium in glass flasks supporting rhododendron seedlings that originated from tissue-culture. The seedlings, treated and untreated with MBR-52, were incubated for an additional 10 days before transplanting into soil. Five seedlings were transplanted in 20 g of autoclaved soil-mixture of peat moss, perlite, vermiculite, satulite (67:16:16:1), with 100 ml of deionized water in a plastic plant box (6.5 × 6.5 × 10 cm³). To avoid excess humidity in the box, 5 circular openings (6 mm in diameter) in the lid were sealed with filter papers (Millipore Co.). The seedlings were grown in an incubator conditioned at 25°C with 12 hr illumination per day at 11.8 Wm⁻² for 3 weeks. Every one week, 15 seedlings were randomly taken out of the boxes and each seedling was separated into root and terrestrial part. The latter was cut at intervals of every two nodes with a flame-sterilized knife in a laminar flow chamber. Each piece was placed on IMA-2 agar medium then incubated at 30°C for 1 week. The number of test nodes from which MBR-52 grew was recorded.

Effects of MBR-52 on root growth of tissue-cultured seedlings of rhododendron

Number of adventitious roots emerging from stem bases was recorded for all seedlings taken out of the plant boxes in the above experiment to determine effects of MBR-52 on root growth.

In another experiment, the seedlings of cultivars Sakigake and Wedding bouquet, treated and untreated with MBR-52 in flasks as above, were transplanted into a plug tray containing the above soil-mixture that was not autoclaved. The plug-trays were 33 × 27.5 × 5.5 cm³ with 80 cells and individual cell volume was ca. 25 cm³. All transplanted seedlings in the cell-trays were grown in a large plastic box with a transparent lid. Moisture in the box was kept by placing wet cloths at the bottom. A total of 75 seedlings were grown in each plug-tray and 15 seedlings were taken out every week after the first and third weeks for Sakigake and Wedding bouquet, respectively.

Cultural, physiological and morphological characteristics of MBR-52

MBR-52 that had been grown on IMA-2 medium for 2 weeks was transferred to ISP 2–5 media and incubated at 30°C for 3~4 weeks. The visible colony characteristics were recorded as shown in Table 1.
Utilization of various carbon sources by MBR-52 was examined as follows. Flasks with 60 ml of ISP-9 agar medium (Daigo, Nihon Pharmaceutical Co., Tokyo) were autoclaved at 121°C for 20 min. The medium was cooled to 60°C and then the various sugars (D-glucose, L-arabinose, inositol, D-xylose, L-rhamnose, raffinose, D-mannitol, D-fructose or sucrose) were independently added into the medium. Before addition to the ISP-9 medium, 60 mg of each sugar was added to an appropriate amount of ethyl ether in a separate flask and sealed with a silicon plug overnight. The ethyl ether was evaporated and the sugar dried. Mycelia from the stock culture of MBR-52 were streaked onto each plate with a sterilized needle and incubated at 28~32°C for 10~16 days. Growth of the colony on each plate was compared with that on ISP-9 medium with D-glucose and ISP-9 medium without sugars as positive and negative controls, respectively.

Formation of melanin-like pigment by MBR-52 was examined by incubation of this strain on ISP-6 and -7 media at 30°C for 2~4 days. Color of the medium around the colony was compared with a standard color grade. Specimens for scanning electron microscopy were prepared as follows. Mycelia from a stock culture of MBR-52 were suspended in sterilized water. Fifty µl of the suspension was spread on ISP-3 medium and incubated at 30°C for 1 week. Small disks of the culture were fixed in OsO4 vapor overnight and then immersed into liquid nitrogen. The frozen specimens were freeze-dried overnight and observed with a scanning electron microscope (Hitachi Co., S-4000) after gold-coating.

Analysis of whole cell sugars

One ml of 1N H2SO4 was added to 50 mg of freeze-dried MBR-52 in a vial with a screw cap and heated at 120°C for 15 min. The mixture was centrifuged at 3,000 rpm for 10 min. The supernatant was adjusted to pH 5.5 with saturated barium hydroxide, followed by centrifugation at 6,000 rpm for 10 min. The supernatant was mixed with a small amount of ethanol and evaporated with a rotary evaporator until dry. Dried matter which was dissolved in 300 µl of distilled water was used as a sample. One µl of the sample and standard sugar solution (D-glucose, D-galactose D-mannose, L-arabinose, D-fructose, D-xylose, or L-rhamnose) were spotted separately onto TLC cellulose plate (cellulose F, Merck). The plate was developed in the solvent (n-buthanol:distilled water:pyridine:toluene = 10:6:6:1) for 5 hr. The plate was dried in a chemical hood for 2 hr and sprayed with aniline phthalate, followed by heating with a hair dryer.

Analysis of 16S rDNA full nucleotide sequence of MBR-52

Analysis of 16S rDNA full nucleotide sequence of MBR-52 was charged to NCIMB Japan Co. Ltd. (Shimizu-city, Japan). Briefly, mycelia were grown on IMA-2 medium at 30°C for 10 days. DNA was extracted using InstaGene Matrix (BIO RAD, USA) by following the manufacturer’s protocol. 16S rDNA (1,500~1,600 bp) was multiplied by the PCR method. The nucleotide sequence was analyzed with ABI PRISM 3100 DNA sequencer (Applied Biosystems, USA). The homology and phylogeny analyses were performed with DNA base sequence database (GeneBank/DDBJ/EMBL) using BLAST.

RESULTS

Selection of the isolates that accelerate root growth of cucumber seedlings

| Table 1. Cultural characteristics of MBR-52 colony on ISP media |
|---------------------|-----------------|----------------|
| Media               | Characters       |                  |
| Yeast malt extract agar (ISP-2 medium) | Substrate mycelium | Good growth, pale reddish yellow (130)\(^a\) |
|                     | Reverse of substrate mycelium | Strong reddish yellow (143) |
|                     | Aerial mycelium | Powdery, beige gray (401) |
|                     | Diffusible pigment | Bright reddish yellow (136) |
| Oatmeal agar (ISP-3 medium) | Substrate mycelium | Good growth, brownish gold (160) |
|                     | Reverse of substrate mycelium | Grayish brown (118) |
|                     | Aerial mycelium | Powdery, light bluish gray (404) |
|                     | Diffusible pigment | None |
| Inorganic salts-starch agar (ISP-4 medium) | Substrate mycelium | Good growth, pale reddish yellow (126) |
|                     | Reverse of substrate mycelium | Soft reddish yellow (146) |
|                     | Aerial mycelium | Powdery, beige gray (401) |
|                     | Diffusible pigment | None |
| Glycerol-asparagine agar (ISP-5 medium) | Substrate mycelium | Good growth, soft reddish yellow (146) |
|                     | Reverse of substrate mycelium | Gold (162) |
|                     | Aerial mycelium | Powdery, beige gray (400) |
|                     | Diffusible pigment | None |

\(^a\) : color no. as cited in reference\(^29\).
Length of main roots from cucumber germlings grown in deionized water (control) was 83.3 mm on average. Diluted A-3M medium extract suppressed elongation of main roots, giving their average length only 4.7 mm. Among 73 test isolates, 40 suppressed root growth of the germlings as similarly as A-3M medium extract. However, the main root-lengths of cucumber germlings that were grown in culture media of 7 isolates (MBR-50, 52, 54, 58, 59, 60, 66) were similar to or more than that of water control. Among them, three isolates (MBR-52, 58, 60) enhanced the root-growth up to more than 90 mm significantly (P<0.05). Therefore, these seven isolates were selected for the secondary screening of the target candidate.

**Root-growth accelerating effects of the selected isolates at varied dilutions of their culture extract**

Effects of diluted acetone extract of culture medium of 7 selected isolates on root growth were tested. Average length of the main roots from cucumber germlings grown in control water was 81.0 mm. As indicated in Fig. 1A, 1/10 diluted extracts of most isolates except MBR-52 suppressed root growth. The 1/1000 diluted extracts of MBR-50 and 52 enhanced root growth significantly (P<0.01), giving average root lengths 104.5 mm and 115.9 mm, respectively. These two isolates gave 2.8 and 3.0 µg of dry weight of roots, respectively, while water control did 2.3 µg (Fig. 1B). Thus, MBR-52 was selected as a final target candidate for the next experiment using tissue-cultured seedlings of rhododendron.

**Colonization of MBR-52 in tissue-cultured seedlings of rhododendron**

Colonization of the target candidate, MBR-52, in tissue-cultured seedlings of rhododendron is one of the keys for a successful goal of the current research. Tissue-cultured seedlings were treated with MBR-52 in flasks for 10 days and then transferred to autoclave-sterilized soil, followed by 1 week incubation. Within 1 week after placing their segments on IMA-2 medium, mycelia of MBR-52 expanded from roots and the first through third stem segments of the seedlings of cultivars, Sakigake and Wedding bouquet (Fig. 2). Similar positive results were obtained when the seedlings grown in soil for additional 2 weeks. Because the autoclaved-soil was used, only one type of mycelia colonies which was similar in appearance to that of the original MBR-52 was expanding from the segments. Thus it is plausible that MBR-52 spread on tissue-culture medium colonized in the seedlings and remained surviving there at least 3 weeks after transplanting in soil.

**Acceleration of emergence of adventitious roots from tissue-cultured seedlings of rhododendron**

When tissue-cultured seedlings of rhododendron were grown in autoclaved soil in plant boxes, adventitious roots emerged from the stem bases that had been buried in soil (Fig. 3A, B). Fifteen seedlings of cultivar Sakigake were examined from 1 to 2 weeks after transplantation. As indicated in Fig. 4, the average numbers of adventitious roots from the seedling treated with MBR-52 reached 0.4, 1.1 and 2.7 on 1, 2 and 3 weeks after transplanting, respectively. By contrast, those from the MBR-52-untreated seedlings were 0.1, 0.3 and 0.7 on the same weeks, respectively. Until 3 weeks after transplanting, MBR-52 apparently accelerated emergence of adventitious roots. However, regardless of the treatment and untreatment, the number of adventitious roots reached 4.4 on 5 weeks. Thus, the MBR-52 treatment evidently accelerated emergence of adventitious roots from transplanted tissue-cultured seedlings at the initial stage of transplanting. Similar results with no significant differences were obtained when cultivar Wedding bouquet was used, although emergence of the roots delayed almost 1 week when compared with that of Sakigake.

Emergence of the roots was accelerated in MBR-52-treated seedlings which were grown in unautoclaved soil in plug-trays (Fig. 5A, B). Rooting from the treated seedlings of Sakigake initiated by 2 weeks after transplantation and the average root number reached 6.2 by 3 weeks, while that in the untreated seedlings was only 0.5 by 3 weeks. As similarly as in autoclaved soil, the number of adventitious roots from MBR-52-untreated seedlings caught up that of the treated seedlings by 5 weeks. Thus, the MBR-52 treatment significantly accelerated rooting from the seedlings of Sakigake (P<0.05) within 3 weeks after transplanting. Rooting from the seedlings of Wedding bouquet did not occur before 3 weeks, as similarly as in autoclaved soil in plant boxes. Although the average root number was always greater in MBR-52-treated seedlings than in untreated ones until 5 weeks (Fig. 5B), the comparative numbers were not significantly different (P>0.05).

**Characterization of MBR-52**

On all test ISP 2~5 media, substrate mycelia grew actively (Table 1). Abundant, powdery aerial mycelia formed and turned bluish to beige gray when mature on all test media. Colors of substrate mycelia and the reverse of colonies varied on the respective media (Table 1). Melanin-like pigment was produced on ISP-6 medium but not on ISP-7.

Substrate mycelia grew slightly on ISP-9 medium without any sugar (negative control), whereas they grew actively on the medium containing D-glucose (positive control). Substrate mycelia grew more actively on ISP-9 media containing inositol, D-xylose or sucrose than the positive control, whereas their growth was not beyond that of the positive control in ISP-9 media containing any of other test sugars.

Light microscopy revealed that 10 to 50 spores (average 25) with a smooth surface formed in a slightly wavy chain (rectiflexibles type). Aerial hyphae with a smooth surface were 0.5~0.7 µm wide. Scanning electron
microscopy demonstrated that ellipsoidal spores (0.6 × 1.2 µm on average) were smooth and in slightly spiral chains (Fig. 6).

Galactose was a major sugar component among whole-cell sugars (data not shown)

**Analysis of 16S rDNA full nucleotide sequence of MBR-52**

The analysis revealed that 16S rDNA of only *Streptomyces* spp. occupied the top 20 species having a higher homology with that of MBR-52. Table 2 shows the top five species with identity. From the table it is evident that the DNA showed a highest homology, 99.6%, with that of *S. ciscaucasicus*. Although it is most likely that MBR-52 belongs to this species, another possibility still remains that this isolate belongs to a phylogenically different strain, because the DNA sequence of MBR-52 did not perfectly match that of *S. ciscaucasicus*. Thus, we had better treat it as *Streptomyces* sp. tentatively until other evidences are available.

**DISCUSSION**

As was expected, more than 100 isolates of actinomycetes were obtained from roots, stems and leaves of rhododendron. They took at least 10 days to grow out of epidermal cracks of these organs, as Nishimura *et al.*14 and Shimizu *et al.*11 reported. Since the mycelial mass of all isolates pushed the epidermis of the organs outwards, all these strains were considered as endophytic rather than ectophytic microbes. Because some isolates had the same colony color and appearance and reverse of substrate mycelium, they probably belong to the same or closely related strain.

Among 73 test isolates, 7 isolates (MBR-50, 52, 54, 58, 59, 60, 66) accelerated rooting from cucumber germlings.

![Fig. 1. Effects of diluted acetone-extracts of culture broth of test isolates on length of main roots (A) from cucumber seedlings and dry weight (B) of their roots.](image-url)
Because these isolates had diverse cultural appearances, each of them seemed to belong to different strains. Subsequent detailed experiment revealed that MBR-52 most prominently enhanced elongation and dry weight of cucumber roots even at 1/1000 dilution (Fig. 1A, B). Thus this isolate was selected as a final candidate of root-growth accelerator. Sardi et al.\textsuperscript{21} obtained 499 isolates, most of them being \textit{Streptomyces} spp., from roots of 13 plant species. They classified these isolates into 72 groups based on recorded characteristics. Major groups of actinomycetes that can be isolated from plant materials are \textit{Streptomyces} spp. and \textit{Microbispora} spp.\textsuperscript{3,10,22}. The current analysis of 16S rDNA of MBR-52 demonstrated that the top 20 strains being highly homologous belonged to only the genus \textit{Streptomyces}. SEM images and the cultural (Table 1), chemical and morphological characters (Fig. 6) supported

Fig. 2. Re-isolation of MBR-52 from segments of roots and terrestrial parts of tissue-cultured seedlings of rhododendron that were transplanted to autoclaved soil in plant boxes. One week after placed on IMA-2 medium. In each frame, left = MBR-52 untreated seedlings and right = treated. A: root segment, B, C & D: the first, second & third-node segments of terrestrial part from their base. Note that MBR-52 mycelia emerged only from the treated seedlings.

Fig. 3. Emergence of adventitious roots from the stem base of tissue-cultured seedlings of rhododendron that were treated or untreated with MBR-52 previously in flasks. A, B: untreated and treated seedlings, 3 weeks after transplanting in autoclaved soil in plant boxes. C, D: untreated and treated seedlings, 3 weeks after transplanting in un-autoclaved soil in plug-trays. Greater numbers of the roots emerged from the treated seedlings than the untreated.
that MBR-52 belonged to genus *Streptomyces* described by Hatano\(^2\)). Furthermore, the analytical data by NCIMB Japan Co. Ltd. showed that the nucleotide sequence of 16S rDNA of MBR-52 had a 99.6% homology to *Streptomyces ciscaucasicus*. However, the BLAST database analysis suggested another possibility that MBR-52 could be a phylogenically close strain of *S. ciscaucasicus*. Thus, the species of MBR-52 has not been determined yet.

MBR-52 was added to tissue-culture flasks in which seedlings of rhododendron were growing. These seedlings were transplanted to autoclaved soil 10 days later and grown at least 1 week. As illustrated in Fig. 2, it was reisolated from the seedlings within 1 week after their segments had been placed on IMA-2 medium, as similarly as endophytic strains R-5 of *Streptomyces galbus*\(^{12}\) and AOK-30 of *S. padanus*\(^{15}\). This result plausibly shows that MBR-52 can colonize in rhododendron, a host plant. However, it is needed to prove that re-isolated colonies are certainly identical with those of the original MBR-52 before a final conclusion.

As shown in Figs. 4 and 5, emergence of adventitious roots from stem bases were accelerated when tissue-cultured seedlings treated with MBR-52 in flasks had been transplanted in autoclaved and unautoclaved soils. Such an accelerating effect was more apparent in cultivar Sakigake than Wedding bouquet (Fig. 5). Regardless of MBR-52 treatment and untreated, the Sakigake seedlings produced similar numbers of the roots by 5 weeks after transplanting into soil. However, when the seedlings were treated with MBR-52, adventitious roots emerged 2~3 weeks earlier. Such an acceleration of rooting has a significant advantage to tissue culture technique. Tissue-cultured seedlings are routinely acclimatized in warm and humid spaces such as green houses or plastic tunnels until their newly-born roots start to absorb water from soil. However, such an environment is very often beneficial for disease pathogen’s activity. Thus, if the seedlings can be transferred to an open-air space even a week earlier, they can escape from the risk of disease infection. In this sense, acceleration of rooting by colonization of MBR-52 in tissue-cultured seedlings gives a great practical benefit for them to avoid disadvantageous environment.

Not only plants but also microorganisms can synthesize...
plant-growth promoters such as auxins and cytokinins\textsuperscript{24–28}). The role of plant hormone biosynthesis by microorganisms is not fully understood: in several cases of pathogenic fungi and bacteria these compounds are involved in pathogenesis on plants. Auxin and cytokinin production may also be involved in root growth stimulation by beneficial bacteria and associate symbiosis\textsuperscript{24). Manulis et al.\textsuperscript{25) reported that various Streptomyces spp. including S. violaceus, S. scabies, S. griseus, S. exfoliates, S. coelicolor and S. lividans secreted indole-3-acetic acid (IAA) when fed with L. tryptophan. Their additional studies proved that S. violaceus and S. exfoliates synthesized indole-3-acetamide (IAM), indole-3-lactic acid (ILA), indole-3-ethanol (IEt) and IAA from tryptophan and that their cells catabolized IAM, ILA and IEt into IAA. Igarashi et al.\textsuperscript{8,20) isolated pteridic acids A and B from the culture broth of Streptomyces hygroscopicus TP-A0451 as plant growth promoters with auxin-like activity. They showed that these acids induced the formation of adventitious roots in hypocotyls of kidney beans at 1 nM as effectively as auxin (IAA), natural plant growth hormone. These acids were extracted from the culture broth using aqueous acetone as similarly as the current rooting accelerator of MBR-52. As indicated in Fig. 1A, the acetone extract of MBR-52 culture broth showed the accelerative effect of rooting from cucumber germlings even at 1/1000 dilution level. Because we have not succeeded in purification and identification of the key component of rooting accelerator that MBR-52 produces, the minimum effective dose of this component remains unsolved. Recently we found two active fractions of culture broth of MBR-52 that accelerate rooting of several plant species. The trials to purify and identify the putative component of MBR-52 are now going on.

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