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ORIGINAL ARTICLE

Glutathione S-transferase pi (GST-pi) inhibition and anti-inflammation activity of the ethyl acetate extract of *Streptomyces* sp. strain MJM 8637

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Abstract To investigate the anti-cancer properties of soil-borne actinobacteria, MJM 8637, the glutathione S-transferase pi (GST-pi) assay, anti-tumor necrosis factor (TNF)-α assay, the level of antioxidant potential by DPPH radical scavenging activity, NO scavenging activity, and ABTS radical scavenging activity in ethyl acetate extract were determined. The 16S rDNA sequencing analysis revealed that *Streptomyces* sp. strain MJM 8637, which was isolated from Hambak Mountain, Korea, has 99.5% similarity to *Streptomyces atratus* strain NBRC 3897. The physiological and the morphological characteristics of the strain MJM 8637 were also identified. The ethyl acetate extract of MJM 8637 inhibited TNF-α production approximately 61.8% at concentration 100 μg/ml. The IC_{50} value of the strain MJM 8637 extract on GST-pi was identified to be 120.2 ± 1.6 μg/ml. In DPPH, NO, and ABTS radical scavenging assays, the IC_{50} values of the strain MJM 8637 extract were found to be 977.2 μg/ml, 1143.7 μg/ml, and 454.4 μg/ml, respectively. The ethyl acetate extract of the strain MJM 8637 showed 97.2 ± 1.3% of cell viability at 100 μg/ml in RAW 264.7 cell viability assay. The results obtained from this study suggest that the ethyl acetate extract of *Streptomyces* sp. strain MJM 8637 could be considered as a potential source of drug for

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the cancers that have multidrug resistance with its GST-pi inhibition and anti-inflammation activities, and low cytotoxicity.

1. Introduction

Today, cancer is a growing worldwide problem. The GLOBOCAN reports that the number of new cancer cases was 14.1 million and deaths caused by cancer were 8.2 million in 2012 (Wild, 2014). In the countries of the Eastern Mediterranean Region (EMR) of the World Health Organization (WHO), the increase of new cancer cases is estimated that from 555,000 to 819,000 by 2025 (Hofseth and Ying, 2006). For prevention and treatment of cancer, a large number of anti-cancer agents have developed from natural plants, microorganisms and marine life (Wang et al., 2012). However, there is no perfect anti-cancer agent against disseminated cancer, because of resistance to a broad range of anti-cancer drugs of cancer cells. The most common reason for acquisition of drug resistance is active transporter systems that detect and emit anti-cancer drugs from cancer cells, but induction of drug-detoxifying mechanisms also play a crucial role in acquired anti-cancer drug resistance (Gottesman, 2002).

Glutathione transferases (GSTs; also known as glutathione S-transferase; EC 2.5.1.1.8) are a multi-gene superfamily of isoenzymes ubiquitously present in most living organisms. These enzymes catalyze the nucleophilic addition of glutathione (γ-Glu-Cys-Gly; GSH) to a variety of electrophilic compounds (Hayes et al., 2005; Laborde, 2010) and the resulting GSH conjugated compounds are exported from cells by ATP-binding cassette (ABC) transporters (Gong et al., 2012). The GSTs are classified as three major families, cytosolic, mitochondrial, and microsomal, and cytosolic GSTs are sorted into seven classes, α (*alpha*), μ (*mu*), π (*pi*), σ (*sigma*), θ (*theta*), ω (*omega*), and ζ (*zeta*) (Mannervik et al., 2005). Recent studies revealed that expression level of GST-pi is elevated in various cancer cells that have multidrug resistance to anti-cancer drugs, and suggested inhibition of GST-pi can be a solution to overcome multidrug resistance of cancer cells (Backos et al., 2012; Lu et al., 2011; Mukanganyama et al., 2002; Rolland et al., 2010; Wang et al., 2011).

Recent studies revealed that chronic inflammation increases genomic damage, disruption of DNA repair systems, inhibition of apoptosis, and the promotion of angiogenesis and invasions, and all of these processes may lead to the initiation and progression of cancers (Hofseth and Ying, 2006), and also displayed that anti-inflammatory agents are useful in the treatment of a variety of cancers (García-Lafuente et al., 2009; Liu et al., 2010, 2014; Rayburn et al., 2009; Wang and DuBois, 2013).

Streptomyces, which is the largest genus belonging to the phylum actinomycetes, is Gram-positive filamentous bacteria and they have very high genomic G+C contents (Euzéby 2008; Kämpfer 2006). Most *Streptomyces* species show a complex life cycle. They form substrate hyphae, which are multicellular and filamentous, and in the next stage, aerial hyphae, which are a second filamentous cell type, are produced into the air from the substrate hyphae. The aerial hyphae are divided into prespore compartments, and these prespores developed to mature spores via maturation steps (Elliot et al., 2008).

The actinomycetes is a dominant group in the soil and a large number of actinomycetes have already been isolated from soil and studied (Kuster, 1968; Muiru et al., 2008). Over 75% of all antibiotics have been produced from actinomycetes, and among them, approximately 80% are discovered from the genus *Streptomyces* (Kieser et al., 2000; Thakur et al., 2009; Tiwari and Gupta, 2012). Moreover, many anti-cancer drugs (migrastatin, bleomycin, doxorubicin, etc.) have been discovered from *Streptomyces* strains (Arcamone et al., 1969; Shan et al., 2005; Umezawa et al., 1966). So, the genus *Streptomyces* can be a useful natural resource for screening of novel anti-cancer agent.

In this study, we isolated one *Streptomyces* strain MJM 8637 from mountain forest soil in Korea and identified the cultural and phylogenetic characteristics of the strain MJM 8637. To investigate anti-cancer properties of the strain MJM 8637, GST-pi enzyme inhibition assay and TNF-α production assay, which is for anti-inflammation test, were conducted. Additionally, antioxidant activities of the ethyl acetate (EtOAc) extract of strain MJM 8637 were also tested through a series of *in vitro* tests such as reducing power measurement, nitric oxide (NO) scavenging activity, ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging activity), and DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging activity.

2. Materials and methods

2.1. Chemicals

The following reagents and solvents were purchased from Sigma–Aldrich: ascorbic acid, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium ferricyanide, trichloroacetic acid, ferric chloride, sodium nitroprusside, sulfanilamide, phosphoric acid, naphthyl ethylenediamine dihydrochloride, sodium nitrite, and Folin–Ciocalteu's phenol.

2.2. Isolation of Streptomyces from mountain forest soil

The soil samples were collected from Hambak mountain, Gyeonggi-do province, Korea (latitude 37° 12′ 51.00″ N, longitude 127° 11′ 20.00″ E), in September, 2007. To collect the soil samples, 3 cm of the soil surface was removed and the samples were dug up from a depth of 20 cm. For isolation of *Streptomyces* from the soil samples, 1000 mg of the soil was suspended in 50 ml of deionized water, and then it was vortexed for 5 min. The soil–water suspension was allowed to settle, and serially diluted up to 10^{-4}. Then, an aliquot of 0.2 ml of each dilution was spread on glucose-yeast extract–malt agar

(GYM; medium 65; DSMZ) and Bennet's agar (BN; medium 548; DSMZ), and incubated at 28 °C for 7 days. Repeated streaking on GYM and BN agar plates led to purified bacterial colonies that showed an actinomycetes-like appearance.

2.3. Morphological identification of the strain MJM 8637

Morphological identification of the strain MJM 8537 was performed according to the traditional morphological criteria including characteristics of colonies on the plate, the presence of aerial mycelium, spore mass color, distinctive reverse colony color, and diffusible pigment (Goodfellow and Cross, 1984). The cultural characteristics of the strain MJM 8637 were observed after incubation for 7 days on 4 different media, yeast extract–malt extract agar (ISP-2), oatmeal agar (ISP-3), salt-starch agar (ISP-4), and glycerol–asparagine agar (ISP-5).

2.4. Physiological characteristics

Carbon source utilization pattern of the strain MJM 8637 was identified according to the methods of Gottlieb (1961). The isolated strain was grown on the basal medium, which was composed of the following: KH_2PO_4, 2.38 g; $KH_2PO_4{\cdot}3H_2O$, 5.65 g; $MgSO_4{\cdot}7H_2O$, 1 g; $(NH4)_2SO_4$, 2.64 g; trace element solution, 6.25 ml; agar, 15 g; and distilled water, 1 l. The trace element solution contained the following: $CuSO_4{\cdot}3H_2O$, 102 mg; $FeSO_4{\cdot}7H_2O$, 176 mg; $MnCl_2{\cdot}4H_2O$, 126 mg; $ZnSO_4{\cdot}7H_2O$, 24 mg; and distilled water, 100 ml.

2.5. DNA isolation and 16S rDNA sequencing

Genomic DNA of the strain MJM 8637 was isolated using Bacterial Genomic DNA isolation kit (Corebio, Korea), following the manufacturer's manual. PCR amplification of the 16S rDNA gene was conducted with primer set 27F/1492R (Lane, 1991). Amplified PCR products were sequenced using an automatic sequencer (ABI 3730XL; Applied Biosystems) at Solgent, Daejeon, Korea.

2.6. Phylogenetic analysis

For identification of taxonomic position of the strain MJM 8637, the 16S rDNA gene sequence (1417 bp) of strain MJM 8637 was aligned with sequences of related type strains by using the CLUSTAL_X program (Thomson et al., 1997). Phylogenetic analysis was performed with MEGA software version 6.0 (MEGA6) (Tamura et al., 2013). Phylogenetic trees were generated by Neighbor-Joining method (Saitou and Nei, 1987) using Tajima–Nei model (Tajima and Nei, 1984). The topology of the phylogenetic tree was evaluated by bootstrap resampling method of Felsenstein with 500 replicates (Felsenstein, 1985).

2.7. Extraction of the secondary metabolites

For flask fermentation, the spore suspensions of MJM 8637 were inoculated into 50 ml of BN medium and cultivated at 28 °C for 48 h as seed culture. Then, 500 µl of seed culture broth was collected and inoculated into 50 ml of BN medium and cultivated at 28 °C and 180 rpm for 6 days. The culture broth was filtered through filter paper (Whatman No. 1) and the filtered broth was extracted with two-volumes of ethyl acetate twice. The EtOAc extract was dried in vacuum at 38 °C and 200 mbar for various biological activity tests.

2.8. Cultivation of RAW 264.7 cells

RAW 264.7 macrophage cells were obtained from the Korea Cell Line Bank (Seoul, Korea). The cells were cultivated in DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, Logan, UT, USA) and 1% penicillin/streptomycin (10,000 U/100 µg/ml, Gibco Life Technologies, Carlsbad, CA, USA) in a 5% CO_2 humidified atmosphere incubator at 37 °C.

2.9. GST-pi enzyme assay

GST-pi activity was measured by a photometric assay according to previous report with few modifications (Müller et al., 2008). To measure the rate of coupling of glutathione to chlorodinitrobenzene (CDNB), 50 µl of MJM 8637 EtOAc extract was mixed with phosphate buffer and distributed into 96-well plate. Ten microliters of GST-pi, 50 µl of reduced glutathione solution (1 mM as final concentration) and 50 µl of CDNB (0.5 mM) were added to the each well, and incubated for 5 min. Absorbance was measured at 340 nm and the GST-pi activity was calculated in µM CDNB conjugated/min/well with extinction coefficient (e = 9.6 mM^{-1} cm^{-1}).

2.10. TNF-α production assay

The inhibitory effect of MJM 8637 EtOAc extract on TNF-α production was determined using Enzyme Immuno Assay Kits (BD Biosciences, USA) according to manufacturer's instructions. The RAW 264.7 macrophage cells (1×10^5 -cells/well) were cultured in 96-well plates, and then pre-incubated with different concentrations of MJM 8637 EtOAc extract for 2 h. After pre-incubation, LPS was treated to each well to stimulate TNF-α production and incubated for 6 h. The supernatant was transferred to the well plates, which are coated with TNF-α antibody, and absorbance was measured at 450 nm with an ELISA plate reader (Tecan; Infinite pro 2000, Switzerland).

2.11. Antioxidant assays

2.11.1. Reducing power assay

The reducing power measurement was conducted according to a method of Oyaizu (1986) with slight modifications. The EtOAc extract of MJM 8637 was diluted at various concentrations and 1 ml of each dilution was mixed with 0.1 ml of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After cooling, 0.1 ml of 1% trichloroacetic acid was added to the mixture, and then the upper layer of mixture was mixed with 0.1% ferric chloride. Finally, absorbance of the mixtures was measured at 700 nm using spectrophotometer. In this assay, the higher absorbance values of the reaction mixture indicate an increase in the reducing power.

2.11.2. DPPH radicals scavenging assay

The DPPH radical scavenging assay was carried out according to the modified method of Diaz et al. (2012). EtOAc extracts of the strain MJM 8637 were diluted with ethanol, 10 µl of dilution was distributed into a 96-well plate. To each well, 190 µl of DPPH ethanol solution was added and allowed to react at room temperature for 30 min. The absorbance was measured at 550 nm by microplate reader.

The DPPH radical scavenging capability was calculated by the following equation: DPPH radical scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0, and A_1 are the absorbance of control (blank) and the reaction system of sample or ascorbic acid.

2.11.3. Nitric oxide (NO) scavenging activity

The nitric oxide scavenging activity of MJM 8637 EtOAc extract was determined according to the method of Marcocci et al. (1994) with slight modifications. One milliliter of EtOAc extract was mixed with 1 ml of sodium nitroprusside (5 mM) and the mixture was incubated at 25 °C for 3 h. After the incubation, 50 µl of Griess reagent (1% sulfanilamide, 2% phosphoric acid, and 0.1% of napthylenediamine dihydrochloride) was added to the sample solution. The NO scavenging activity was measured at 540 nm and referred to the absorbance of standard solutions of sodium nitrite salt treated in the same way with Griess reagent.

The NO radical scavenging capability was determined by using the same equation that was used to calculate DPPH scavenging activity.

2.11.4. ABTS free radical scavenging assay

ABTS free radical scavenging assay was performed using the method by Zhishen et al. (1999) with modifications. ABTS, 2 ,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was dissolved in deionized water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS solution with 2.45 mM potassium persulfate ($K_2S_2O_8$) and storing in the dark at room temperature for 12–16 h. The ABTS radical solution was diluted to an absorbance of 0.7 at 734 nm. 10 µl of MJM 8637 EtOAc extracts were distributed into 96-well plate. To each well, 190 µl of ABTS radical solution was added and incubated for 3 min at room temperature. The absorbance was measured at 734 nm by microplate reader. $ABTS^{\cdot+}$ scavenging activity was determined using the same equation that was used to calculate DPPH scavenging activity.

2.12. Cytotoxicity assay

Cytotoxicity of the EtOAc extract was determined by MTT cell viability assay, following the method by Mosmann et al. (1983). RAW 264.7 cells were pated in a 96-well plate (5×10^4 cells/well), and then incubated at 37 °C in 5% CO_2. After 24 h, cells were treated with MJM 8637 EtOAc extracts (final concentration: 25, 50, and 100 µg/ml^{-1}) and incubated for 24 h, and then the cells were treated with MTT solution (5 mg/ml in PBS) for 4 h. The medium was removed and the remaining cells were dissolved in DMSO. Then, the optical density was measured at 570 nm by using microplate reader. The control (untreated) optical density of cells was taken as 100% of viability.

2.13. Statistical analysis

The data of all experiments were represented as Means ± SD and were analyzed with Sigmaplot (version 12.5). Differences were considered significantly at $p < 0.05$.

3. Results

3.1. Morphological identification of the isolated strain MJM 8637

The strain MJM 8637 formed dark gray colored aerial mass on ISP-2, ISP-3, and ISP-4 agar medium. Color in media: soluble pigments were not formed in ISP-2 and ISP-5 agar medium. In ISP-3 and ISP-4 agar, dark grayish brown and yellowish gray exopigments were found (Table 1).

3.2. Carbon source utilization of the isolated strain MJM 8637

Eight carbon sources (glucose, arabinose, rhamnose, fructose, raffinose, mannitol, sucrose, and inositol) were tested in this study, and the strain MJM 8637 efficiently utilized fructose, glucose, raffinose, and rhamnose for growth and showed fair growth with sucrose. Growth was not observed with arabinose, inositol, and mannitol.

3.3. Molecular identification of the isolated strain MJM 8637

The phylogenetic analysis revealed that the strain MJM 8637 belongs to the genus *Streptomyces*. The rooted phylogenetic tree for MJM 8637 and related and representative type strains of the genus *Streptomyces* demonstrated that this strain is most closely related to *Streptomyces atratus* NBRC 3897 (Fig. 1). The 16S rDNA gene sequence similarity between the strain MJM 8637 and the type strain *S. atratus* NBRC 3897 was 99.5%.

3.4. GST-pi inhibition activity of the MJM 8637 EtOAc extract

The activity of GST-pi was inhibited with increasing concentrations of EtOAc extract of MJM 8637 as follows: 62.7 ± 15.8% at 7.8 µg/ml, 56.6 ± 6.8% at 15.6 µg/ml, 52.55 ± 11.4% at 31.25 µg/ml, 51.74 ± 12.6% at 62.5 µg/ml, 41.7 ± 1.8% at 125 µg/ml, 32.2 ± 3.1% at 250 µg/ml, 30.5 ± 6.6% at 500 µg/ml, and 24.6 ± 4.3% at 1000 µg/ml. The IC_{50} value was found to be 120.2 ± 1.6 µg/ml (Fig. 2).

3.5. Anti-inflammation activity of the MJM 8637 EtOAc extract

Anti-inflammation activity of the EtOAc extract of MJM 8637 was determined via TNF-α production assay. For this assay, 1 µM of dexamethasone was used as a control reagent. The TNF-α production was inhibited approximately 23.8 ± 5.2% by 1 µM of dexamethasone, and 50 µg/ml and 100 µg/ml of the EtOAc extract showed the inhibition rate 37.8 ± 15.3% and 61.8 ± 2.4%, respectively (Fig. 3).

Table 1 Culture characteristics between the isolated strain MJM 8637 on different media.

Medium	Growth	Arial mycelium	Substrate mycelium	Pigmentation	Aerial mass color
ISP-2	Moderate	Poor	Moderate	No pigment	Dark gray
ISP-3	Abundant	Abundant	Abundant	Dark grayish brown	Dark gray
ISP-4	Abundant	Abundant	Abundant	Yellowish gray	Dark gray
ISP-5	Abundant	Abundant	Abundant	No pigment	Gray

*ISP-2–5: International *Streptomyces* Project medium (Shirling and Gottlieb, 1966).

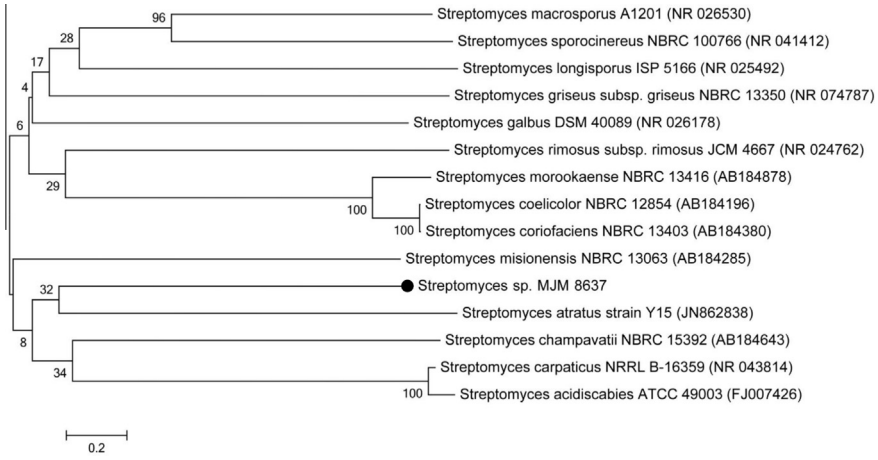

Figure 1 Phylogenetic tree based on the 16S rDNA sequences of showing affiliation of the strain MJM 8637 with closely related members in GenBank. Phylogenetic trees were generated using MEGA version 6.0 with default parameters, Tajima–Nei model and the Neighbor-Joining algorithm. The numbers at the branching prints are the percentages of occurrence in 500 bootstrapped tree.

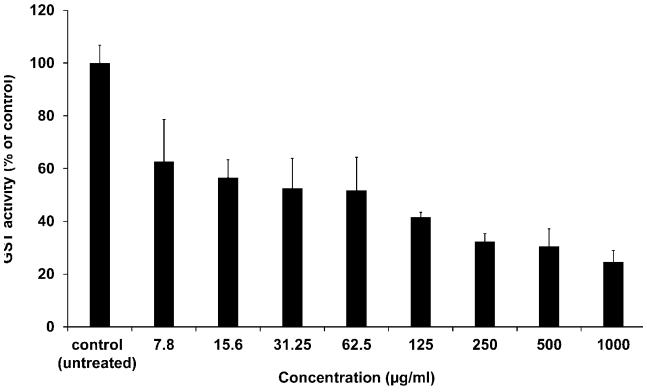

Figure 2 GST-pi inhibition activity of the EtOAc extract from *Streptomyces* sp. strain MJM 8637. Results represent the average of three replicates ($n = 3$). Error bars represent standard deviation.

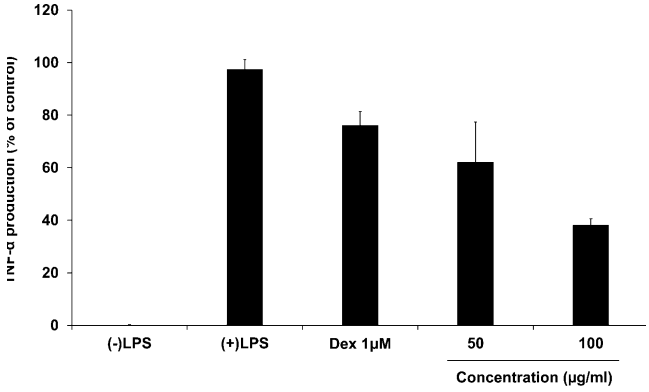

Figure 3 Inhibition activity of the EtOAc extract from *Streptomyces* sp. strain MJM 8637 on TNF-α production. Dexamethasone was used as a positive control. Results represent the average of three replicates ($n = 3$). Error bars represent standard deviation.

3.6. Reducing power

The potential antioxidant capacity of the extract can be measured by reducing power assay and it is indicated by changing color. The color of the solution is changed into various shades from green to blue, depending on the reducing power of antioxidant agents, and it can be measured at 700 nm. Fig. 4a shows the reductive capabilities of the *Streptomyces* sp. strain MJM 8637 extract, and the highest reducing power and IC_{50} value were found to be 1000 μg/ml and 1271.5 μg/ml, respectively.

3.7. DPPH radical scavenging activity

The DPPH radical is scavenged by antioxidant compounds via the donation of hydrogen ion, forming the non-radical DPPH. The color of solution is changed from purple to yellow after the reaction. The EtOAc extract of MJM 8637 showed the scavenging activities with increasing concentrations of extract as follows: 8.0 ± 2.7% at 31.25 μg/ml, 13.6 ± 1.7% at 62.5 μg/ml, 16.7 ± 3.4% at 125 μg/ml, 32.2 ± 2.9% at 250 μg/ml,

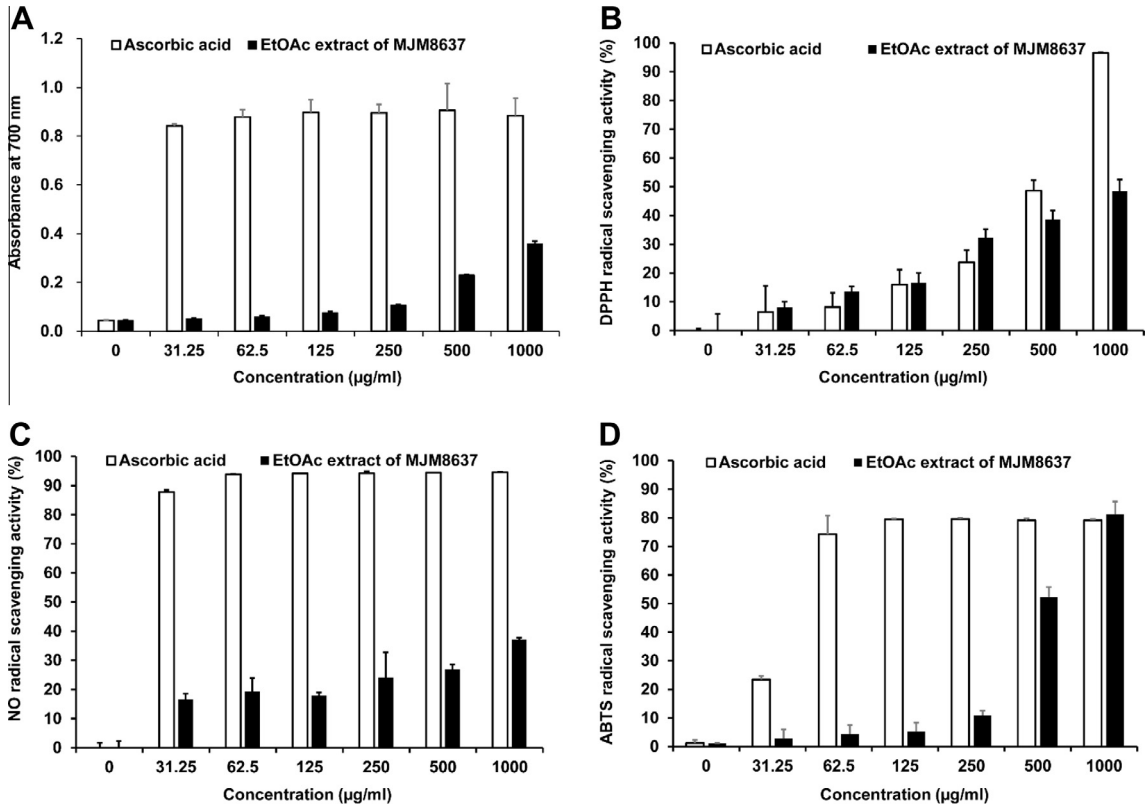

Figure 4 Results of antioxidant tests of the EtOAc extract from *Streptomyces* sp. strain MJM 8637 on (a) reducing power assay, (b) DPPH radical scavenging assay, (c) NO scavenging assay, (d) ABTS free radical scavenging assay. Results represent the average of three replicates ($n = 3$). Error bars represent standard deviation.

38.6 ± 3.2% at 500 μg/ml, and 48.5 ± 4.0% at 1000 μg/ml (Fig. 4b). The IC_{50} value was found to be 977.2 μg/ml.

3.8. NO scavenging activity

The EtOAc extract of MJM 8637 showed NO scavenging activities with increasing concentration of extract as follows: 16.6 ± 1.9% at 31.25 μg/ml, 19.2 ± 4.6% at 62.5 μg/ml, 17.9 ± 1.1% at 125 μg/ml, 24.1 ± 8.7% at 250 μg/ml, 26.9 ± 1.6% at 500 μg/ml, and 37.2 ± 0.6% at 1000 μg/ml (Fig. 4c). The IC_{50} value was found to be 1143.7 μg/ml.

3.9. ABTS free radical scavenging activity

The antioxidant capacity of the EtOAc extract of MJM 8637 was also evaluated according to the ABTS decolorization method. The extract showed the scavenging activities with increasing concentrations of extract as follows: 2.8 ± 3.2% at 31.25 μg/ml, 4.3 ± 3.1% at 62.5 μg/ml, 5.2 ± 3.1% at 125 μg/ml, 10.9 ± 1.6% at 250 μg/ml, 52.2 ± 3.6% at 500 μg/ml, and 81.2 ± 4.5% at 1000 μg/ml (Fig. 4d). The IC_{50} value was found to be 454.4 μg/ml.

3.10. MTT cell viability test

The cytotoxicity of the EtOAc extract of MJM 8637 at various concentrations on RAW 264.7 cells was determined by the MTT assay. Cell viabilities were 100 ± 12.6% at control

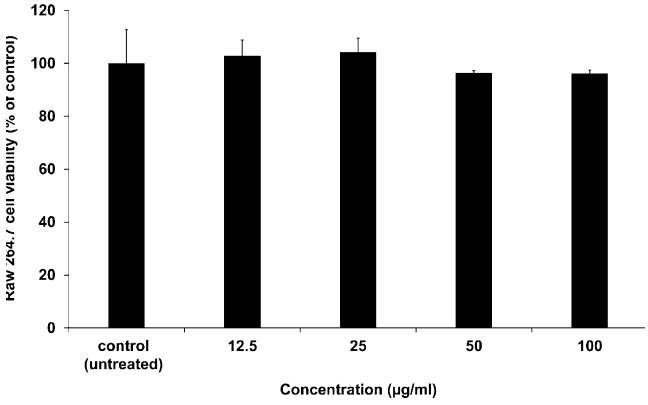

Figure 5 Cytotoxicity of the EtOAc extract from *Streptomyces* sp. strain MJM 8637 on RAW 264.7 cells. Results represent the average of three replicates ($n = 3$). Error bars represent standard deviation.

(untreated), 102.9 ± 5.9%, 104.2 ± 5.2%, 96.8 ± 0.9%, and 97.2 ± 1.3% at 12.5 μg/ml, 25 μg/ml, 50 μg/ml, and 100 μg/ml of the EtOAc extract of MJM 8637, respectively (Fig. 5).

4. Discussion

Recent studies have revealed that GST-pi has critical roles in detoxification of anti-cancer drugs in cancer cells and GST-

pi could be considered as a target to disable multidrug resistance of cancer cells. The close correlation between chronic inflammation and the initiation of cancer has also been studied well and the suppression of inflammation can be an effective way for cancer prevention (Backos et al., 2012; García-Lafuente et al., 2009; Hofseth and Ying, 2006; Laborde, 2010; Liu et al., 2010, 2014, 2011; Mukanganyama et al., 2002; Rayburn et al., 2009; Rolland et al., 2010; Wang et al., 2011).

The increasing of cancer incidence and drug resistance of cancer cells led us to investigation of novel anti-cancer compound that has anti-GST-pi and anti-inflammation activities from actinobacteria and *Streptomyces* strains. For the screening, we isolated actinomycetes from mountain forest soil in Korea. The isolated actinobacteria, *Streptomyces* sp. strain MJM 8637, exhibited GST-pi inhibition activity against and reduction activity on the production of TNF-α. The 16S rDNA sequencing revealed that *Streptomyces* sp. strain MJM 8637 has 99.5% similarity with *S. atratus* NBRC 3897. The strain MJM 8637 showed differences in carbon source utilization to that of the strain NBRC 3897. A previous study reported that the strain NBRC 3897 showed heavy growth with glucose and raffinose, good growth with rhamnose, fair growth with fructose, and no growth with arabinose, mannitol, sucrose, inositol (Shibata et al., 1961). The isolated strain MJM 8637 showed good growth with fructose, glucose, raffinose, and rhamnose, fair growth with sucrose, and no growth with inositol, mannitol, and sucrose.

The antioxidant activity assays demonstrated that the antioxidant properties of the strain MJM 8637 EtOAc extract are not powerful. The extract showed IC_{50} values of 977.2 μg/ml, 1143.7 μg/ml, and 454.4 μg/ml for DPPH, nitric oxide, and ABTS free radical scavenging assays, respectively, and in all antioxidant tests, the ascorbic acid, which was used as a control, displayed IC_{50} values of 4.2 μg/ml, 8.0 μg/ml, and 31.7 μg/ml for DPPH, nitric oxide, and ABTS free radical scavenging assays, respectively. According to these results, the inhibition activities of the strain MJM 8637 EtOAc extract on GST-pi and TNF-α do not seem to be related to the antioxidant activities.

The cytotoxicity of the strain MJM 8637 EtOAc extract was determined by MTT assay on RAW 264.7 cells, and it was measured as low cytotoxicity (97.2% of cell viability at 100 μg/ml).

In this study, *Streptomyces* sp. strain MJM 8637, a putatively novel, was isolated from mountain forest soil and identified that the EtOAc extract of the strain MJM 8637 has strong GST-pi inhibition and anti-TNF-α production activity. Moreover, the low cytotoxicity of the extract gives a possibility to develop the extract to an anti-cancer agent that can disable the multidrug resistance of cancer cells. Further studies are being performed for the purification of single compound that has anti-cancer activities from the EtOAc extract of *Streptomyces* sp. strain MJM 8637.

5. Conclusions

In this study, we isolated and characterized Streptomyces sp. strain MJM 8637, and have found the ethyl acetate extract of the strain MJM 8637 has good glutathione S-transferase pi inhibitory and anti-inflammatory activities, and low cytotoxicity. The results could help to develop the drugs for treating cancers with multidrug resistance.

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