Abstract: Microorganisms are regarded as a sustainable source of biologically active molecules. Among them, *Trichoderma* spp. have been an attractive source of biological compounds. However, the study of marine-derived *Trichoderma* has developed slowly because of the difficulty in isolating the fungi. In our study, 30 strains of marine-derived *Trichoderma* were identified through the translation elongation factor 1-alpha (EF1α) sequences, and their biological activities, such as antioxidant activity by ABTS and DPPH assays, antifungal activity against *Asteromyces cruciatus* and *Lindra thalassiae*, and tyrosinase inhibition activity, were investigated. As a result, the 30 marine *Trichoderma* species were classified into 21 taxa, including three new species candidates. Three strains of *T. asperellum* showed the highest ABTS radical scavenging activity and antifungal activity. *T. bissettii* SFC20170821-M05 and *T. guizhouense* SFC20180619-M23 showed notable DPPH radical scavenging activity and tyrosinase inhibition activity, respectively. This study showed the potential of marine-derived *Trichoderma* as a source of bioactive compounds.

Keywords: antagonistic activity; biological control; phylogenetic analysis; radical scavenging; skin whitening agents

1. Introduction

In a specific environment, biodiversity is the most essential information for sustainable development and is important in discovering biological resources [1–6]. In particular, microorganisms have been considered as a sustainable source of various bioactive compounds as well as useful enzymes [7–14]. Traditional sources of natural products have been terrestrial plants, fungi, and bacteria. The idea of natural products from the ocean has recently been of interest, but the need for additional efforts, such as scuba diving or instruments for collecting or culture, have made the development of marine natural compounds slow in comparison with its terrestrial counterpart [15,16]. Nevertheless, the structural uniqueness and profound effect of marine-derived compounds have been of interest to the pharmaceutical and cosmetic industry [17,18]. A total of 200 million microorganisms in the ocean covering 71% of the surface of the earth have been presumed, although the exact number of microorganisms is still open to debate [19,20]. For this reason, marine organisms, especially microorganisms, have attracted the attention of the pharmaceutical and cosmetic industries as a new reservoir of novel natural compounds [17,20].
The genus *Trichoderma* is well known for its ability to produce antibiotic compounds and parasitize other fungi [21–23]. The actions of antifungal secondary metabolites (i.e., 6-pentyl-α-pyrone and trichodermaketones) and cell wall hydrolytic enzymes (i.e., β-1,3-glucanases and β-1,6-glucanases) secreted by *Trichoderma* spp. can cause the death of prey [21,24–26]. Several *Trichoderma* provide a beneficial effect to host plants by activating plant defense mechanisms, preventing pathogen attacks and promoting plant growth [21,27]. To date, more than 250 species of the genus *Trichoderma* have been reported [28]. Approximately 78 metabolites have been described from marine-derived *Trichoderma* so far, and most of them showed a variety of industrially useful biological activities, such as antifungal, antibacterial, and antioxidant activity [29,30]. Reliable phylogenetic information is important to discover the diversity of secondary metabolites of microorganisms, and it is difficult to distinguish *Trichoderma* spp. by morphology alone, because they share many morphological features [31–33]. Thus, a molecular biological analysis is essential for the accurate identification of *Trichoderma* [34]. The internal described spacer (ITS) is the most universal fungal molecular barcode [35,36]. However, the ITS has low species resolution in the genus *Trichoderma* [32]. Instead of ITS, translation elongation factor 1-alpha (EF1α) sequences were recommended for phylogenetic analysis of this genus [32].

The aim of this study was to investigate the diversity of *Trichoderma* spp. in marine environment in South Korea using phylogenetic analysis and evaluate the marine *Trichoderma* spp. as a source of bioactive secondary metabolites by investigating the antifungal, antioxidant, and tyrosinase activities of the fungal extracts.

2. Materials and Methods

2.1. Preparation of Trichoderma Cultures

A total of 30 marine *Trichoderma* cultures were obtained from the Marine Fungal Resource Bank (http://mfrb.snu.ac.kr; Seoul, Korea). A list of the fungal species with their general information is shown in Table 1. Their sampling sites are indicated in Figure 1. They were subcultured on potato dextrose agar (PDA) media in room temperature: 20–25°C.

![Figure 1. The map of sampling sites: (A) Hwado-myeon, and (B) Gilsang-myeon, Ganghwa-gun, Incheon; (C) seaside, and (D) mudflat in Hyeongyeong-myeon, Muan-gun, Jeollanam-do; (E) Hyeonnae-myeon, and (F) Jugwang-myeon Goseong-gun; (G) Sokcho Beach in Sokcho-si; (H) Hyeonam-myeon, Yangyang-gun; and (I) Jumunjin-eup, and (J) Yeongok-myeon, Gangneung-si, Gangwon-do; (K) Byeollyang-myeon, Suncheon-si, Jeollanam-do; (L) Andeok-myeon, Seogwipo-si, Jeju-do, Korea.](image-url)
### Table 1. General information of 30 marine-derived Trichoderma spp.

| Identity               | ID                  | Sampling Date | Sampling SITE * | Isolation Source                  |
|------------------------|---------------------|---------------|-----------------|-----------------------------------|
| *Trichoderma afroharzianum* | SFC20160907-M20   | January 2015  | Hyeonnae-myeon, Goseong-gun, Gangwon-do | Eggs of *Arctospus japonicus* |
|                        | SFC20180619-M25   | January 2017  | Gilsang-myeon, Ganghwa-gun, Incheon | Mudflat                           |
|                        | SFC20190312-M15   | July 2016     | Gilsang-myeon, Ganghwa-gun, Incheon | Mudflat                           |
| *T. asperelloides*     | SFC20180619-M20   | January 2015  | Hyeonnae-myeon, Goseong-gun, Gangwon-do | Eggs of *Arctospus japonicus* |
| *T. asperellum*        | SFC20160907-M21   | January 2015  | Yeongok-myeon, Gangneung-si, Gangwon-do | Eggs of *Arctospus japonicus* |
|                        | SFC20180619-M22   | October 2016  | Hyeongyeong-myeon, Muan-gun, Jeollanam-do | Mudflat                           |
| *T. atroviride*        | SFC20190312-M11   | January 2015  | Hyeonnae-myeon, Goseong-gun, Gangwon-do | Eggs of *Arctospus japonicus* |
| *T. atriviride*        | SFC20161110-M05   | December 2015 | Jugwang-myeon, Goseong-gun, Gangwon-do | *Agarum clathratum*               |
| *T. bissetti*          | SFC20170821-M05   | October 2016  | Byoollyang-myeon, Suncheon-si, Jeollanam-do | Mudflat                           |
| *T. capillare*         | SFC20180619-M19   | January 2015  | Sokcho-si, Gangwon-do | *Agarum clathratum*               |
| *T. citrinoviride*     | SFC20180510-M16   | July 2016     | Hwado-myeon, Ganghwa-gun, Incheon | Sea sand                          |
| *T. gamsii*            | SFC20160907-M22   | January 2015  | Yeongok-myeon, Gangneung-si, Gangwon-do | Eggs of *Arctospus japonicus* |
| *T. guizhouense*       | SFC20160907-M23   | January 2015  | Yeongok-myeon, Gangneung-si, Gangwon-do | Eggs of *Arctospus japonicus* |
|                        | SFC20180619-M21   | August 2015   | Jumunjin-eup, Gangneung-si, Gangwon-do | *Agarum clathratum*               |
| *T. hamatum*           | SFC20180510-M09   | July 2016     | Gilsang-myeon, Ganghwa-gun, Incheon | Mudflat                           |
| *T. longibrachiatum*   | SFC20171019-M03   | July 2016     | Gilsang-myeon, Ganghwa-gun, Incheon | Mudflat                           |
| *T. orientalis*        | SFC20170718-M02   | January 2017  | Byeoollyang-myeon, Suncheon-si, Jeollanam-do | Mudflat                           |
| *T. paraviroides*      | SFC20160907-M24   | January 2015  | Yeongok-myeon, Gangneung-si, Gangwon-do | Eggs of *Arctospus japonicus* |
| *T. pyramidal*         | SFC20160907-M25   | January 2015  | Yeongok-myeon, Gangneung-si, Gangwon-do | Eggs of *Arctospus japonicus* |
| *T. songyi*            | SFC20171120-M04   | October 2016  | Hyeongyeong-myeon, Muan-gun, Jeollanam-do | *Agarum clathratum*               |
| *T. subviride*         | SFC20170919-M07   | July 2016     | Andeok-myeon, Seogwipo-si, Jeju-do | Sea sand                          |
| *T. virens*            | SFC20180817-M24   | October 2016  | Hyeongyeong-myeon, Muan-gun, Jeollanam-do | *Agarum clathratum*               |
| *Trichoderma* sp. 1    | SFC20190312-M13   | January 2015  | Yeongok-myeon, Gangneung-si, Gangwon-do | Eggs of *Arctospus japonicus* |
|                        | SFC20190312-M14   | July 2016     | Gilsang-myeon, Ganghwa-gun, Incheon | Mudflat                           |
| *Trichoderma* sp. 2    | SFC20190312-M17   | November 2015 | Hyeonnae-myeon, Yangyang-gun, Gangwon-do | *Agarum clathratum*               |
| *Trichoderma* sp. 3    | SFC20161110-M06   | September 2015| Jumunjin-eup, Gangneung-si, Gangwon-do | *Agarum clathratum*               |

* The sampling sites in Figure 1 were indicated by A–L.
2.2. Phylogenetic Analysis

EF1α sequences of marine Trichoderma were obtained from Marine Fungal Resource Bank. The closely related sequences for references were downloaded from GenBank using nucleotide BLAST. Type specimens were chosen for reference sequences except for Trichoderma virens. EF1α sequence data of the CBS 249.59, type specimen of T. virens has only 200 bp in GenBank. Protocrea illinoensis GJS 94-54 (EU703904) was downloaded as outgroup of the genus Trichoderma. The obtained sequences were aligned using MAFFT 7.388 [37]. The aligned dataset was proofread and modified manually using MacClade 4.08 [38]. The aligned dataset contained 113 taxa and 752 characters. A neighbor joining tree was constructed with PAUP 4.0b10 [39]. The Kimura 2-parameter model was applied [40]. To indicate branch stability, 1000 replications of bootstrap analysis were carried out.

2.3. Preparation of Fungal Extracts

All of the fungal species were cultivated on 50 mL of PDA at 25 °C for seven days in darkness. After 7 days of cultivation, the solid media were extracted with 200 mL of methanol for 24 h. The methanol solution was filtered with Whatman No. 1 filter paper. The filtrated solutions were evaporated at 37 °C under a vacuum. The condensed residues were dissolved in 20 mL of ethyl acetate and 20 mL of distilled water. After 6 h, the supernatant, the partitioned ethyl acetate fraction, was evaporated. The extracts were stored at 4 °C. The fungal cultures were incubated in triplicate, and all the subsequent biological assays were performed in triplicate.

2.4. Measurement of Antioxidant Activity by ABTS Scavenging Ability

The 2.2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS, Sigma-Aldrich, Inc., St. Louis, MO) solution dissolved in PBS (7 mM) was oxidized with potassium persulfate (2.45 mM) for 24 h in darkness at room temperature. The ABTS•+ solution was diluted with PBS to an absorbance of 0.70 (± 0.02) at a wavelength of 734 nm. Then, 990 µL of the ABTS•+ solution and 10 µL of each fungal extract sample (10 mg/mL in DMSO) were mixed in the cuvette and measured at 734 nm after 6 min.

2.5. Measurement of Antioxidant Activity by DPPH Radical Scavenging Ability

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich Inc., St. Louis, MO, USA) was dissolved in methanol (80%) at 150 µM. An aliquot (22 µL) of the fungal extracts (10 mg/mL) were each mixed with 200 µL of DPPH solution in the 96-well plate. The mixture was stored at room temperature for 30 min and measured at 520 nm.

2.6. Measurement of Antifungal Activities

Asteromyces cruciatus SFC20161110-M19 was obtained from Marine Fungal Resource Bank (MFRB) at Seoul National University as a marine bioresource bank of Korea by the Ministry of Oceans and Fisheries, and Lindra thalassiae NBRC106646 was purchased from Biological Resource Center under National Institute of Technology and Evaluation. Asteromyces cruciatus SFC20161110-M19 and Lindra thalassiae NBRC106646 were tested as the target fungi and antifungal activity was determined in a 96-well plate. The 25 µL of spore suspensions (4 × 10^5 conidia/mL) of the target fungi was added to each well containing 49 µL of potato dextrose broth. The 2 µL of Trichoderma extracts solubilized in dimethyl sulfoxide (DMSO) to a final concentration of 100 µg/mL was added to each well at the last. The 96-well plates were incubated at 25 °C for 3 days and the fungal growth was detected by measuring the absorbance at the wavelength of 595 nm [41]. The minimum inhibitory concentration (MIC) values were determined in the concentration range of 6.25-50 µg/mL.

2.7. Tyrosinase Inhibition Activity

The assay was modified from the method described by Lai et al. [42]. An amount of 40 µL of each fungal extract samples dissolved in 50% DMSO at the same concentration (2.5 mg/mL), 70 µL
of a 0.1 M potassium phosphate buffer (pH 6.8), and 30 µL of 0.02 mg/mL of tyrosinase were mixed in 96-well plate. The mixture was heated to 30 °C for 5 min and mixed with 100 µL of 2.5 mM L-dihydroxyphenylalanine (L-DOPA). After 30 min, to terminate the reaction put the plate in the ice and the absorbance was measured at 492 nm. Kojic acid was used as a positive control and all mixtures without L-DOPA was used as a blank.

3. Results and Discussion

3.1. Identification and Phylogeny

The marine Trichoderma were identified by phylogeny based on the EF1α sequences (Figure 2). A tree containing 86 taxa of Trichoderma, including 83 known species and three new species candidates, was constructed. Among them, 25 of the sequences obtained were identified as eighteen previously reported species. Eleven of the sequences were grouped in the Harzianum clade: SFC20190312-M14, SFC20190312-M15, SFC20180619-M23, SFC20190312-M12, SFC20180619-M25, SFC20190312-M13, SFC20160907-M23, SFC20160907-M25, SFC20160907-M20, SFC20180619-M21, and SFC20190312-M17 [32,43]. The morphology of the tree was similar to that in other studies [32,43]. SFC20190312-M15, SFC20180619-M25, and SFC20160907-M20 were identified as *T. afroharzianum* with a high bootstrap value (100%). SFC20180619-M23, SFC20190312-M12, SFC20160907-M23, and SFC20180619-M21 were grouped with *T. guizhouense*, but the bootstrap value was not very high (62.9%). SFC20190312-M14, SFC20190312-M13, and SFC20190312-M17 had their own group without a close reference and were regarded as new species candidates. In this study, these cultures were named *Trichoderma* sp. 1. Their closest sister group contains *T. afarasin*, *T. harzianum*, and *T. camerunense*.

SFC20180817-M24 was placed in the Virens clade [43] and identified as *T. virens* (bootstrap value: 97.7%). Its closely related species were *T. crassum* [43]. SFC20180510-M16, SFC20170109-M03, SFC20170821-M05, SFC20170718-M02, and SFC20160907-M2190 were placed in the Longibrachiatum clade [44,45]. SFC20171019-M03 was identified as *T. longibrachiatum* with high support (bootstrap value: 98.3%). SFC20170718-M02 was grouped with *T. orientale* with a bootstrap value of 100%. SFC20180510-M16 was identified as *T. citrinoviride* (bootstrap value: 100%). SFC20160907-M2190 was grouped with *T. capillare* (bootstrap value: 100%). Though SFC20170821-M05 was placed in a paraphyletic group with *T. bissettii*, it showed 100% similarity with the type specimens of *T. bissettii* based on a BLAST search. Thus, it was regarded as *T. bissettii*.

Eleven sequences were placed in the Viride clade: SFC20180510-M09, SFC20170919-M07, SFC20180619-M22, SFC20171120-M04, SFC20180619-M04, SFC20180619-M24, SFC20160907-M21, SFC20160907-M22, SFC20180619-M20, SFC20190312-M11, SFC20161110-M06, and SFC20161110-M05 [46–48]. Among them, SFC20160907-M22 was grouped with *T. gamsii* (bootstrap value: 100%). SFC20190312-M11 was identified as *T. paratroviride* with a bootstrap value of 100%. SFC20161110-M05 made a monophyletic group with *T. atroviride* (bootstrap value: 99.9%). SFC20170919-M07 was placed in a monophyletic clade with high support (bootstrap value: 100%). SFC20171120-M04 was grouped with *T. songyi* with a bootstrap value of 100%. This species is known to be associated with *Tricholoma matsutake* [48]. SFC20180619-M22, SFC20180619-M24, and SFC20160907-M21 were in a monophyletic clade with *T. asperellum* (bootstrap value: 99.9%). SFC20180619-M20 was identified as *T. asperelloides* (bootstrap value: 100%). SFC20180510-M09, and SFC20161110-M06 were not assigned to a clade through close references; therefore, we regarded them as new species candidates. These cultures were named *Trichoderma* sp. 2 and 3, respectively.
Figure 2. The neighbor joining tree of the EF-1α sequences dataset. Numbers above branches indicate bootstrap values. Bootstrap values less than 50 were not shown. Fungal cultures collected in this study are in bold and colored. GenBank accession numbers are in parentheses.
3.2. Antioxidant Activity

ABTS and DPPH radical scavenging assays were used to evaluate the antioxidant activity of 30 methanol crude fungal extracts of marine *Trichoderma*, and many of the marine *Trichoderma* extracts contained antioxidative compounds. *Trichoderma asperellum* SFC20160907-M21, SFC20180619-M22, and SFC20180619-M24 showed over 70% ABTS radical scavenging activity, and *T. bissettii* SFC20170821-M05 and *T. longibrachiatum* SFC20171019-M03 exhibited over 70% DPPH radical scavenging activity (Table 2). Other extracts showed lower inhibition rates from 2.3% to 69%. Only four strains, *T. bissettii* SFC20170821-M05, *Trichoderma* sp. 1 SFC20190312-M13, *T. longibrachiatum* SFC20171019-M03, and *T. subviride* SFC20170919-M07, exhibited over 50% antioxidant activities in both the ABTS and DPPH radical scavenging tests. Numerous studies have shown that various marine-derived natural compounds have radical scavenging properties that could be used as raw materials in the cosmetic and pharmaceutical industries [18,49,50]. For example, the major antioxidant in the food supplement Seanol is phlorotannins extracted from *Ecklonia Cava* [51]. Several natural compounds that have antioxidant ability, such as polyphenols and vitamins, also have other biological activities, such as antiaging and skin-whitening [52,53]. Some antioxidants have the ability to inhibit tyrosinase by eliminating reactive quinone products [52]. In this study, the extracts of *T. atroviride* SFC20161110-M05, *T. gamsii* SFC20160907-M22, *T. guizhouense* SFC20180619-M23, and *T. songyi* SFC20171120-M04, which showed a remarkable ability to inhibit tyrosinase (IC<sub>50</sub> < 100 µg/mL), exhibited low radical scavenging activity (<50%), indicating that they have other mechanisms for inhibiting tyrosinase rather than scavenging reactive quinone products. For examples, they may be the competitive inhibitors such as copper chelators that inhibit this metalloenzyme or suicide inhibitors that inactivate tyrosinase by changing the tertiary and quaternary structures of the enzyme [54].

3.3. Antifungal Activity

The antifungal activity was determined using the fungal extracts of marine *Trichoderma* on *Asteromyces cruciatus* and *Lindra thalassiae* as target fungi. *A. cruciatus* is a ubiquitous marine fungus and has the ability to degrade alginate, which is the major material of the brown algal construct; therefore, it is regarded as a potentially harmful fungus to brown algae [55]. *Lindra thalassiae* is a well-known pathogen of brown algae and seagrasses and causes raisin disease [56]. In this study, eight strains showed remarkable growth inhibitory ability against both *A. cruciatus* and *L. thalassiae*: *T. afroharzianum* SFC20180619-M25, *T. asperelloides* SFC20180619-M20, *T. asperellum* SFC20160907-M21, SFC20180619-M22, and SFC20180619-M24, *T. capillare* SFC20160907-M2190, *T. citrinoviride* SFC20180510-M16 and *T. virens* SFC20180817-M24. *T. asperellum* SFC20180619-M21, SFC20180619-M22, and SFC20180619-M24 showed a high inhibitory effect against both target fungi (Table 2). In particular, *T. asperellum* SFC20160907-M21 and SFC20180619-M24 showed the highest inhibitory effect, as they could inhibit *L. thalassiae* at 6.25 µg/mL. Since *Trichoderma* spp. are well known producers of antifungal compounds, such as 6-pentyl-α-pyrone and trichodermaketones, it was expected that *Trichoderma* species could exhibit antagonistic ability against *A. cruciatus* and *L. thalassiae*, which are regarded as pathogenic fungi to algae [25,26]. Similarly, *T. asperellum* has already been investigated for its antagonistic ability against pathogenic fungi in several previous studies [57–59]. In addition, the synergistic effects of antifungal secondary metabolites and enzymes from *Trichoderma* could provide support to the plant rhizosphere attacked by other pathogenic fungi [21,27]. The results of this antifungal activity suggested that the antifungal compounds from *Trichoderma* could also support marine algae defense systems that are related to symbiosis, similar to the action of the plant rhizosphere.
Table 2. Biological activities of the marine *Trichoderma* spp. extracts.

| Fungal Name       | ID                | Radical-Scavenging Activity (%) | Antifungal Activity (MIC \(3\), \(\mu g/mL\)) | Tyrosinase Inhibition (IC\(_{50}\) \(4\), \(\mu g/mL\)) |
|-------------------|-------------------|-------------------------------|-----------------------------------------------|-------------------------------------------------|
| \(T.\) afroharzianum | SFC20160907-M20   | 27.16                         | N.D.                                         | 100                                             |
|                   | SFC20190312-M15   | 69.92                         | 24.47                                         | N.D. \(>100\)                                   |
|                   | SFC20180619-M25   | 66.70                         | 33.77                                         | \(>100\)                                       |
| \(T.\) asperelloides | SFC20180619-M20  | 47.70                         | 2.41                                          | \(>100\)                                       |
| \(T.\) asperellum  | SFC20160907-M21   | 73.38                         | 30.62                                         | \(>100\)                                       |
|                   | SFC20180619-M22   | 74.70                         | 37.83                                         | \(>100\)                                       |
|                   | SFC20180619-M24   | 72.63                         | 32.34                                         | \(>100\)                                       |
| \(T.\) atroviride  | SFC20161110-M05   | 32.18                         | 38.03                                         | N.D. \(>100\)                                  |
|                   | SFC20190312-M11   | 48.48                         | 34.80                                         | N.D. \(>100\)                                  |
| \(T.\) bissettii   | SFC20170821-M05   | 57.17                         | 79.60                                         | \(>100\)                                       |
| \(T.\) capillare   | SFC20180619-M19   | 29.37                         | 49.62                                         | \(>100\)                                       |
| \(T.\) citrinoviride | SFC20180510-M16  | 25.52                         | 48.27                                         | \(>100\)                                       |
| \(T.\) guizhouense | SFC20160907-M23   | 51.18                         | 31.27                                         | \(>100\)                                       |
| \(T.\) hamatum     | SFC20180819-M21   | 14.65                         | 18.62                                         | \(>100\)                                       |
|                   | SFC20180619-M23   | 18.17                         | 23.41                                         | \(>100\)                                       |
| \(T.\) longibrachiatum | SFC20180510-M09 | 23.29                         | 36.76                                         | \(>100\)                                       |
| \(T.\) orientalis  | SFC20170119-M03   | 66.36                         | 72.72                                         | \(>100\)                                       |
| \(T.\) paraviridescens | SFC20170718-M02  | 28.62                         | 32.22                                         | \(>100\)                                       |
| \(T.\) pyramidal  | SFC20160907-M24   | 49.59                         | 38.32                                         | \(>100\)                                       |
| \(T.\) songgi     | SFC20171120-M04   | 27.14                         | 14.60                                         | \(>100\)                                       |
| \(T.\) sulviride   | SFC20170919-M07   | 61.71                         | 53.43                                         | \(>100\)                                       |
| \(T.\) virens     | SFC20180817-M24   | 42.20                         | 45.33                                         | \(>100\)                                       |
| \(T.\) virens sp. 1 | SFC20190312-M14  | 42.09                         | 44.05                                         | \(>100\)                                       |
| \(T.\) virens sp. 2 | SFC20190312-M13  | 61.57                         | 55.03                                         | \(>100\)                                       |
| \(T.\) virens sp. 3 | SFC20190312-M17  | 40.11                         | 55.81                                         | \(>100\)                                       |
| \(T.\) virens sp. 4 | SFC20190312-M16  | 43.96                         | 33.57                                         | \(>100\)                                       |
| Ascorbic acid *    | 13.70             | 6.80                          |                                               |                                                |
| Kojic acid *       |                   |                               |                                               |                                                |

\(\text{ABTS}^1\), \(\text{DPPH}^2\), \(\text{MIC}^3\), \(\text{IC}_{50}^4\), \(\text{N.D.}^5\)

*positive controls.

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1. 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; 2. DPPH, 2,2-diphenyl-1-picrylhydrazyl; 3. minimum inhibitory concentration; 4. half maximal inhibitory concentration; 5. not detected.
3.4. Tyrosinase Inhibition Activity

Among the fungal extracts, 14 extracts showed tyrosinase inhibition activity (Table 2). Three strains of *T. asperellum* (SFC20160907-M21, SFC20180619-M22, and SFC20180619-M24) showed weak inhibitory activity. *T. guizhouense* SFC20180619-M23, *T. atroviride* SFC20161110-M05, and *T. songyi* SFC20171120-M04 exhibited the highest tyrosinase inhibitory effect. Interestingly, the extracts of *T. guizhouense* SFC20180619-M23, *T. atroviride* SFC20161110-M05, and *T. songyi* SFC20171120-M04 exhibited even higher activities than kojic acid, the most widely studied tyrosinase inhibitor from fungi. Kojic acid, which is produced by various species of *Aspergillus* and *Penicillium*, inhibits the formation of melanin and supports skin whitening [60–62]. Considering that the fungal extracts used in the experiments, unlike the single compound kojic acid, were crude extracts, these three *Trichoderma* species are expected to produce highly active tyrosinase inhibitors or produce multiple compounds with synergistic effects. In addition to kojic acid isolated from mushrooms, other tyrosinase inhibitors from fungi have been found recently, including 1β,5α,6α,14-tetraacetoxy-9α-benzyloxy-7βH-eudesman-2β,11-diol and 4α,5α-diacetoxy-9α-benzyloxy-7βH-eudesman-1β,2β,11,14-tetraol [63]. Moreover, the novel tyrosinase inhibitor from marine *Trichoderma* was purified. The structure of the compound is the same as antibiotics from *T. koningii* and *T. harzianum* [64]. Many antibiotics and antioxidants also have potent tyrosinase inhibition activity, although the mechanism of various tyrosinase inhibitors is different [52]. In this study, the strains that exhibited high potent tyrosinase inhibition activity (*T. guizhouense* SFC20180619-M23, *T. atroviride* SFC20161110-M05, and *T. songyi* SFC20171120-M04) did not directly relate to the radical scavenging activities and antifungal activity. However, some strains that exhibited relatively weak tyrosinase inhibition activity showed radical scavenging activity and antifungal effects (*T. asperellum* SFC20160907-M21, SFC20180619-M22, and SFC20180619-M24; *T. bissettii* SFC20170821-M05; *Trichoderma* sp. 1 SFC20190312-M13; *T. longibrachiatum* SFC20171019-M03).

4. Conclusions

We investigated the diversity of marine-derived *Trichoderma* spp. and provided reliable DNA information of 30 marine *Trichoderma* species isolated in South Korea as well as their exploitable biological activities. Based on the phylogenetic analysis, the 30 marine *Trichoderma* species were classified into 21 taxa, including three new species candidates. Among them, three species—*Trichoderma* sp. 1, *T. asperellum*, and *T. longibrachiatum*—showed remarkable abilities in most of the biological activities investigated in this study. The potent bioactive compounds of these species will be studied in the near future. This study proved that marine-derived *Trichoderma* can be a useful source of bioactive compounds.

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