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Diversity of *Streptomyces* spp. from mangrove forest of Sarawak (Malaysia) and screening of their antioxidant and cytotoxic activities

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Diversity of *Streptomyces* spp. from mangrove forest of Sarawak (Malaysia) and screening of their antioxidant and cytotoxic activities

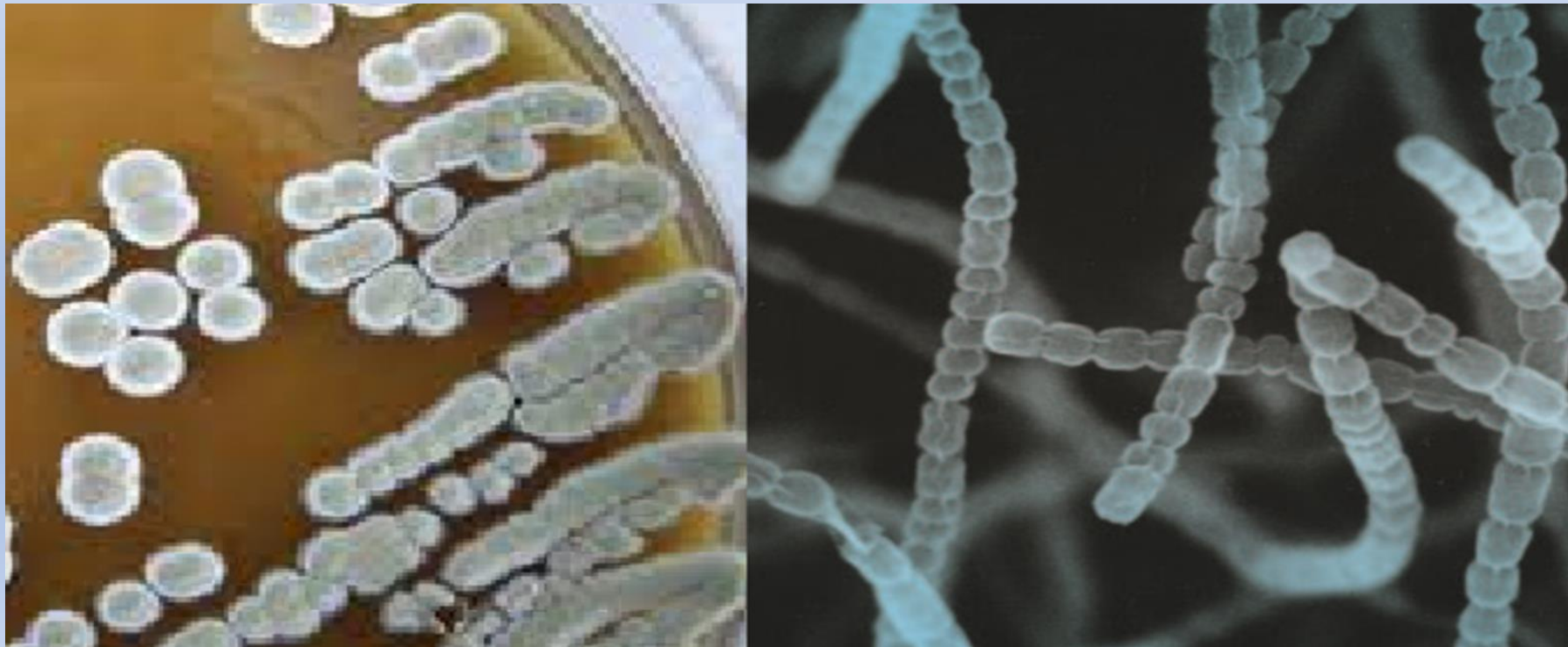
Jodi Woan-Fei Law^{1,2}, Kok-Gan Chan ^{3,4*}, Ya-Wen He⁵, Tahir Mehmood Khan^{6,7}, Nurul-Syakima Ab Mutalib ⁸, Bey-Hing Goh^{6,9*} & Learn-Han Lee^{2,5*}

Introduction

- Bacteria belonging to the genus *Streptomyces* are known to produce many bioactive compounds.
- Streptomyces are complex gram-positive bacteria with a fungus-like morphology.
- Studies have shown the presence of more than 20 biosynthetic gene clusters related to the biosynthesis of secondary metabolites in the large genome of streptomyces.

Introduction

- Due to the production of various beneficial compounds from streptomycetes including enzymes, pigments and compounds with biological activities antimicrobial, anticancer, antioxidant, immunosuppressive and other important biological activities, these bacteria are widely Have been studied for a wide range of applications.



Introduction

- ✓ The aim of this study was to investigate the diversity of *Streptomyces* spp. Mangroves in Sarawak and their screening to determine potential sources for antioxidant and cytotoxic secondary metabolites.
- ✓ Sarawak mangrove forest is predicted to provide a rich source of actinobacteria.
- ✓ This is the **first report** on the diversity and bioactive properties of streptomycetes from mangrove habitats in Sarawak

Materials and Methods

- ✓ Environmental sampling
- ✓ Selective isolation and purification of *Streptomyces* spp
- ✓ Molecular identification of *Streptomyces* isolates
- ✓ Fermentation and preparation of *Streptomyces* crude extracts
- ✓ Bioactivities screening of *Streptomyces* crude extracts
- ✓ Determination of total phenolic content
- ✓ Maintenance and growth condition of human derived cancer cell lines
- ✓ Cytotoxic activity screening using 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) assay

statistical analysis:

- ✓ Antioxidant and cytotoxic screening assays were performed in this four-replicate study.
- ✓ Data were expressed as mean standard deviation (SD). One-way analysis of variance (ANOVA) was performed by Tukey post hoc analysis using **SPSS** statistical analysis software version 22 to investigate significant differences between groups.
- ✓ The difference when $p < 0.05$ was considered statistically significant.

Results

➤ **Diversity of Streptomyces isolates:**

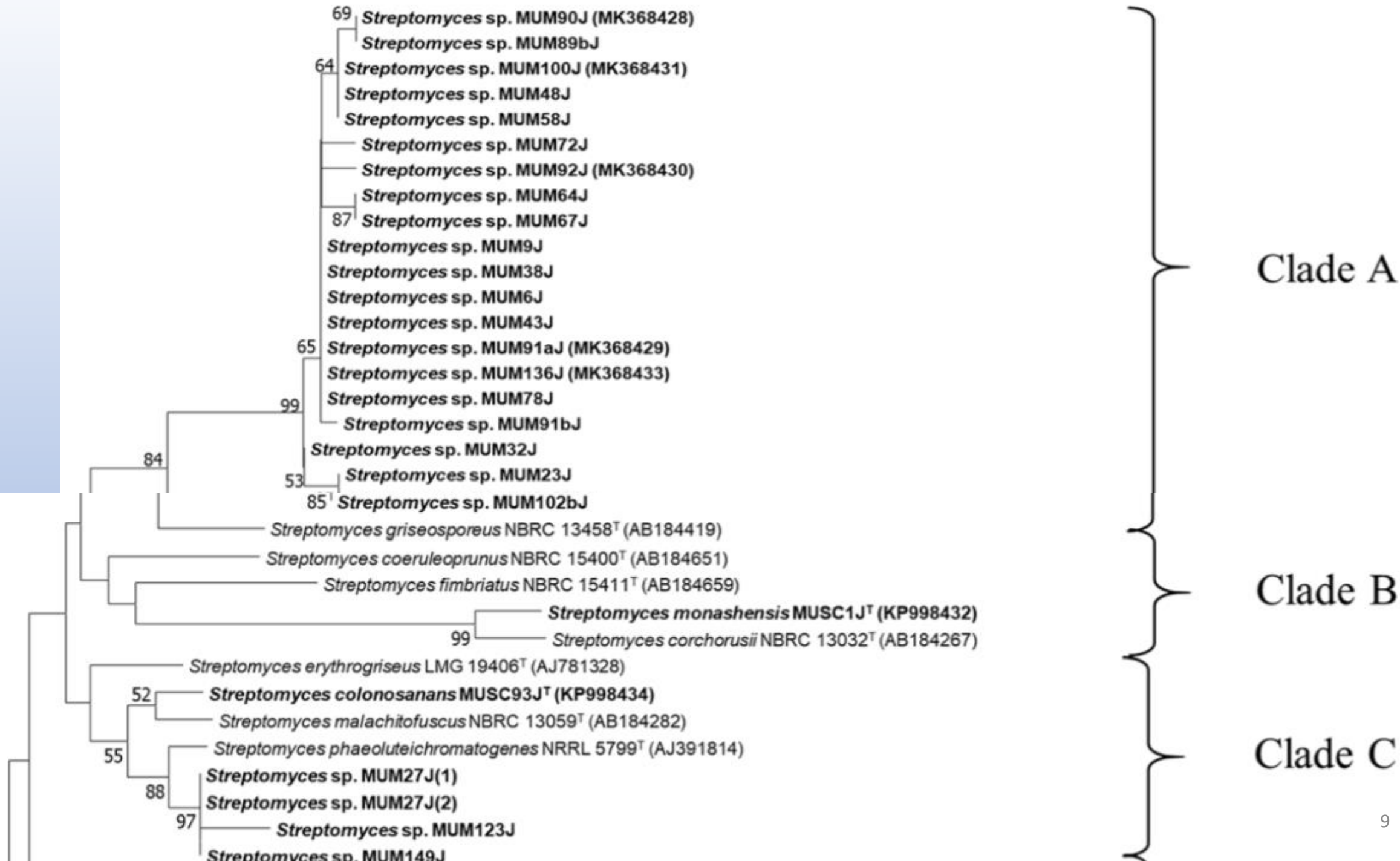


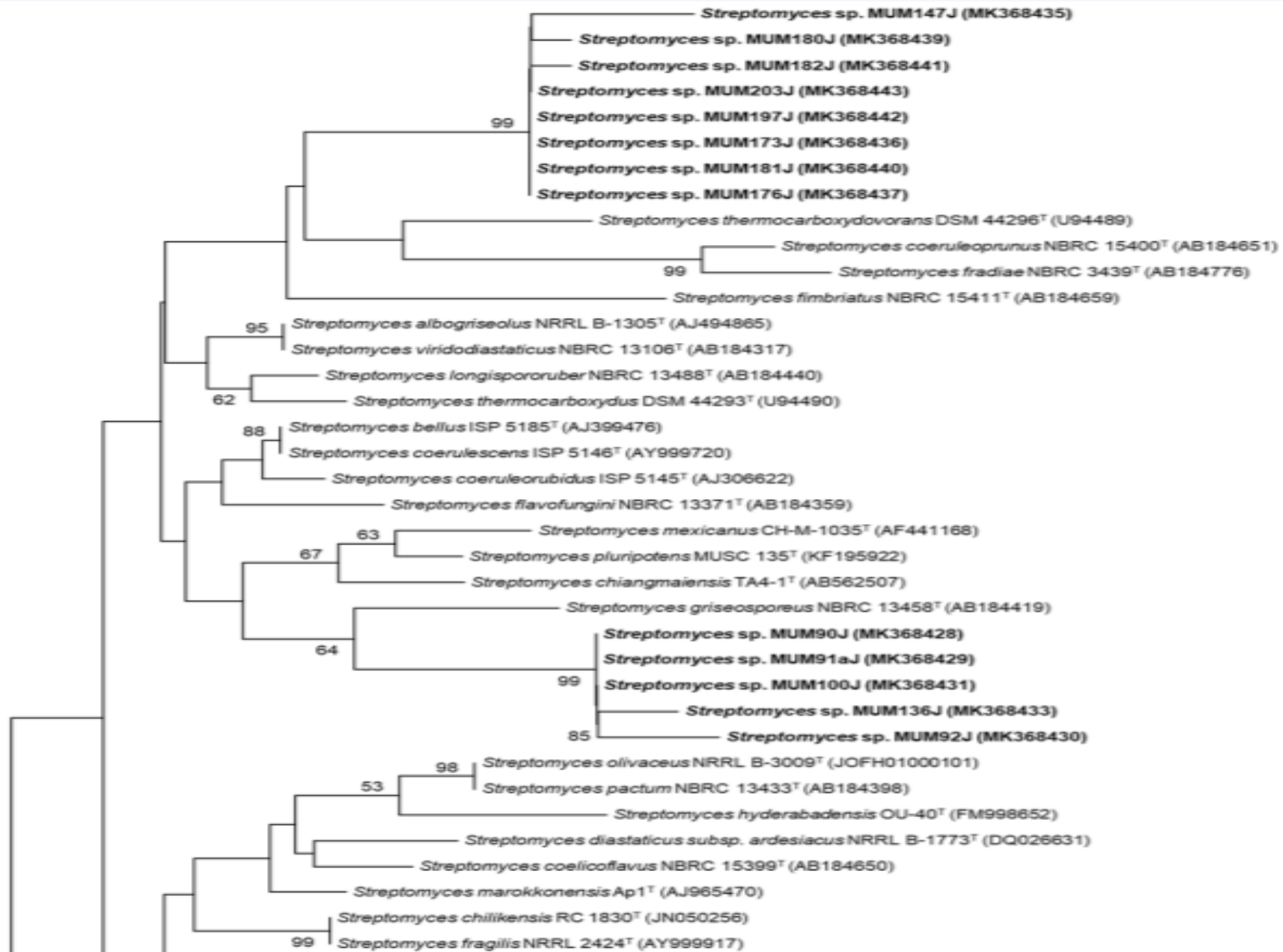




Figure 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the 88 *Streptomyces* sp. isolates and their closely related type strains. Bootstrap value based on 1000 resampled datasets are shown at branch nodes. Bar, 0.005 substitutions per site.

Results

- ✓ **Diversity and phylogeny of potentially novel Streptomyces isolates:**



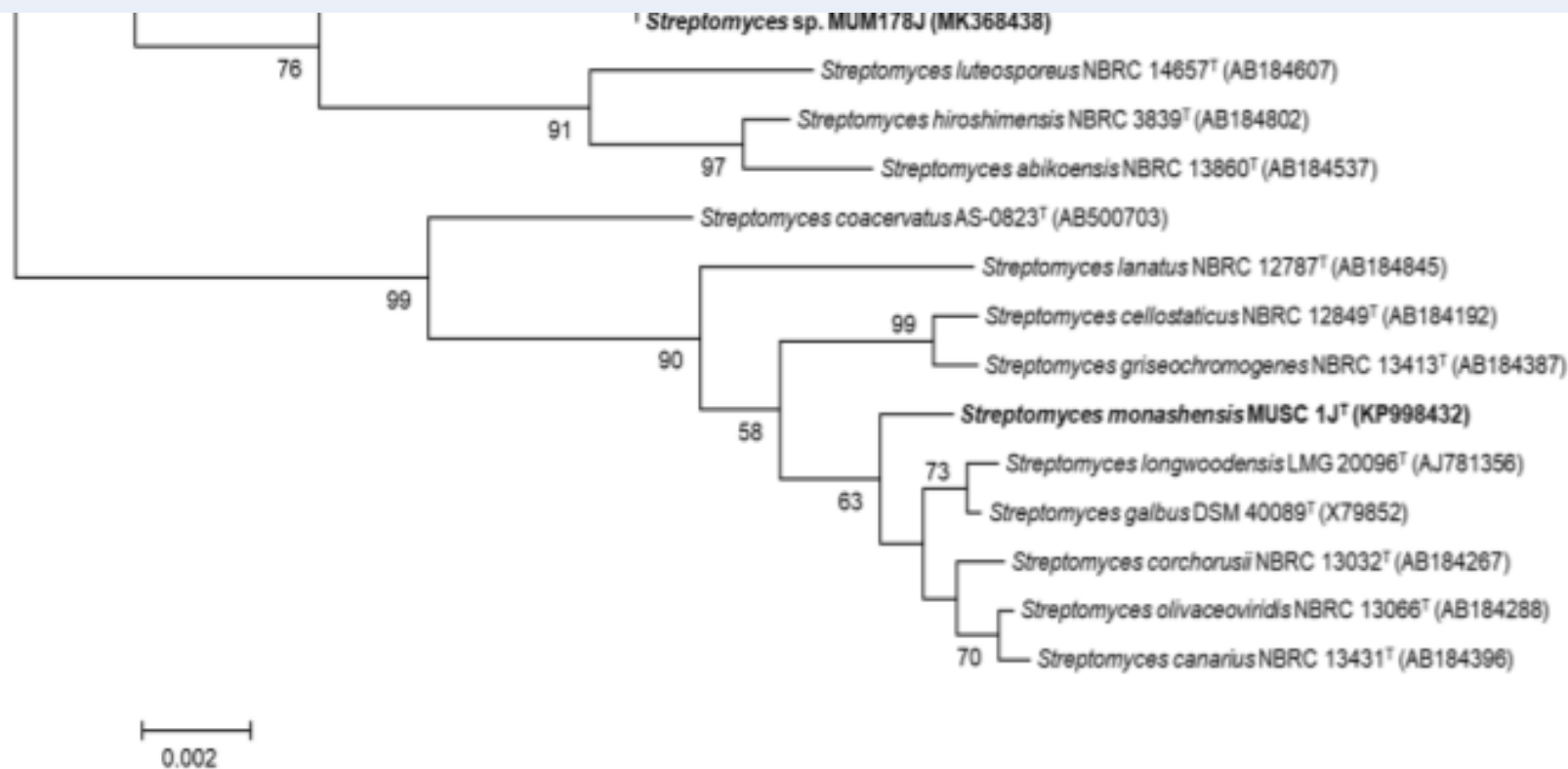


Figure 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing relationship between 18 potentially novel *Streptomyces* sp. and their closely related type strains. Bootstrap value based on 1000 resampled datasets are shown at branch nodes. Bar, 0.002 substitutions per site.

Results

- ✓ **Antioxidant activity of extracts from potentially novel Streptomyces isolates**

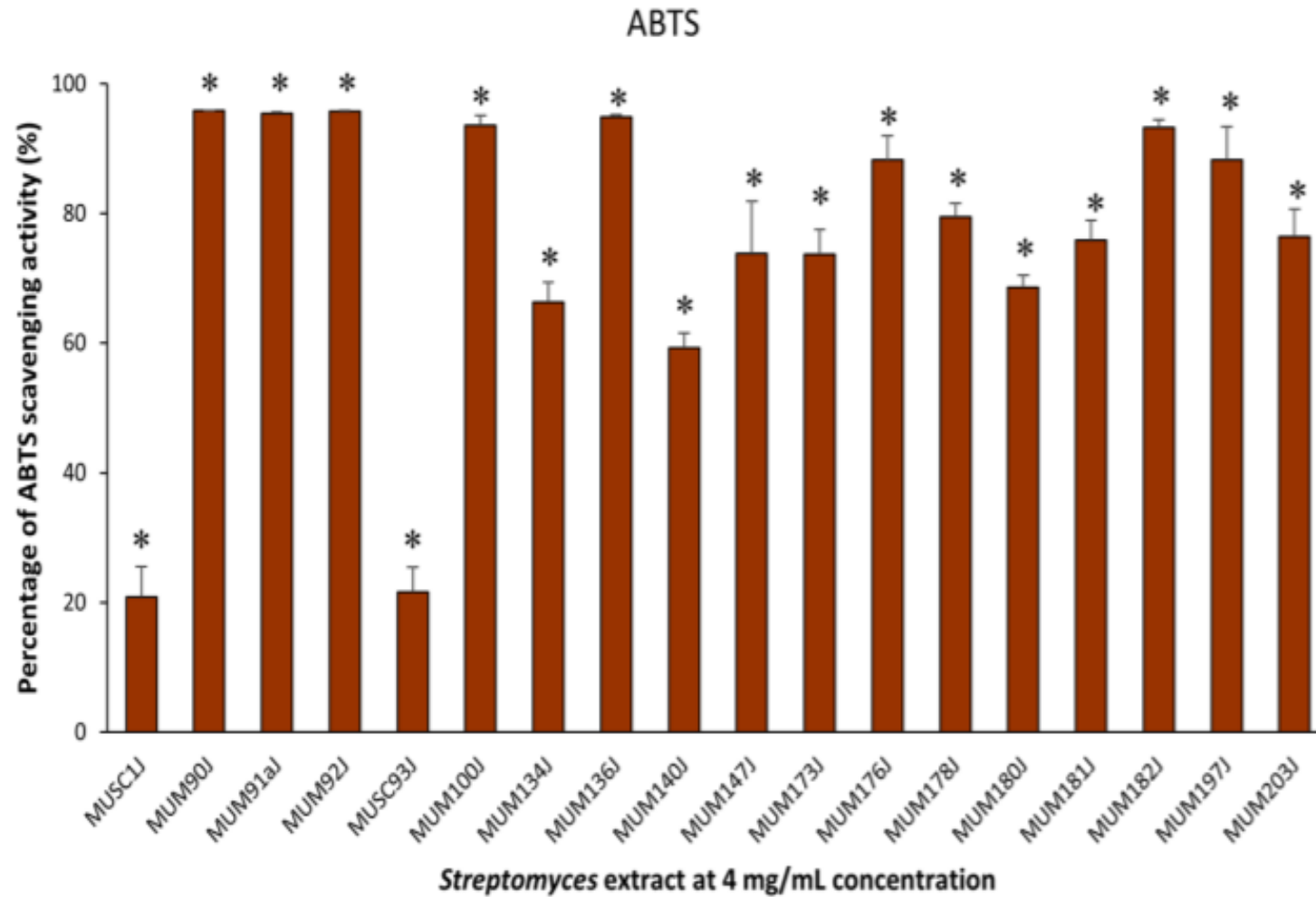


Figure 3. ABTS radical scavenging activity of extracts from potentially novel *Streptomyces* sp. isolates. Symbol (*) indicates $p < 0.05$ significant difference between the extract and control (without extract).

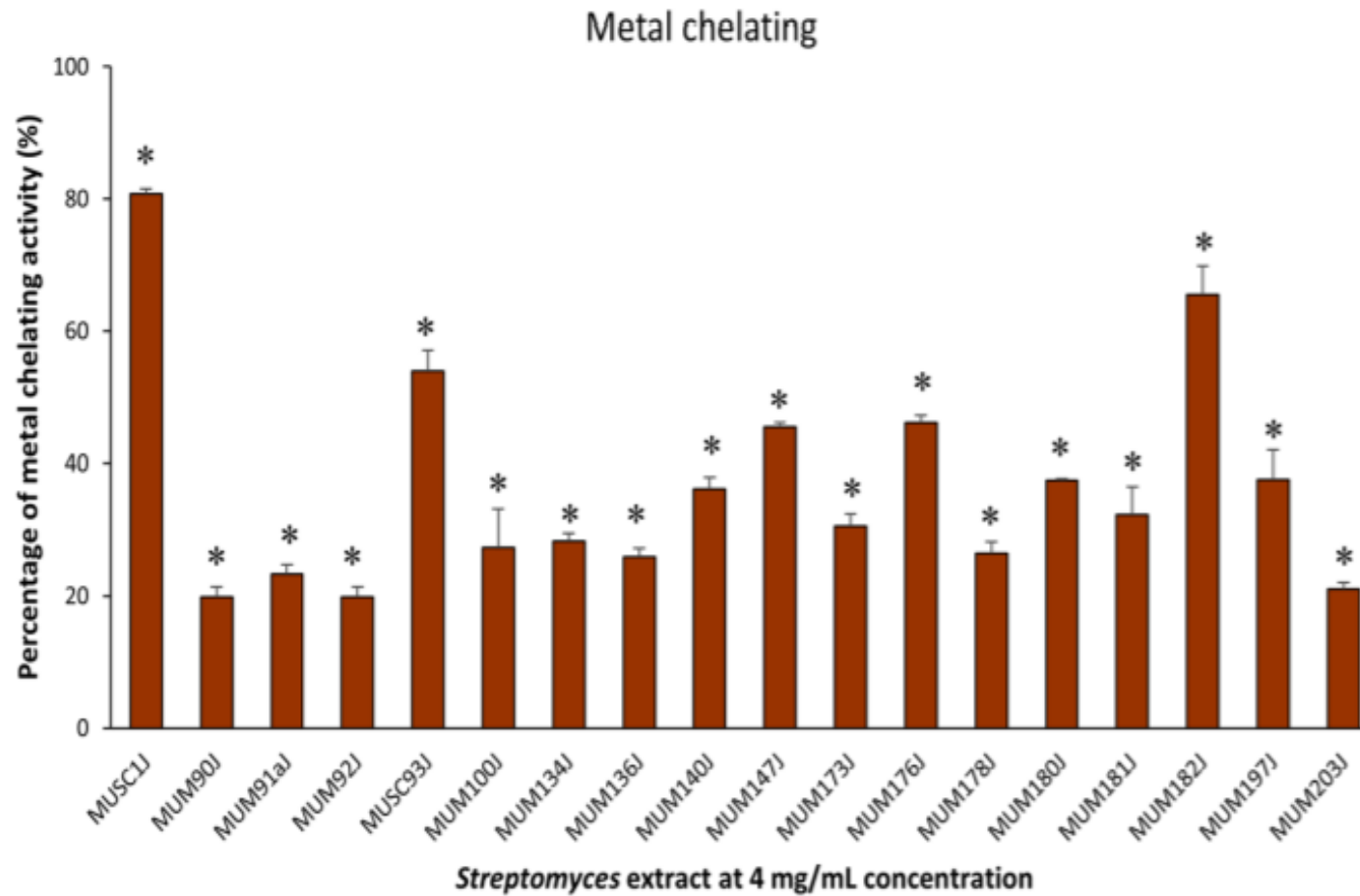


Figure 4. Metal chelating activity of extracts from potentially novel *Streptomyces* sp. isolates. Symbol (*) indicates $p < 0.05$ significant difference between the extract and control (without extract).

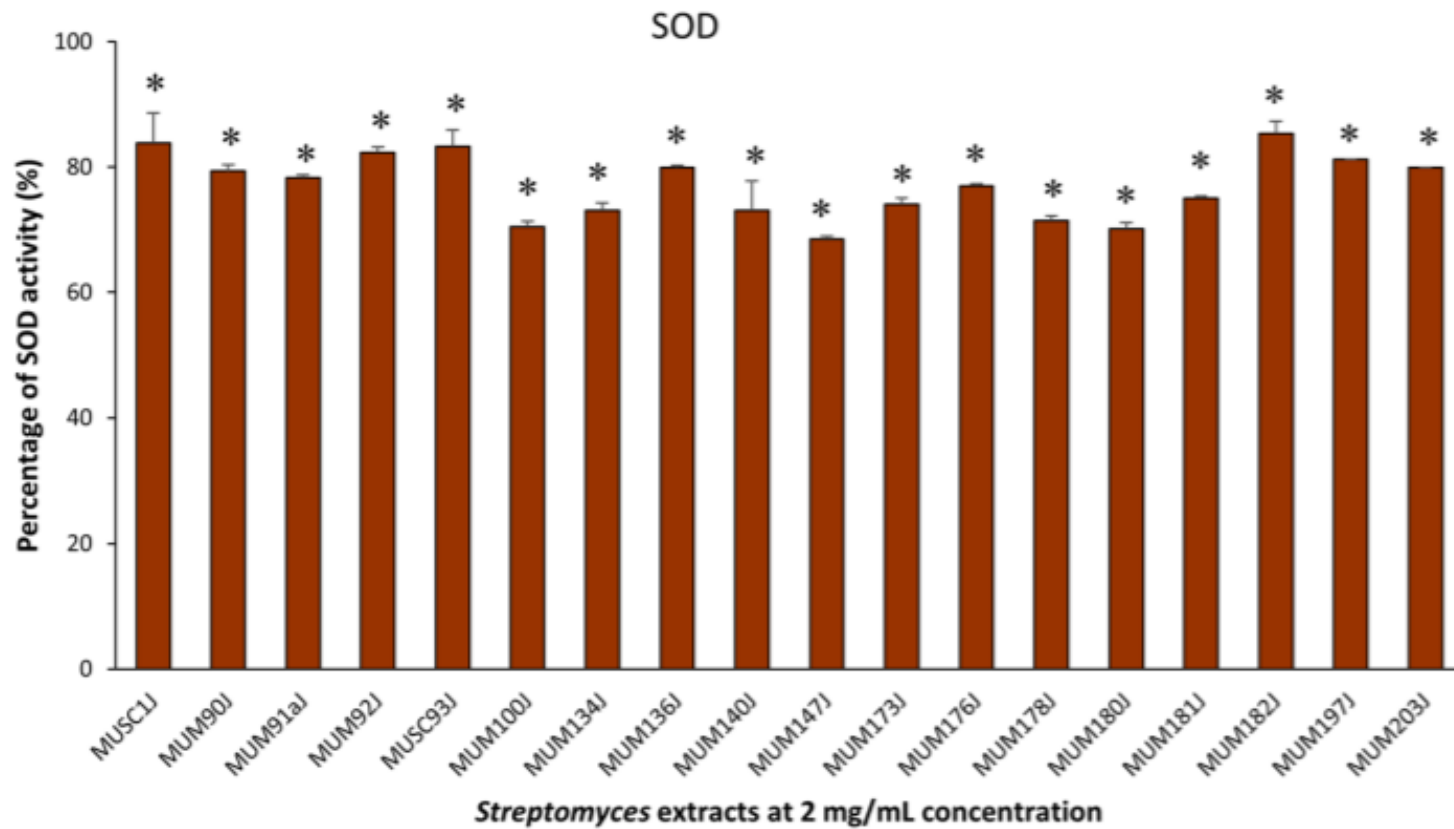


Figure 5. SOD-like activity of extracts from potentially novel *Streptomyces* sp. isolates. Symbol (*) indicates $p < 0.05$ significant difference between the extract and control (without extract).

Results

- ✓ **Total phenolic content (TPC) of methanolic extracts and antioxidant activity**

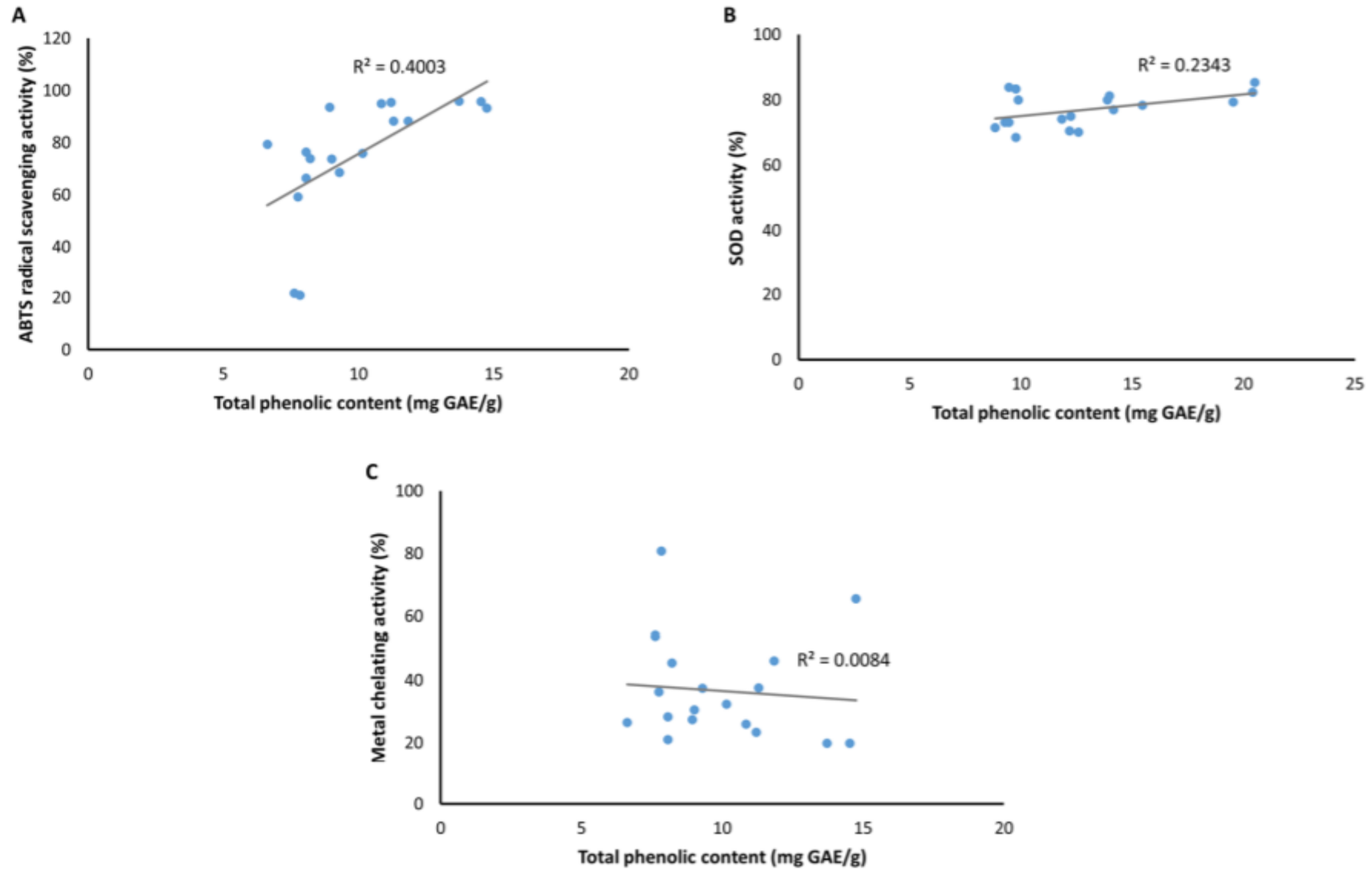


Figure 6. Correlation between total phenolic content and antioxidant capacity of 18 *Streptomyces* methanolic extracts. The relationship was observed in three different antioxidant assays: (A) ABTS, (B) SOD, and (C) metal chelating.

Results

- ✓ **Cytotoxic activity of extracts from potentially novel Streptomyces isolates**

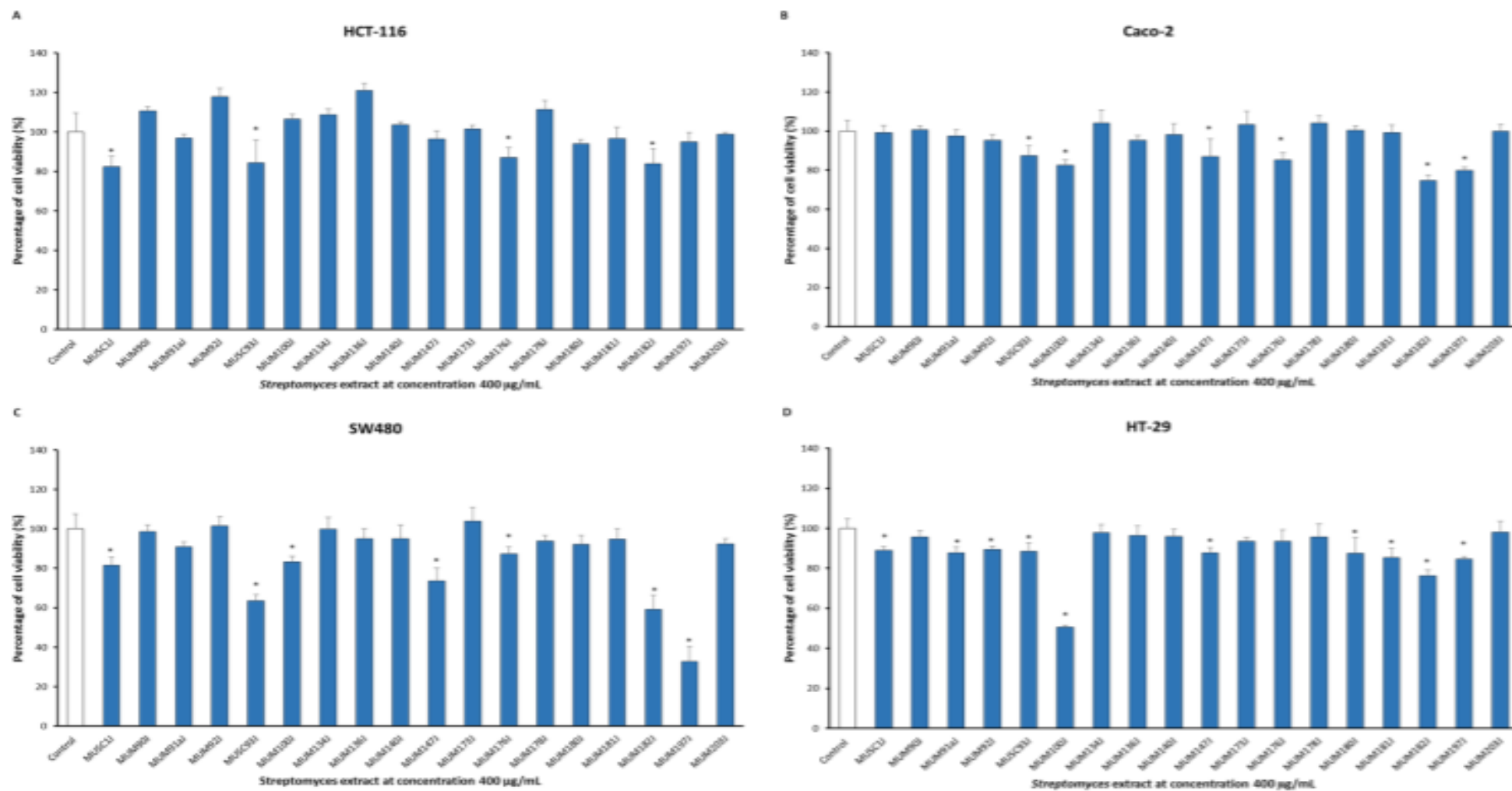


Figure 7. Cytotoxic activity of extracts from potentially novel *Streptomyces* isolates against colon cancer cell lines. The measurement of cell viability was done using MTT assay and the concentration of extract was 400 µg/mL. The graphs show cytotoxicity effect of the extracts against: (A) HCT-116, (B) Caco-2, (C) SW480, and (D) HT-29. All data are expressed as mean ± standard deviation and significance level are set as 0.05. Symbol (*) indicates $p < 0.05$ significant difference between the cells treated with extract and control (without extract).

Discussion

- ✓ The findings of this study indicate that there is a high diversity of the genus *Streptomyces* in Sarawak mangroves.
- ✓ These strains have been shown to produce beneficial bioactive compounds.

Discussion

✓ This study was performed using three different antioxidant methods in vitro: ABTS, metal chelating and SOD.

These assays operate on a variety of principles, for which ABTS measures the ability to inhibit free radicals, metal chelating assesses the ability to chelate metal ions, and SOD determines the inhibition of the production of superoxide anion radicals.

In addition, ABTS, SOD, and metal chelating assays are commonly used in many studies to evaluate the antioxidant capacity of extracts.

Discussion

- ✓ ABTS measurement offers advantages such as rapid reaction with samples, greater flexibility in which it can operate at different pH levels as well as soluble in aqueous and organic solvents.
- ✓ Meanwhile, metal chelating is performed because chelation of metal ions is considered as one of the main mechanisms of antioxidant activity.
- ✓ In the case of SOD measurement, this is a simple test that can provide repeatable results.

Conclusion

- ✓ As a result, the present study demonstrates the diversity of Streptomyces species. Sarawak mangrove based on comprehensive genotypic and phylogenetic analysis.
- ✓ Isolates showed promising antioxidant and cytotoxic activity through high-potency bioactivity screening.
- ✓ This study demonstrates the untapped potential of the Sarawak mangrove forest and is a valuable source of streptomyces that could be used for future biological exploration studies.
- ✓ In addition, the antioxidant and cytotoxic abilities provided by these mangrove-derived streptomyces are a sign that they can produce interesting bioactive compounds that may help discover the drug in the future.

thank you for your attention

Strong people
don't put others
down...
They lift them up.