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ARTICLE

Soil-derived actinobacteria with antibacterial and antiparasitic activities

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Abstract: Microorganisms synthesise a wide range of bioactive compounds that can serve as drugs to treat various infections. Toxoplasmosis is a widespread zoonotic infection caused by the protozoan parasite Toxoplasma gondii, for which no existing drugs can completely eliminate latent tissue cysts. Thus, actinomycetes were isolated from soil samples in Mongolia and evaluated for their antibacterial and antiparasitic activities. All the crude extracts inhibited the growth of T. gondii by more than 50%. Notably, the crude extract from MNL-50 exhibited anti-protozoal activity with an IC50 of 21.9 µg/ml. Moreover, bioactive compounds derived from natural resources represent a promising approach for combating bacterial infections. Therefore, we examined these compounds for their antibacterial properties against Escherichia coli and Staphylococcus aureus. The enzymatic activities of actinomycetes were also assessed. Among the tested isolates, all strains showed the ability to degrade amylase and lipase, indicating broad starch-degrading potential. Phylogenetic analysis of the selected strain MNL-50 revealed that it shares the greatest similarity with Streptomyces bacillaris, as determined by 16S rRNA gene sequencing analysis. This study highlights the potential of soil actinomycetes as sources of antiprotozoal and antibacterial compounds, and suggests that further investigation is warranted.

Keyword: *Actinomycetes, Toxoplasmosis, soil, enzyme, bioactive compounds;*

INTRODUCTION

Natural resources are critically important in modern drug discovery due to their broad pharmaceutical relevance, providing a broad range of structurally diverse and biologically potent compounds. Microorganisms and medicinal plants are major sources of these bioactive compounds. Among these microorganisms, actinobacteria, which is one of the most metabolically variable and ecologically significant microorganisms in the world, is characterised by a high G+C genome content and a remarkable ability to produce secondary metabolites. Actinobacteria play major roles in soil nutrient cycling, organic matter decomposition, and interactions with plants and other microorganisms [1].

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Among actinobacteria, the genus *Streptomyces* is particularly noteworthy for its capacity to produce a wide variety of secondary metabolites, including antibiotics, antifungals, antiparasitic agents, anticancer compounds, immunosuppressants, and numerous agrochemicals [2]. In fact, actinobacteria are responsible for producing more than two-thirds of clinically used antibiotics discovered to date [3]. Theextraordinary biosynthetic richness of this group has made it central to natural product discovery and biomedical research.

Furthermore, actinobacteria, especially *Streptomyces* species, produce a variety of extracellular and intracellular enzymes that have significant ecological, industrial, and pharmaceutical importance. These enzymes are capable of degrading complex organic matters [4]. Actinomycetes produce various industrially important enzymes, including amylases, proteases, lipases, cellulases, xylanases, chitinases, gelatinases, and keratinases, many of which have important biotechnological applications [5].

Toxoplasmosis is caused by the intracellular protozoan parasite *Toxoplasma gondii*, which can infect various warm-blooded animals, including humans. Members of the family *Felidae* serve as the definitive hosts, shedding oocysts that facilitate efficient transmission to humans and other hosts. Human infection commonly occurs through ingestion of contaminated food or water, the consumption of undercooked meat containing tissue cysts, or vertical transmission during pregnancy [6]. Current treatments have notable side effects and limited efficacy, particularly against chronic infections, highlighting the urgent need for safer and more effective therapy. Currently, no ideal drugs are available. Toxoplasmosis remains a critical challenge due to the parasite's complex intracellular lifestyle and its ability to form persistent tissue cysts that are refractory to most medications. Current therapeutic strategies primarily target the rapidly dividing tachyzoite stage and rely on a combination of pyrimethamine and sulfadiazine, along with folinic acid, to reduce hematologic toxicity [7]. Although this regimen is considered the gold standard, it is associated with significant side effects, limited availability, and diminished efficacy against chronic infection.

Mongolia, one of the largest landlocked countries, which is characterised by its extreme and variable climate, has preserved ecosystems rich in microbial biodiversity. Therefore, it is important to continue the research in this field to identify microorganisms in Mongolia that can be used to control infectious diseases. Globally, the identification of bioactive compounds from microorganisms for drug development remains a major challenge [9]. Therefore, there is a critical need to screen for more effective drugs targeting protozoan infections. The objective is to screen and evaluate active compounds from natural sources such as actinobacteria (*Streptomyces* sp.), for potential therapeutic use.

MATERIAL AND METHODS

Sampling and isolation

Soil samples were collected from Selenge *aimag (*province), Mongolia, by removing surface litter and taking samples at a depth of 5–10 cm. The samples were air-dried, and appropriate aliquots of the dilutions were prepared in saline. These were spread on Gauze No. 1 and ISP media, as previously described [9].

Fermentation

The cultures were grown in ISP2 broth at 28°C for 7 days with shaking at 200 rpm. Afterward, the supernatant and mycelia were separated through centrifugation, and the supernatant was then extracted with an equal volume of ethyl acetate ($C_4H_8O_2$), and evaporated. Crude extracts were dissolved in dimethyl sulfoxide (DMSO) at a maximum concentration of 100 µg/ml.

Enzymatic activities

Urease activity was assessed using a 20% stock solution of urea, resulting in a final urea concentration of 2%.

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The tubes were observed regularly for enzyme production over a period of 14 days. For the protease activity, the skimmed milk utilisation test was conducted using nutrient agar medium, and 5% skimmed milk was autoclaved separately. Each strain was streaked across the plates, which were then incubated for 14 days. The plates were observed daily for clear zone. For amylase activities, nutrient agar media prepared with 5% starch solution were streaked with the strains and incubated for 14 days. After incubation, the plates were flooded with Gram's iodine and observed for clear zone formation. For lipase hydrolysis, tween agar (Peptone 10g, NaCI 5g, $CaCI_2$ 0.1g, Tween-80 10 ml) medium was prepared as mentioned earlier [9].

Antibacterial and antiparasitic activities

Antibacterial activity was determined by the agar diffusion method, and the results were evaluated based on the presence of clear zones. For antiprotozoal activities, human foreskin fibroblast (HFF) cells were grown in a 96-well plate at 37°C for 48 hours, and then infected with 100 µl of *T. gondii* (RH-GFP) strain. The evaluation was conducted using fluorescence intensity measured with a microplate reader, and IC_{50} values were determined based on dose–response curves analysed in GraphPad Prism 8 software.

***In vitro* cytotoxicity assay**

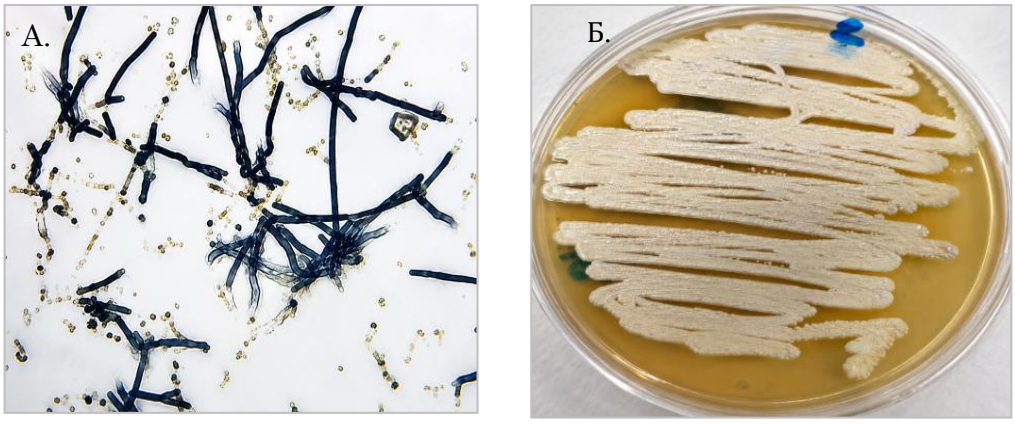
HFF were seeded in 96-well plates with 100 µl of Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (1×10^5 cells per well),allowed to adhere overnight at 37 °C, 5% CO_2 for 48 hours. Then, crude extracts were prepared by DMEM at the desired concentration and incubated for 72 hours. Cytotoxicity was assessed using the cell counting kit-8 CCK-8 assay on the HFF cell line.

Phylogenetic analysis

Total DNA was isolated from an overnight culture using the Zanaspex bacterial DNA isolation kit, following the manufacturer's instructions. For molecular identification, the 16S rRNA gene sequence was amplified through PCR using the universal bacterial primers 27F and 1492R.

RESULTS AND DISCUSSION

In the present study, 6 actinomycete cultures were isolated from soil, and crude extracts were obtained using organic solvents. Morphological characterisation was then evaluated.



Picture 1. Morphological characterization A. Actinomycetes cell (MNL-12) B. Colony of actinomycetes (MNL-12, MNL.-50).

The crude extracts from 6 isolates demonstrated antibacterial activity against the pathogenic bacteria S.aureus and E.coli. Actinomycete isolates displayed a wide range of extracellular enzymatic activities, reflecting their strong biodegradation potential. All strains produced amylase,

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while the activities of lipase and protease varied among isolates (shown in Table 1).

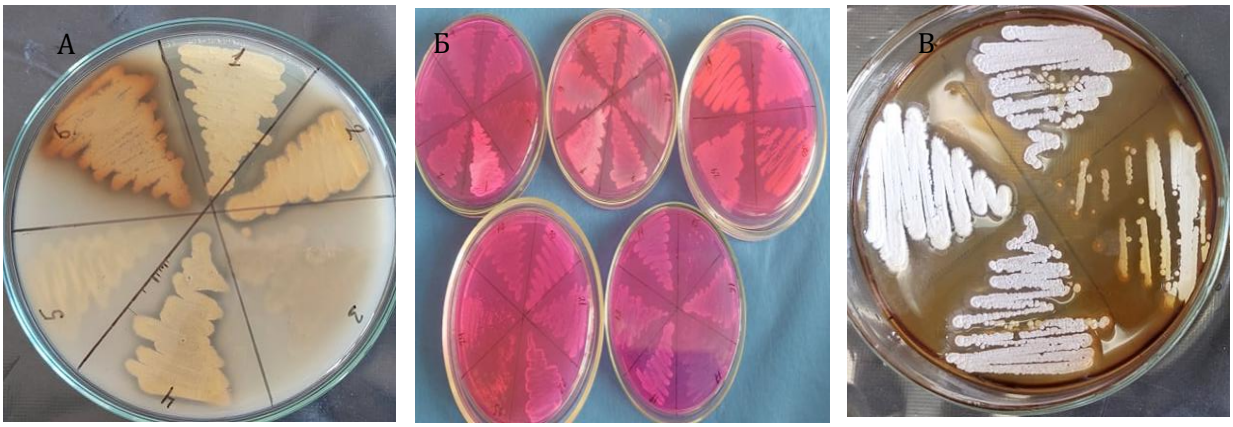
Table 1. Enzymatic and antibacterial activities of actinomycetes.

Code	Enzymatic activities				Antibacterial activities	
	Amylase	Urease	lipase	Protease	*E coli*	*S. aureus*
MNL-2	+++	-	-	+	+++	+++
MNL-50	+++	+	+++	+++	+++	+++
MNL-24	+++	-	+	+	++	++
MNL-21	+++	-	+	+	++	++
MNL-54	+++	-	+	+++	+++	+++
MNL-7	++	+	+	+	+++	+++

+++ Good activity, ++ Moderate activity, +Low activity, -No activity

Notably, every tested isolate was capable of degrading amylase, which indicates a broad starch-degrading potential. Isolates MNL-50 and MNL-54 exhibited high levels of lipase, and protease activities. Urease activity was present only in MNL-50 and MNL-7, and it was relatively weak.

In terms of antibacterial activity, MNL-50, MNL-54, MNL-7, and MNL-2 showed strong activity against both E. coli and S. aureus, indicating their potent broad-spectrum antimicrobial potential. Meanwhile, MNL-24 and MNL-21 exhibited moderate antibacterial activities.



Picture 2. Enzymatic activities of tested isolates A. Protease activity B. Urease activity C. Amylase activity.

The 16S rRNA gene sequences of the isolated actinomycete strains were analysed to determine their phylogenetic relationships (Picture 3).

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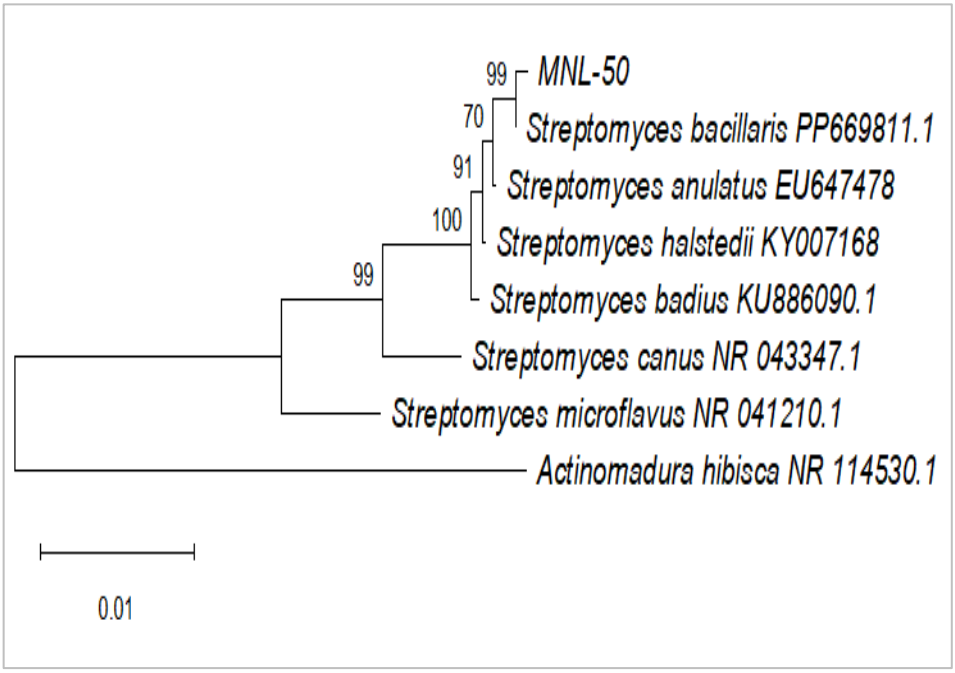

Picture 3. Rooted neighbor joining tree of strain MNL-50 based 16S rRNA gene sequences.

Nucleotide sequencing was performed by Tsingke Biotechnology Company, and the resulting sequences were compared for similarity with those in the NCBI GeneBank database. A phylogenetic tree was constructed using MEGA-12, employing the neighbour-joining method with 1000 bootstrap replications.

The *in vitro* cytotoxicity of MNL-50 was evaluated to determine its toxicity. The extract was tested on HFF cells at a concentration of 100 µg/ml, and cell viability was assessed. The *In vitro* cytotoxicity testing of MNL-50 on HFF cells exhibited minimal cytotoxicity with cell viability ranging from 65.9% to 99.5% at serial dilution concentrations of 6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, respectively (Diagram 1).

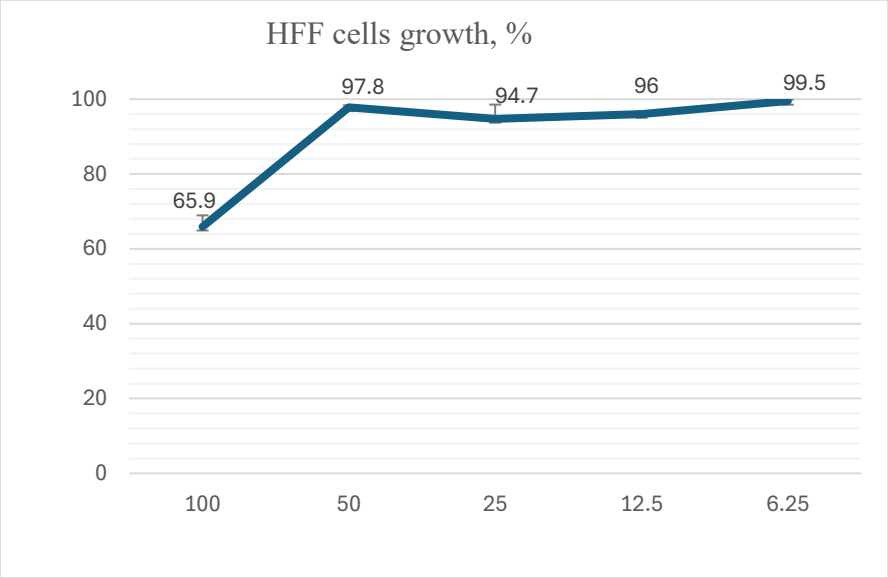

Diagram 1. HFF cells viability, %.

Strain MNL-50 showed minimal cytotoxicity, as indicated by the cell morphology (see Picture 4).

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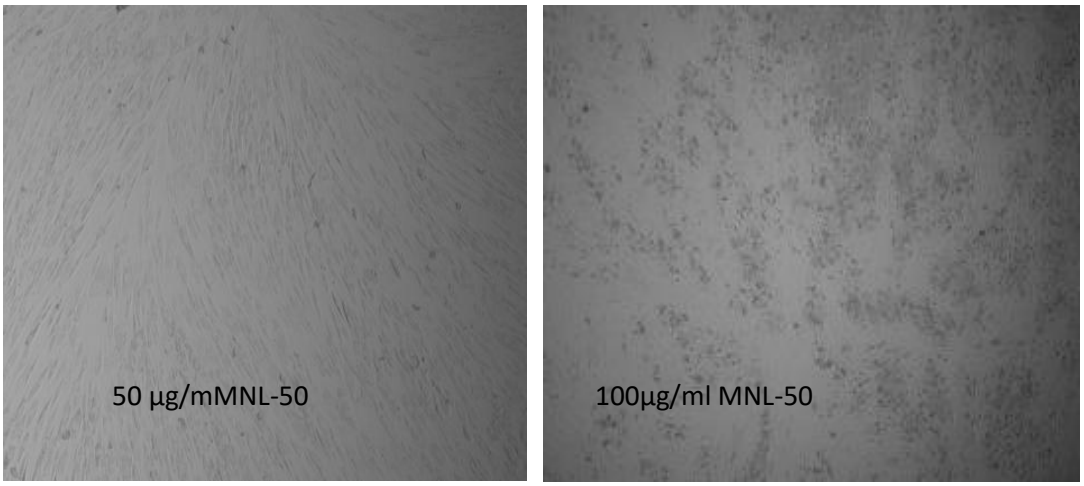
***In vitro* cytotoxicity of MNL-50 strain.**



Picture 4. HFF cell growth in concentration of 100 µg/ml crude extract.

The results demonstrated that isolate MNL-50 not only had low cytotoxicity in concentation of 50 µg/ml, it also showed mild morphological changes in concentration of 100 µg/ml crude extracts on the cell lines. These findings indicate low cytotoxicity at the tested concentrations.

All crude extracts were tested for their inhibitory effect on the growth of T.gondii (RH-GFP) in vitro. Among the tested crude extracts, 6 showed an inhibition of T. gondii growth by more than 50%, suggesting moderate antiparasitic activity. The crude extract of MNL-50 exhibited the most potent activity, with an IC50: 21.9 µg/ml (as shown in Diagram 2 and Picture 5).

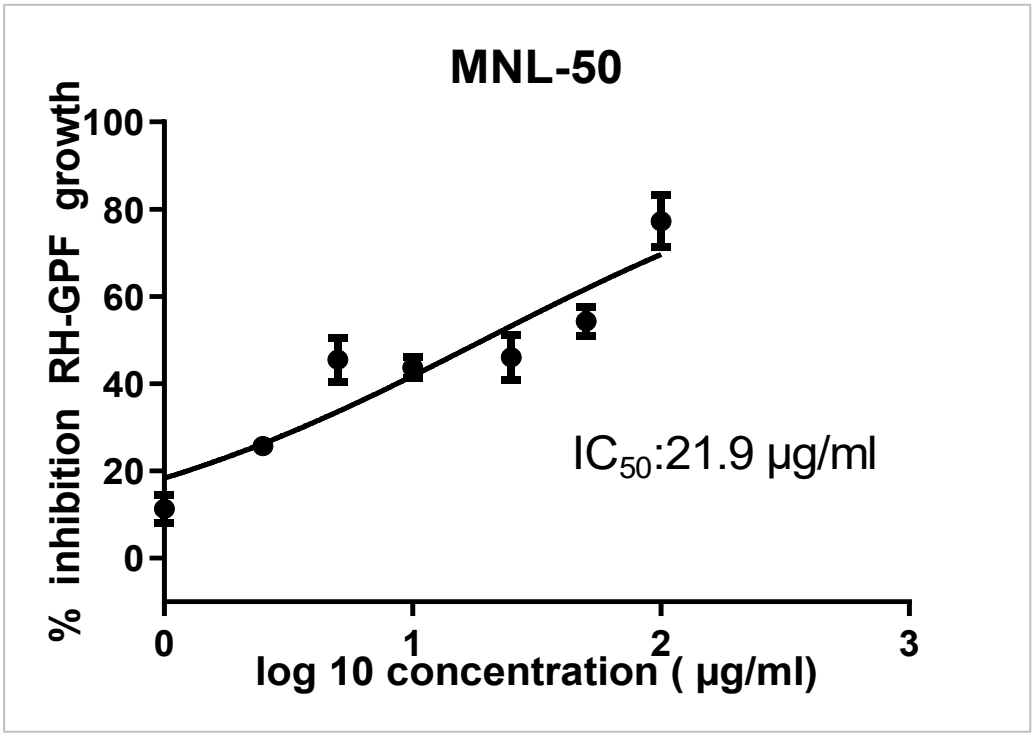


Diagram 2. *In vitro anti-Toxoplasma* activity.

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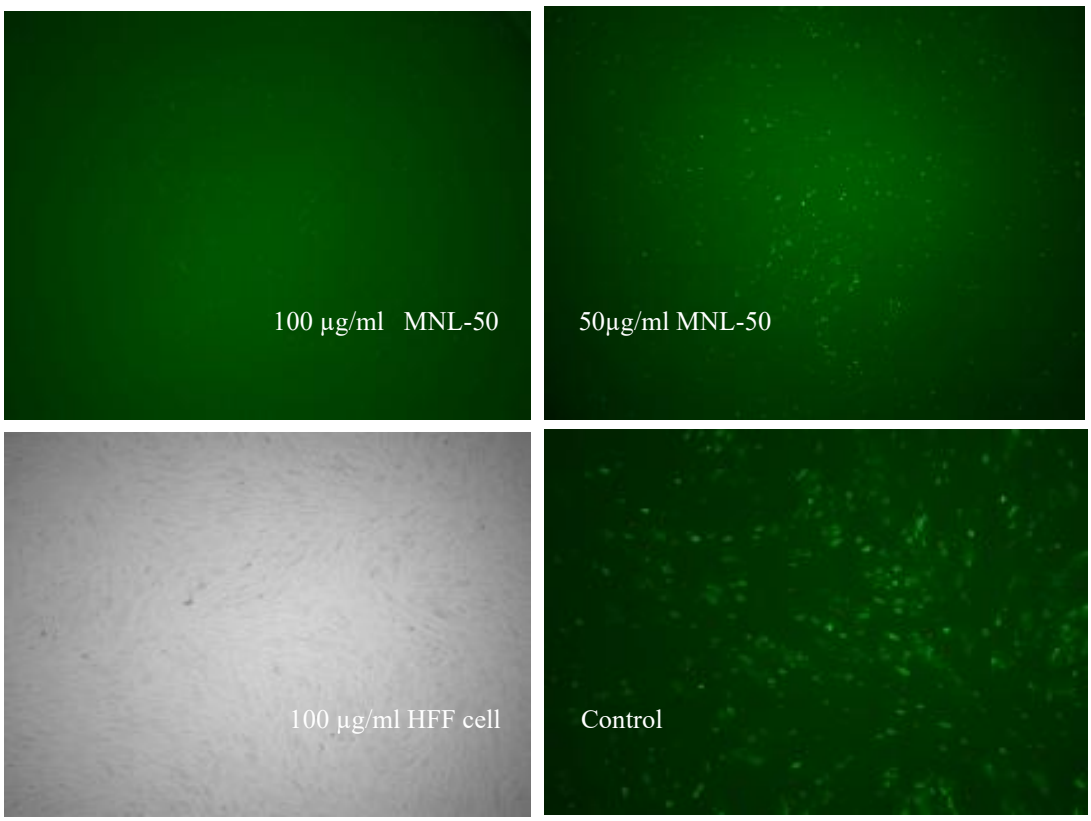

Picture 5. ***Fluorescence intensity of T.gondii RH-GFP.***

In the present research, actinomycetes from Mongolia were isolated, and investigated for their antibacterial, enzymatic, and antiparasitic activities, with particular consideration against *T. gondii*.

In our previous study, actinomycetes were isolated from soil in Mongolia and then evaluated for anti-*Toxoplasma* and antimalarial activities. Based on these results, phenazine-1-carboxylic acid (PCA) was detected in strain N25 (*Streptomyces canus),* and PCA possesses antiprotozoal activity against *T. gondii* (IC_{50}: 55.5 µg/ml) and *Plasmodium falciparum* (IC_{50}: 6.4 µg/ml) *in vitro.* The results reveal that actinomycetes are potential sources of antiprotozoal compounds, and that PCA merits further investigation as an anti-protozoal agent [8].

The present study showed that soil-derived actinomycetes from Mongolia exhibited both antibacterial and antiprotozoal activities, with the MNL-50 extract showing significant activity against *T. gondii* (IC_{50}: 21.9 µg/mL) with low toxicity. These results are consistent with previous studies, such as the evaluation of Lactoquinomycin A from *S.bacillaris* isolated from Jeju Island sediments, which displayed strong antibacterial activity [10].

Enzymatic activities of the isolates showed differences in extracellular enzyme production that can contribute to ecological importance and industrial applications [11]. This time, although enzymatic activities were not directly linked to antiparasitic effects, such properties may indicate that they synthesise secondary metabolites.

The isolate MNL-50 demonstrated effective antibacterial and antiparasitic activities and was characterised further through the 16S rRNA sequencing method. When comparing the nucleotide sequence of MNL-50 with entries in the NCBI database, a 99% sequence similarity was observed with *Streptomyces bacillaris* (PP669811.1).

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CONCLUSIONS

This study demonstrates that soil-derived actinomycetes from Mongolia are promising sources of bioactive metabolites with antiprotozoal and antibacterial activities. Among the isolates, strain MNL-50 exhibited the strongest antiprotozoal activity against *T.gondii* RH-GFP strain, and showed the highest sequence similarity to *Streptomyces bacillaris*. Further, it is important to continue the research in this field to discover microorganisms that can be used for the control of infectious diseases.

Acknowlegement

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Conflict of interest

The authors declare no conflict of interestrelated to this work.

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Author contribution

The authors confirm contribution to the paper as follows: P.B. conceived and designed the study. P.B., E.S., B.O., and R.T. conducted the experiments and collected data. P.B. analysed the data and drafted the manuscript. Y.N. provided supervision, methodological guidance. All authors reviewed the results and approved the final version of the article.

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