Streptomycetes as a source of novel antimicrobial and antiaging products

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Abstract

The genus *Streptomyces* consists of filamentous Gram positive bacteria found mainly in soil that contain an average of 30 secondary metabolite biosynthetic gene clusters and which are responsible for the production of nearly 80% of bioactive molecules. This makes investigating streptomycetes for the production of novel secondary metabolites that can serve as novel antimicrobial or antiaging compounds of great interest.

Our culture collection contains over 1000 streptomycete isolates originating from a wide variety of Greek locations and biomes, the majority of which have never been studied. For this reason, we decided to screen them for antimicrobial activity using a cross plate methodology involving the growing of streptomycetes on an agar plate in the presence of two Gram positive bacteria, two Gram negative bacteria and two fungi of the class Saccharomycetes selected based on their frequent use as indicator strains. Of the currently examined streptomyetes 45% showed inhibitory activity against Gram positive bacteria, 33% against Gram negative bacteria and 28% against saccharomycete fungi. The most promising of these streptomycete strains will have their active compounds extracted and isolated in order to determine whether they are novel compounds.

In addition, secondary metabolites will be extracted from solid cultures in order to evaluate their antiaging activity. Extracts will be screened in vitro and in cell-based assays for their antioxidant activity and their inhibitory activity against the elastase (anti-wrinkle) and tyrosinase (whitening activity) enzymes, as well as their ability to activate known anti-aging pathways such as proteasome and autophagy.

Methods

To screen our collection we used a cross-streak protocol that utilized two Gram positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), two Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and two saccharomycete fungi (*Saccharomyces cerevisiae*, *Candida albicans*) as markers of antibiotic or antifungal production.

Liquid cultures of each strain to be tested were streaked out on one third of an Arginine Glycerol Salt (AGS) medium plate for 7 days at 30°C. The bacterial marker strains were grown n liquid culture for 1 day and the fungal markers for 2 days, followed by the streaking them perpendicularly to the streptomycete isolate and incubating them for 2 days at 30°C along with a control that contained only the marker strains.

Visual inspection was used to determine whether there was inhibition and the level of inhibition was quantified by subtracting the length of each microbial marker growth line from that of the control plate.

Results

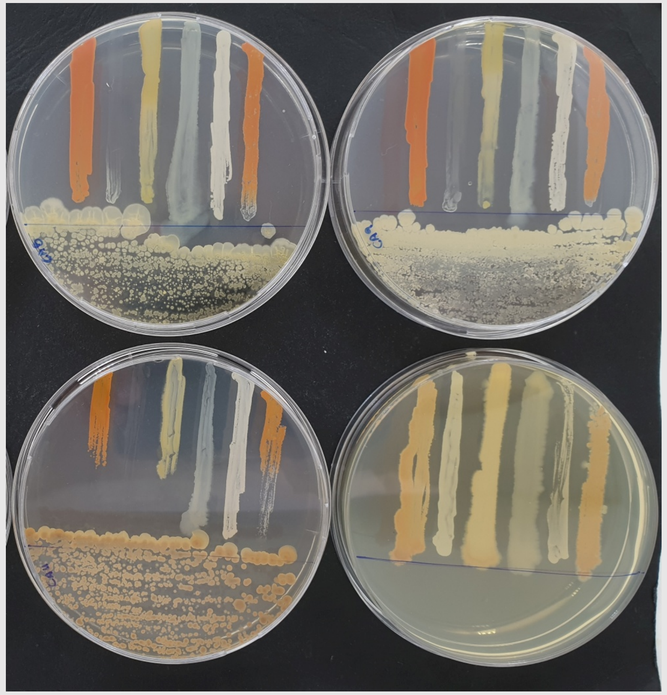

Figure 1. Example of screened actinobacteria

Marker	Inhibited	Not inhibited	Percent
B. subtilis	129	165	43.88%
S. aureus	28	266	9.52%
E. coli	18	276	6.12%
P. aeruginosa	89	205	30.27%
S. cerevisiae	52	242	17.69%
C. albicans	44	250	14.97%

Table 1. Number of streptomycete isolates able to inhibit each marker

Number of Markers Inhibited	Number of strains	Percent	Number of Markers Inhibited	Number of strains	Percent
1	48	16.33%	4	13	4.42%
2	39	13.27%	5	3	1.02%
3	45	15.31%	6	1	0.34%

Table 2. Number of streptomycete with antimicrobial activity against multiple markers

Discussion

- The protocol we used provided a simple and effective method to examine the production of antibiotic and antifungal compounds by the strains in our collection.
- A large percentage of our strains had antibiotic or antifungal activity and many of them were able to inhibit multiple different organisms, suggesting they were producing multiple antimicrobials.
- This demonstrates that the streptomycetes in our collection are prolific antimicrobial producers.
- The strains that inhibit multiple markers are of great interest and will be investigated further.

Future Work

- Streptomycetes produce a wide variety of secondary metabolites, the majority of which do not have an antimicrobial function; examining what functions these other compounds have is of great research interest .
- Extracts from solid cultures will be prepared to evaluate their antiaging activity.
- Tests will include *in vitro* and in cell-based assays for the extracts' antioxidant and inhibitory activity against the elastase and tyrosinase enzymes, as well as their ability to activate known anti-aging pathways such as proteasome and autophagy.
- The individual natural products with antiaging activities will then be isolated.