NISR Research GRANT
2005 Research Grant

Antibiotic Production in Streptomyces Induced in Co-culturing System

Yasuhiro IGARASHI

Department of Engineering, Toyama Prefectural University

Research aims

Pure culture is the fundamental basis in modern microbiology. Except for a part of symbiotic bacteria, it is widely accepted that microorganisms should be purely cultured both for scientific and industrial purposes and the contamination should be eliminated. Actinomycetes have a great ability of producing diverse bioactive compounds, many of which are industrially produced as pharmaceuticals and agrochemicals. In the use of actinomycetes, it is normal to culture them purely as same as other microorganisms. However, it was unexpectedly found in our laboratory that a contaminated bacterium induced the pigment production by *Streptomyces lividans* on the agar plate. We initiated this research to investigate the potential utility of the bacterium in the induction of antibiotic production by actinomycetes.

Methods

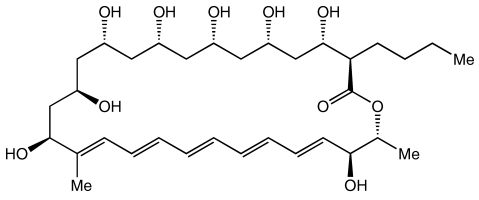
The bacterium used for the co-culture with *Streptomyces* was isolated as a contaminant from the culture plate of *Streptomyces lividans*. It is a Gram-positive bacillus, and was identified as *Tsukamurella pulmonis* TP-B0596 based on the 16S rDNA analysis. The actinomycete strains used in this study were isolated from the soil and plant samples in our laboratory, and identified as *Streptomyces* based on the taxonomic study. The seed and production fermentation was carried out in V-22 and A-3M medium, respectively[1]. The medium composition is as follows: V-22; soluble starch 1%, glucose 0.5%, NZ-case 0.3%, yeast extract 0.2%, tryptone 0.1%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, $CaCO_3$ 0.3% (pH 7) and A-3M; soluble starch 2%, glucose 0.5%, glycerol 2%, Pharmamedia 1.5%, yeast extract 0.3%, Diaion HP-20 1% (pH 7). The HPLC analysis was performed on an Agilent HP-1100 system with a photodiode array detector.

Results

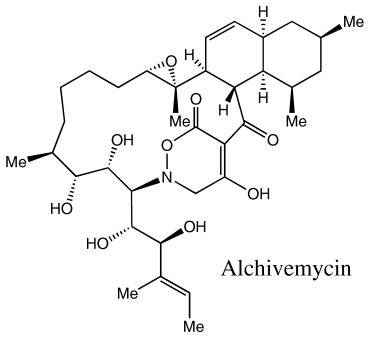
The co-culture of actinomycetes and *T. pulmonis* was carried out in the following manner. Actinomycete strains were cultured in V-22 medium for 4 days and *T. pulmonis* for 1 day, separately. To the A-3M medium were transferred 3% (v/v) of the seed actinomycete culture and 0.3% (v/v) of the seed *T. pulmonis* culture, and the production fermentation was carried out for 6 days at 30°C on a rotary shaker. The whole broth was extracted with 1-butanol, the organic layer was concentrated *in vacuo*, and the extract was subjected to antimicrobial assay. Similarly, the extract was prepared from the pure-cultured broth of actinomycetes and subjected to the bioassay. Among the 122 *Streptomyces* strains examined, 8 strains were identified to show antibacterial activity when co-cultured with *T. pulmonis* but not when purely cultured. In addition, production of secondary metabolites was detected in the co-cultured extract by the HPLC analysis, that were not found in the pure culture extract. Then, we isolated the secondary metabolites produced in the co-culture and analyzed their structures. Spectroscopic analysis using NMR and MS revealed that in *S. albogriseolus* S430 was induced the production of antibiotic A33853 and in *S. panayensis* TT1712 the production of aclacynomycins. Furthermore, two new compounds were isolated from the co-cultured broth: antibiotic TPU-0043 is the polyene macrolide from *S.* sp. TP-A0625[2] and alchivemycin is the antimicrobial macrocyclic compound from *S.* sp. S522[3], both of which are produced in the co-culture with *T. pulmonis*.

Conclusion

We found an interesting and unusual phenomenon that the co-culture of *Streptomyces* with a bacterium *T. pulmonis* induced the production of secondary metabolites which were not produced in a pure culture of *Streptomyces*. Although some metabolites produced in the co-culturing sys-



Antibiotic TPU-0043



Alchivemycin

tem are known compounds, two new compounds were found from this novel fermentation method. One of the new compounds is antibiotic TPU-0043, a stereoisomer of the pentaene macrolide chainin, and the other is alchivemycin which possesses the unprecedented heterocyclic structure revealed by X-ray crystallography. It is noteworthy that the secondary metabolism which is dormant under the normal condition is activated by 'contamination' and the new bioactive compounds are produced.

Recently, the frequency of finding a new compound from actinomycetes is decreasing[4]. However, an innovative fermentation technology such as the co-culturing system shown in this study may provide a new insight into the screening program from actinomycetes. The secondary metabolism is not induced by other bacteria, *e.g*. *Bacillus subtilis*, *Escherichia coli*, except for *T. pulmonis*. Further analysis on the mechanism of the co-culture and secondary metabolism induction in rare actinomycetes is under investigation.

References

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