The Expression of Pleiotropic and Pathway-Specific Regulators of Secondary Metabolism in Clorobiocin Producer *Streptomyces Roseochromogenes* NRRL3504

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Abstract – Clorobiocin is potent aminocoumarin against resistant pathogens. We describe attempts to improve clorobiocin production via expression of pleiotropic (adpA) and pathway-specific regulatory (cloG, novG) genes in Streptomyces roseochromogenes NRRL3504. Positive influence of adpA on clorobiocin production was revealed. The effects of cloG and novG are under investigation.

Keywords – Streptomyces, clorobiocin, AdpA, StrR-like regulators, gyrase inhibitors.

Introduction

Streptomyces roseochromogenes NRRL3504 produces an aminocoumarin antibiotic clorobiocin. This antibiotic inhibits bacterial topoisomerases type II (or DNA gyrases) and topoisomerase IV [1]. The dual mechanism of action might prove beneficial in slowing the development of resistance against this antibacterial compound, so there is an interest in the development of new drugs based on clorobiocin. The level of clorobiocin synthesis by the strain NRRL3504 is relatively low, so there is a need to generate its overproducers. This would greatly accelerate the study of the properties of the antibiotic and its transformation into a medical product. The synthesis of clorobiocin in *S. roseochromogenes* is controlled by the positive path-specific regulator CloG [2]. The orthologue of *cloG* in *S. spheroides*, a producer of the aminocoumarin antibiotic novobiocin, is *novG*, the overexpression of which leads to the significant increase of novobiocin production [3]. It is also known that the global transcription regulator AdpA often participates in positive regulation of secondary metabolites [4]. Therefore, the introduction of additional copies of the *cloG*, *novG* or *adpA* into the strain-producer of clorobiocin might have an effect on the synthesis level of the latter.

Results and Discussion

We have constructed a number of strains containing additional copies of the above-mentioned regulatory genes. The *cloG*, *novG*, *adpA* were cloned into a vector pmoeE5script under the control of the promoter of hexose isomerase gene *moeE5*. Thus, the plasmids pSM2097, pSM2025, pOOB95d, respectively, were constructed. Also, previously described plasmid pOOB92a, in which the *adpA* was controlled by the strong constitutive promoter *ermEp*, was used.

The conjugative transfer of these plasmids from the F+ strain *E. coli* WM6026 to strain *S. roseochromogenes* NRRL3504 was carried out. The recipient was grown in a liquid media. In a 50 ml flask, 8 ml of SG1 or TSB medium and a 1 cm^2 of NRRL 3504 , grown for 4-7 days in oatmeal, were added. The flask was incubated on a shaker (200 rpm) at 30 °C for 2-3 days. 100 μl of the resulting culture was transferred to a new flask with 8 ml of SG1 or TSB medium and grown for 14-19 hours. 1 ml of the mixture was centrifuged, the supernatant was poured out and resuspended in 300 μl of TSB. After that, the donor cells (from 1/2 cup) were added to the recipient, mixed and plated on the dishes (+25 mM $MgCl_2$). The dishes were incubated at 30 °C and were flooded with aqueous solution of apramicyn (for selection of transconjugants) and phosphomycin (to kill the donor cells) after 24 h. The growth of transconjugants was observed after 72h of selection. For the comparison of the antibiotic activity of transconjugants and parent

strain they were grown in a liquid medium of GYM, chlorobiocin was extracted using ethyl acetate from a whole fermentation medium. Antibiotic activity of the extracts was detected by the diffusion of antibiotics from disks into agar. The results indicate that the level of antibiotic activity of some recombinant clones containing the *adpA* of *S. ghanaensis* ATCC14672 is higher than that of the parent strain. An analysis of the antibiotic activity of *cloG*+ and *novG*+ strains is currently under investigation.

We have used liquid chromatography coupled to mass spectrometry to monitor secondary metabolites in culture extracts of NRRL3504 and recombinant strains (MORT1, SM3). In the wild type strain, clorobiocin (695.20 Da, $[M-H]^-$ ion) was revealed with Rt 12.12 min as a dominant product (Fig.1). In MORT1 and SM3 strains clorobiocin mass peak was revealed at a approximately the same retention time. Hence, all the analyzed strains indeed produce clorobiocin, and their quantitative analysis is underway in our laboratory.

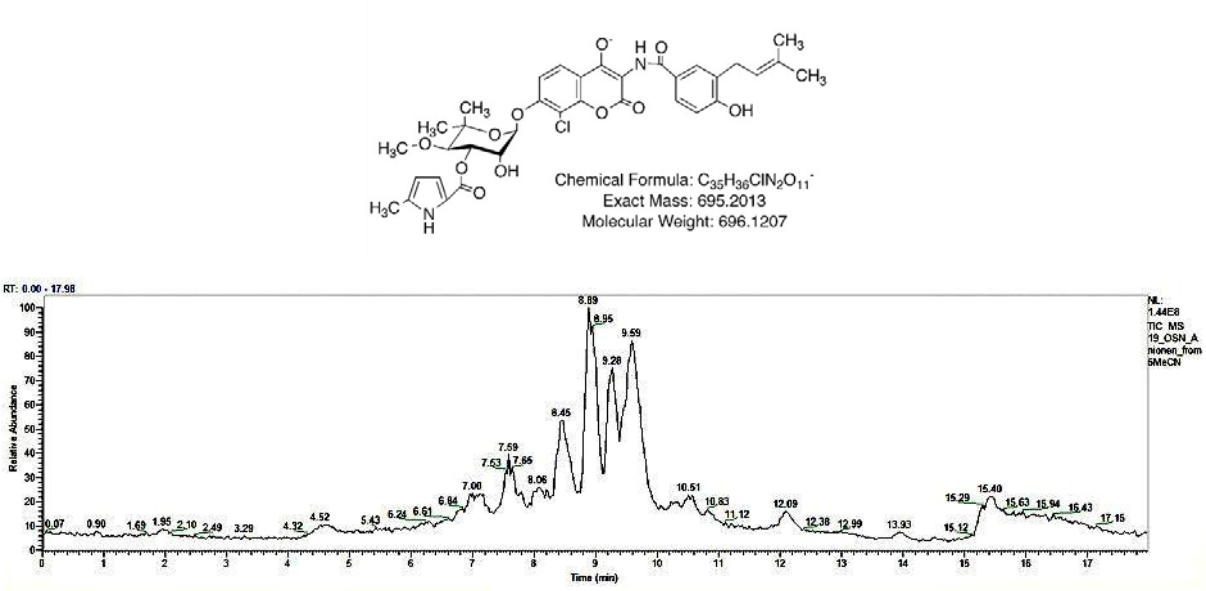

Fig.1. Chemical formula and results of mass spectrometry of clorobiocin (695.20 Da, [M-H]- ion).

Conclusion

A number of strains carrying additional copies of genes cloG, novG and adpA were constructed. Experiments have showed positive influence of adpA on the biosynthesis of clorobiocin. The effects of cloG and novG are under investigation.

References

[1] S. Alt, L.A. Mitchenall, A. Maxwell and L. Heide. “Inhibition of DNA gyrase and DNA topoisomerase IV of *Staphylococcus aureus* and *Escherichia coli* by aminocoumarin antibiotics”. *J. Antimicrob. Chemother.*, vol. 66, pp. 2061-2069, 2011.

[2] F. Pojer, S.M. Li, and L. Heide. “Molecular cloning and sequence analysis of the clorobiocin biosynthetic gene cluster: new insights into the biosynthesis of aminocoumarin antibiotics”. *Microbiology*, vol. 148, no. 12, pp. 3901-3911, 2002.

[3] L. Heide. “The aminocoumarins: biosynthesis and biology”. *Nat. Prod. Rep.*, vol. 26, no. 10, pp. 1241-1250, 2009.

[4] G. Liu, K.F. Chater, G. Chandra, G. Niu and H. Tan. “Molecular regulation of antibiotic biosynthesis in Streptomyces”. *Microbiol. Mol. Biol. Rev.*, vol. 77, no. 1, pp. 112-143, 2013.