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SHORT COMMUNICATION

Amicoumacins from a desert bacterium: quorum sensing inhibitor against *Chromobacterium violaceum*

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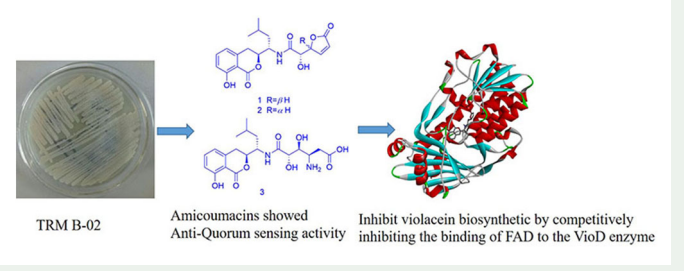
ABSTRACT

In our study, the anti-quorum sensing (QS) activity of fermentation broth from TRM B-02, a bacterium isolated from Taklimakan desert, was investigated using the biosensor bioassay on *Chromobacterium violaceum* ATCC12472. TRM B-02 was 100% similar to *Bacillus subtilis* subsp. Inaquosorum KCTC 13429$^{(T)}$ by genotypic and phenotypic analyses. Based on anti-QS activity tracking, six known amicoumacins, named as AI-77-H (**1**), AI-77-F (**2**), amicoumacin B (**3**), amicoumacin C (**4**), AI-77-C (**5**) and bacilosarcins D (**6**), were isolated and identified. Among them, compounds **1–3** exhibited a better inhibitory effect on *C. violaceum* ATCC12472. Further research suggested that compounds **1**–**3** could significantly down-regulate the expressions of violacein operon A (vioA), vioB, vioD and vioE and up-regulate vioC. Docking experiments indicated that compounds **1–3** may act as an inhibitor of violacein biosynthetic pathway competitively inhibiting the binding of flavin adenine dinucleotide (FAD) with the vioD enzyme.



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Quorum sensing inhibitor; *Bacillus subtilis*; amicoumacins

1. Introduction

Quorum sensing (QS), a bacterial cell-to-cell communication pathway, has been demonstrated in many human opportunistic pathogens. According to the survey, interfering with QS systems was shown to be a promising alternative strategy for controlling

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bacterial infections, especially those caused by traditional antibiotic-resistant strains (Sheng et al. 2015; Bai et al., 2014). *C. violaceum* ATCC12472 is one of the common used bacterial species for QS researching and disrupt the QS system can decrease the secretion of virulence factors. Compared with traditional antibiotics, quorum sensing inhibitors (QSIs), as an antibacterial agent, focus on reducing the virulence of bacteria without killing bacteria or inhibiting bacterial growth. To date, some literature reported that phytol, sponge extracts, freshwater bryozoan Hyalinella punctate extracts have anti-quorum sensing and anti-biofilm activity (Pejin et al. 2014, 2015, 2016). Besides, some microbial-derived compounds, such as tyrosol, piericidin A, glucopiericidin A, cyclo (Trp-Ser), docosanoic acid, borrelidin acid and 1H-pyrrole-2-carboxylic acid (Chang et al. 2019), have been reported to manifest potential QS inhibitory ability, and thus microbes could be an important source of QS inhibitors (QSIs). Extreme environment usually breeds special microbiological resources which can produce new rich secondary metabolites. Therefore, screening candidate bioactive strains from Taklimakan desert was an alternative choice. Base on this consideration, 182 bacteria strains isolated from Taklimakan desert were screened for QS inhibitory activity in preliminary experiment. Among them, a bacterium numbered TRM B-02 manifested anti-QS activity. In present study, taxonomic status and QS inhibition activity of extracts and compounds from TRM B-02 were studied.

2. Results and discussion

2.1. Identification of TRM B-02

TRM B-02 was identified similar to be *B. subtilis* subsp. Inaquosorum KCTC13429$^{(T)}$ by genotypic and phenotypic analyses (Figure S1).

2.2. Identification of six compounds from TRM B-02

AI-77-H (**1**), AI-77-F (**2**) (Gutierrez et al., 2012), amicoumacins B (**3**) and C (**4**) (Itoh et al., 1982), AI-77-C (**5**) (Shimojima et al., 1984), and bacillcoumacin D (**6**) (Bai et al., 2014) were isolated and identified (Figure 1) by comparison of their spectroscopic data with previously reported values.

2.3. Anti-QS activity of extracts and compounds isolated from TRM B-02

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) levels of five fractions ranged from 250 to 500 μg/mL and 500 to 1000 μg/mL, respectively (Table S1). Fractions fermentation broth (FB), petroleum ether extract (EAE) and ethanol extract (EE2) showed the same MIC and MBC value indicated that they had similar anti-QS activity. Besides, Fractions EAE and EE2 were the main active fractions in TRM B-02 crude extracts. Compounds **1**–**3**, isolated from fraction EE2, showed similar MIC value to fraction EE2, which indicated that they were the main active compounds in fraction EE2. All the tested extracts and compounds did not inhibit *C. violaceum* ATCC 12472 growth at subinhibitory concentrations of MIC.

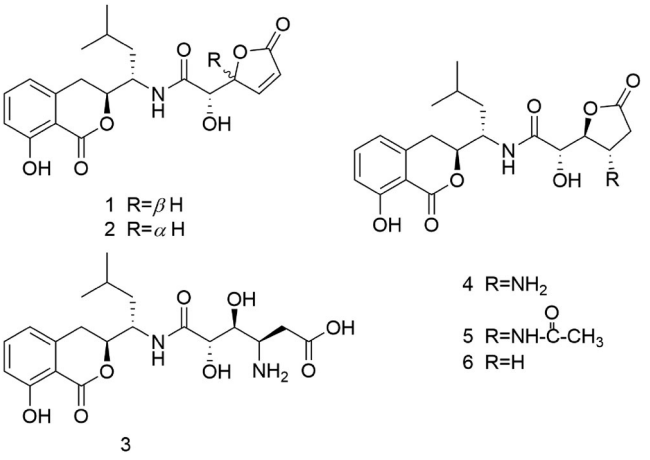

Figure 1. Structure of secondary metabolites isolated from TRM B-02.
Note: AI-77-H (**1**) and AI-77-F (**2**) , amicoumacins B (**3**) and C (**4**), AI-77-C (**5**), and bacillcoumacin D (**6**).

2.4. Reduction of purple pigment production

Further assessment of purple pigment inhibition in *C. violaceum* ATCC12472 of fractions FB, EE2, and compounds 1–**3** were performed. The purple pigment inhibition activity of fractions FB, EE2, and compounds **1–3** were further performed on *C. violaceum* ATCC12472 (Figure S2). The tested extracts and compounds could reduce the formation of purple pigment with the inhibition rate of 32.38%, 16.51%, 20.11%, 21.89% and 16.67%, respectively, at the concentration of 31.25 μg/mL, while no interfering with the growth of *C. violaceum* ATCC12472 was observed. After treated with FB, the reduction rate of purple pigment in *C. violaceum* ATCC12472 was higher than that treated with EE2. Compounds **1**–**3**, three amicoumacin compounds isolated from EE2 fraction, exhibited higher anti-QS activity than EE2, indicating that they were the main anti-QS activity compounds of EE2 fraction. Amicoumacins were mainly produced by microorganisms including *Actinomycete* and *Bacillus*, and exhibit extensive biological activities, such as anti-microbial, anti-inflammatory and antiulcer (Berrue et al. 2009). Our results showed that amicoumacins isolated from TRM B-02 had anti-QS activity, which was reported for the first time.

2.5. Influence on vioABCDE operon expression

The content changing of vioA, vioB, vioC, vioD and vioE genes was observed during the assessment of virulence activity in *C. violaceum* ATCC12472 treated with fractions FB, EE2 and compounds **1–3** at MIC concertrations. As shown in Figure S3, compared with the untreated group, the vioA, vioB, vioD and vioE genes in the treated groups were down-regulated, and the vioC gene was up-regulated.

2.6. Molecular docking analyses

In order to analyse the mechanism of action of compounds **1**–**3**, docking studies were conducted to determine the potential binding interaction between compounds **1**–**3**

and QS receptor, which might draw a possible explanation for the different responses of vioD and vioE genes to compounds **1**–**3**. Results indicated that the predicted binding energies between compounds **1**–**3** and the vioD enzyme were −9.02, −8.89 and −7.91 kcal·mol^{-1}, respectively. The binding energies of compounds **1**–**3** and the vioE enzyme were predicted to be −5.32, −4.04, and −3.88 kcal·mol^{-1}, respectively. Compared to vioE, compounds **1**–**3** were more stable in docking with vioD (Figure S4). Compounds **1**–**3** interact with the same binding residues when docked with vioD, namely Arg97, Tyr163, Phe276, and Trp165 (Figure S5 b,d,f). It indicated that compounds **1**–**3** could inhibit the violacein biosynthetic pathway by competitively inhibiting the binding of FAD to the vioD enzyme, thus deactivating the enzyme and suppressing the formation of 5-hydroxytryptophan of prodeoxyviolacein to the purple pigment violacein.

3. Conclusions

In conclusion, TRM B-02 was isolated from Taklimakan desert and was 100% similar to *Bacillus subtilis* subsp. Inaquosorum KCTC 13429$^{(T)}$. Six compounds were isolated and identified to be amicoumacins. Among them, compounds **1**–**3** exhibited better QS inhibitory effect on *C. violaceum* ATCC12472 and could interfere with the viacein biosynthesis by downregulating vioA, vioB, vioD and vioE genes and upregulating vioC gene. This is the first report that amicoumacins have the potential inhibitory activity against QS. Compounds AI-77-H (**1**), AI-77-F (**2**) and amicoumacin B (**3**) could be used in the development of novel anti-microbial agent to treat pathogenic infections.

Disclosure statement

There was no conflict of interest in all the authors.

Author contributions

Zhong-Bo Zhou and Chuan-Xing Wan designed the experiment and performed the statistical analysis. Wen-Pan Shi carried out the study and Hong Zeng drafted the manuscript. All authors read and approved the final version of the manuscript.

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