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It has previously been reported that some strains of the marine bacterium Pseudoalteromonas luteoviolacea produce the purple bioactive pigment violacein as well as the antibiotic compound indolmycin, hitherto only found in Streptomyces. The purpose of the present study was to determine the relative role of each of these two compounds as antibacterial compounds in P. luteoviolacea S4054. Using Tn10 transposon mutagenesis, a mutant strain that was significantly reduced in violacein production in mannose-containing substrates was created. Full genome analyses revealed that the viobiosynthetic gene cluster was not interrupted by the transposon; instead the insertion was located to the maeA gene encoding the malic enzyme. Supernatant of the mutant strain inhibited Vibrio anguillarum and Staphylococcus aureus in well diffusion assays and in MIC assays at the same level as the wild type strain. The mutant strain killed V. anguillarum in co-culture experiments as efficiently as the wild type. Using UHPLC-UV/Vis analyses, we quantified violacein and indolmycin, and the mutant strain only produced 7-10% the amount of violacein compared to the wild type strain. In contrast, the amount of indolmycin produced by the mutant strain was about 300% that of the wild type. Since inhibition of V. anguillarum and S. aureus by the mutant strain was similar to that of the wild type, it is concluded that violacein is not the major antibacterial compound in P. luteoviolacea. We furthermore propose that production of violacein and indolmycin may be metabolically linked and that yet unidentified antibacterial compound(s) may be play a role in the antibacterial activity of P. luteoviolacea.

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