

Isolation, characterization, anti-MRSA evaluation, and in-silico multi-target anti-microbial validations of actinomycin X₂ and actinomycin D produced by novel *Streptomyces smyrnaeus* UKAQ_23.

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Streptomyces smyrnaeus UKAQ_23, isolated from the mangrove-sediment, collected from Jubail, Saudi Arabia, exhibited substantial antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), including non-MRSA Gram-positive test bacteria. The novel isolate, under laboratory-scale conditions, produced the highest yield (561.3 ± 0.3 mg/kg fermented agar) of antimicrobial compounds in modified ISP-4 agar at pH 6.5, temperature 35 °C, inoculum 5% v/w, agar 1.5% w/v, and an incubation period of 7 days. The two major compounds, K₁ and K₂, were isolated from fermented medium and identified as Actinomycin X₂ and Actinomycin D, respectively, based on their structural analysis. The antimicrobial screening showed that Actinomycin X₂ had the highest antimicrobial activity compared to Actinomycin D, and the actinomycins-mixture (X₂:D, 1:1, w/w) against MRSA and non-MRSA Gram-positive test bacteria, at 5 µg/disc concentrations. The MIC of Actinomycin X₂ ranged from 1.56–12.5 µg/ml for non-MRSA and 3.125–12.5 µg/ml for MRSA test bacteria. An in-silico molecular docking demonstrated isoleucyl tRNA synthetase as the most-favored antimicrobial protein target for both actinomycins, X₂ and D, while the penicillin-binding protein-1a, was the least-favorable target-protein. In conclusion, *Streptomyces smyrnaeus* UKAQ_23 emerged as a promising source of Actinomycin X₂ with the potential to be scaled up for industrial production, which could benefit the pharmaceutical industry.

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