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THE QUORUM SENSİNG INHIBITION ACTIVITY OF THE ETHYL ACETATE EXTRACT OF *Streptomyces griseoflavus* OC. 124-2

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Abstract

Streptomyces griseoflavus OC. 124-2 used in this study was isolated from the field soil of Dalaman Directorate of Agricultural Enterprises Muğla. As a result of phenotypic and molecular characterization, the isolate was identified as Streptomyces griseoflavus and named as OC. 124-2. The fermentation liquid of Streptomyces griseoflavus OC. 124-2 was obtained in optimum fermentation conditions, and then it was filtered and extracted with ethyl acetate (1:1, v/v). The extract containing the active compounds was obtained by evaporating the solvent. Biomonitor strains, Chromobacterium violaceum CV026 for the determination of anti-quorum sensing activity (anti-QS), Chromobacterium violaceum CV12472 for the determination of inhibition of violacein pigment production and Pseudomonas aeruginosa PA01 for the determination of anti-swarming activity were used at MIC and sub-MIC concentrations. The anti-quorum sensing and anti-swarming activities could not be detected for the extract. Violacein production was inhibited by 100%, 74.86%, 65.74% and 31.99% at MIC, MIC/2, MIC/4 and MIC/8 concentrations of the extract treatment, respectively. While the detected inhibition of violacein pigment production did not inhibit the bacterial growth, it was revealed that it inhibited the quorum-sensing-regulated signaling systems. Accordingly, it was shown that the active compounds obtained from ethyl acetate extract of OC. 124-2 constituted a non-selective pressure for the growth of drug resistant pathogen bacteria and they may be used as an alternative at treatment of these bacteria.
Keywords: *Streptomyces, Streptomyces griseoflavus, Anti-quorum sensing, Violacein pigment inhibition, Anti-Swarming*

Streptomyces griseoflavus OC. 124-2 ETİL ASETAT EKSTRAKSİYONU AKTİF BİLEŞİKLERİNİN QUORUM SENSİNG İNHİBİTÖR AKTİVİTESİ

Özet

Çalışmada, kullanılmış olan Streptomyces griseoflavus OC. 124-2 suşu Muğla ili Dalaman ilçesi Tarım İşletme Müdürlüğü topraklarından izole edilmiştir. İzolat fenotipik ve moleküler karakterizasyon sonucu Streptomyces griseoflavus olarak identifiye edilmiş ve OC. 124-2 olarak isimlendirilmiştir. Streptomyces griseoflavus OC. 124-2 suşunun uygun fermantasyon koşullarında elde edilen fermantasyon sıvısı filtre edilmiş ve etil asetat ile 1:1 oranında ekstraksiyona tabi tutulmuştur. Solvent evaporatörde uçurularak aktif bileşikleri içeren ekstrakt elde edilmiştir. Elde edilen ekstraktın Minimum İnhibisyon konsantrasyonu (MİK) ve MİK altı konsantrasyonlarda anti-quorum sensing (anti-QS) aktivite tayini için Chromobacterium violaceum CV026, Violacein pigmenti üretimi inhibisyon aktivite tayini için Chromobacterium violaceum CV12472 ve anti-swarming aktivite tayini için Pseudomonas aeruginosa PA01 biomonitör suşları kullanılmıştır. Elde edilen ekstraktın anti-quorum sensing ve anti-swarming aktivite taşımadığı tespit edilmiştir. Ekstrakt 0.39 mg/mL (MİK) konsantrasyonda %100, 0.2 mg/mL (MİK/2) konsantrasyonda %74.86, 0.1mg/mL (MİK/4) konsantrasyonda %65.74 ve 0.05 mg/mL (MİK/8) konsantrasyonda %31.99 oranında violacein pigment üretimini inhibe ettiğini göstermiştir. Tespit edilen bu violacein pigment üretiminin inhibisyonu bakteri üremesini engellemezken, quorum sensing sinyal moleküllerinin inhibe olduğunu ortaya çıkarmıştır. Buna bağlı olarak OC. 124-2 etil asetat ekstraktından elde edilen aktif bileşiklerin bu etkisi ile dirençli patojen bakterilerin gelişmesinde seçici olmayan bir baskı oluşturacağı ve bu bakterilerin tedavisinde bir alternatif olarak kullanılabileceği görülmüştür.
Anahtar Kelimeler: *Streptomyces, Streptomyces griseoflavus*, Anti-quorum sensing, Violacein pigmenti inhibisyonu, Anti-Swarming

1 Introduction

Streptomyces is a genus of Gram-positive, aerobic, spore-forming, micelle-forming (filamentous), and constituting a significant component of the soil microflora of natural or manmade environments. *Streptomyces* plays a very important role in degradation of hydrocarbons [1-4]. Members of the *Streptomyces* genus are among the most important industrial microorganisms because of their economic importance as producers of antibiotics, enzymes, seconder metabolites for agriculture and food industries and pharmacologically active agents [5-10].

In recent years, alternative pathways against antibiotics were investigated due to increased resistance of bacteria. First observation of the quorum sensing system was defined in a fish pathogen, *Vibrio fisheri,* then it was discovered that many Gram (-) bacteria, human, animal and plant pathogens used the quorum sensing system for various physiological processes

such as bioluminescence, antibiotic biosynthesis, conjugation, production of virulence factors, biofilm production [11-15]. Quorum sensing system depends on the production the small signal molecules to provide the intercellular communication [16-19]. Purpose of the recent works in this area is development of new approaches to inhibit the intercellular communication [20, 21].

Streptomyces have been a major source for anti-quorum sensing researches because of many kinds of secondary metabolites they produced. In this study, anti-quorum sensing activity of the active compounds obtained by extraction of fermentation liquid of *S. griseoflavus* OC. 124-2 with ethyl acetate was determined.

2 Materials and methods

2.1 Bacterium and cultivation

Bacterium used in the study was isolated from field soil of Dalaman Directorate of Agricultural Enterprises, and due to results of phenotypic and molecular characterization, it has been identified as *Streptomyces griseoflavus* OC.124-2. Bacterium was obtained from Ozgur CEYLAN's doctoral thesis. The stock culture was inoculated to ISP2 media and incubated at 30°C for 5-7 days to activate.

2.2 Fermentation

Bacteria spores incubated in sporulation medium (tryptone-yeast extract- glucose broth (TYGB) at 30°C for 3 days at 150 rpm. After incubation an inoculum was transferred from sporulation medium to fermentation medium. Fermentation was performed at 30°C for 6 days at 150 rpm [22]. At the end of the fermentation process, the cells were filtered with Whatman No: 1, in preparation for the extraction process.

2.3 Extraction of the active secondary metabolites

Extraction was performed by mixing fermentation broth with 1:1 ethyl acetate [23]. Ethyl acetate were removed under low vacuum by using rotary evaporation.

2.4 Bacterial strains and culture medium

The strains of *C. violaceum* CV 12472, CV026 and *P.aeruginosa* PA01 were purchased from Spanish Type Culture Collection (CECT). All the bacterial strains used in this study were cultivated and maintained in Luria-Bertani (LB) medium. CV026 and CV12472 were grown in LB broth at 30°C with shaking. PA01 was grown on LB agar plates at 37°C.

2.5 MIC assay

MIC values of the extract against biosensor strains (*C. violaceum* CV12472, CV026 and *P. aeruginosa* PA01) were determined using broth microdilution method [24].

2.6 Anti-QS activity assay

The quorum sensing inhibition potential of the extract was performed by following the method specified by Koh and Tham [25].

2.7 Violacein inhibition assay

The effect of extract on the QS-controlled violacein production in *C. violaceum* ATCC12472 was determined by McLean et al. [26].

2.8 Anti-swarming activity assay

The anti-swarming potential of the extract was performed by following the method specified by Yeo and Tham [27].

3 Results and discussion

MIC results of the extract were determined as 25 mg/mL for *C. violaceum* CV026, 0.39 mg/mL for *C. violaceum* CV12472 and >100 mg/mL for *P. aeruginosa* PA01. Naik et al. [28] were reported that 0.1 mg/mL concentration of methanol extract of marine invertebrate–derived *Streptomyces* sp. strains did not affect the growth of *C. violaceum* CV12472 and *P. aeruginosa* PA01. Ooka et al. [29] were reported that MIC values of three piericidin derivatives isolated from *S. aburaviensis* and *S. phaeofaciens* were more than 100 µg/mL against *C. violaceum* CV026, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC25923 and *P. aeruginosa* ATCC27853.

The anti-quorum sensing activity results of the extract were shown in Table 1. Anti-microbial zones were detected at the 25-0.39 mg/mL concentrations (Fig. 1). Ooka et al. [29] were detected that the half maximal inhibitory concentration (IC50) of piericidin A1 isolated from *S. aburaviensis* and *S. phaeofaciens* was 10 µg/mL.

Table 1. Anti-quorum sensing results of *S. griseoflavus* OC. 124-2.

Concentration (mg/mL)	Antimicrobial activity (mm)	Anti-QS activity (mm)
25	18.5±0.5	-
12.5	16.17±0.29	-
6.25	13.33±0.58	-
3.13	10.5±0.5	-
1.56	9.5±0.5	-
0.78	9.16±0.29	-
0.39	8.33±0.57	-
Ethanol	8.33±0.29	-
C_{10}HSL	-	28.5±1.32

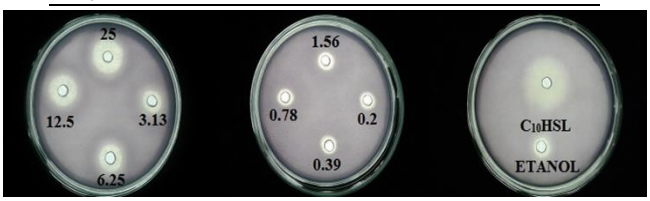

Figure 1. Anti-quorum sensing activity of *S. griseoflavus* OC. 124-2.

Results for the inhibition of violacein pigment production by the extract were shown in Table 2. Violacein inhibition at 0.39 and 0.03 mg/mL concentrations of extract was determined as 100% and 0%, respectively (Fig. 2). Ooka et al. [29] were reported that three piericidin derivatives isolated from *S. aburaviensis* and *S. phaeofaciens* inhibited the violacein production between 1-100 µg/mL concentrations in dose dependent manner.

Table 2. Violacein inhibition results of *S. griseoflavus* OC.124-2.

Concentration (mg/mL)	Violacein inhibition (%)
0.39	100
0.2	74.86±0.006
0.1	65.74±0.005
0.05	31.99±0.077
0.03	0

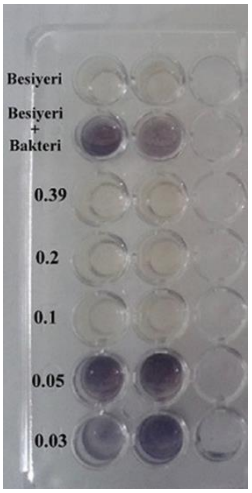

Figure 2. Violacein inhibition activity of OC. 124-2.

It was determined that the extract had no anti-swarming activity (Fig. 3). Naik et al. [28] were detected that 0.1 mg/mL concentration of methanol extract of marine invertebrate–derived *Streptomyces* sp. strains had influence on warming motility by 65-90% and inhibited the violacein production by 15-21 mm inhibition zone.

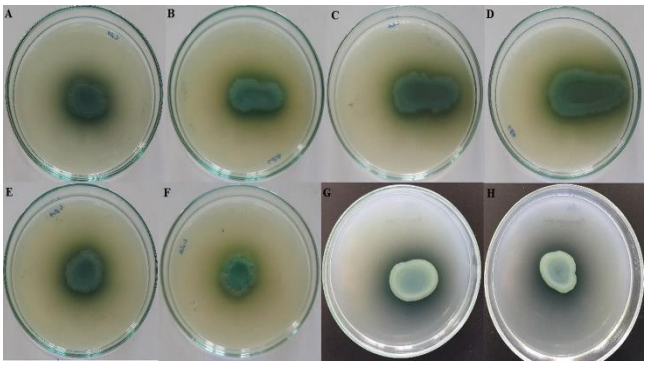

Figure 3. Anti-swarming activity of *S. griseoflavus* OC. 124-2. A: 100 mg/mL, B: 50 mg/mL, C: 25 mg/mL, D: 12,5 mg/mL, E: Control, F: Ampicilin, G: Kanamycin H: Ethanol

4 Conclusion

The results showed that ethyl acetate extract of *Streptomyces griseoflavus* OC. 124-2 strain inhibited the violacein production at 0.39-0.03 mg/mL concentrations on dose dependent manner. It was determined that the extract did not have the anti-quorum sensing and anti-swarming activities.

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