Isfahan University of Medical Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutical Biotechnology

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Title:

Molecular Detection of *Streptomyces griseus* Isolated from Isfahan's Soil

Supervisors:
Dr. Mohhammad Rabbani(Ph.D)
Dr. Hamid Mir Mohammad Sadeghi(Ph.D)

By:
Zahra Karjoo

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Molecular Detection of *Streptomyces griseus* Isolated from Isfahan's Soil

Mohammed Rabbani, Hamid Mir Mohammad Sadeghi, Zahra Karjoo

ABSTRACT:

Introduction: *Streptomycetes* are Gram-positive, filamentous soil bacteria, which undergo morphological differentiation during their life cycle. *Streptomyces* species are known to be producers of many secondary metabolites, which show different biological activities, such as antibacterial, antifungal, antiparasitic, antitumor and immunosuppressive actions. *Streptomyces griseus*, physiologically, is one of the best-studied species among *streptomyces spp.* In this study, *streptomyces griseus* isolated from Isfahan's soil was detected by biochemical, morphological and molecular methods.

Methods: Twenty soil samples were gathered from different places of Isfahan, such as wheat farms, river bed and domestic yards. Six of them were composts. One gram of each was diluted and cultivated in a primary isolation medium. Isolated samples of bacteria from soil were detected by their morphological shapes of spores and vegetative hyphae. Then, a number of biochemical tests were performed to screen *streptomyces* from other related genera. For molecular detections, PCR on the genomic DNA of *streptomyces griseus* was performed using specific primers for aphE and strA genes. After optimising the best condition for PCR, screening for the soil isolates were carried out. Detection of a proper sized band would indicate the presence of aphE and strA genes, representing the bacteria as *S. griseus*.

Results: From 10 colonies undergown molecular detection, 6 colonies (W_1, W_3, W_5, F_4, F_5, F_{26}) showed the presence of the band 750 bp, four of them ($W1$,W_3 , F_5,F_{26}) showed a 900bp band as well. The proper band of 671bp for aphE was detected in *S. griseus*, but none of 10 colonies showed any band of the expected size.

Discussion: With the aid of non-molecular results, the soil isolates up to cluster level were determined, but it was impossible to determine *S. griseus* through its cluster containing 71 members. The results showed that there were some streptomycin producing strains among the isolates, because the presence of 750 and 900bp strA gene, confirming the presence of streptomycin biosynthesis gene clusters. However the absence of 671bp aphE gene showed that these strains owned only one mechanism for resistance.

Key words: *Streptomyces griseus*, aphE gene, strA gene, molecular screening, Isfahan's soil

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شناسائی و بررسی مولکولی استرپتومایسس گریزئوس (Streptomyces griseus) در خاک اصفهان

محمد ربانی ، حمید میرمحمد صادقی ، زهرا کارجو

خلاصه فارسی :

مقدمه :استرپتومایسسها ، باکتری های گرم مثبت فیلامنت دار خاک هستند که در طول دوره زندگی شان دستخوش تغییرات مورفولوژیکی می شوند. این باکتری ها از خانواده اکتینوماسیتها هستند و به خاطر تولید متابولیت های ثانویه ای چون آنتی بیوتیکها، ضد قارچها، مواد ضد انگل، ضد سرطان و سرکوب کننده های ایمنی از جنبه داروئی دارای اهمیت می باشند. استرپتومایسس گریزئوس یکی از گونه های استرپتومایسس ها است که مطالعات فیزیولوژیک بسیاری بر روی آن انجام شده است. در این مطالعه با استفاده از روش های مورفولوژیک ، بیوشیمیائی و مولکولی باکتری تولید کننده استرپتومایسین ، استرپتومایسس گریزئوس ، از خاک جدا شد و مورد شناسائی قرار گرفت.

روش ها : ۲۰ نمونه خاک ازمناطق مختلف اصفهان از جمله بستر رودخانه،مزرعه گندم،یک باغ پنجاه ساله وباغچه های خانگی جمع آوری شد. یک گرم از هر کدام رقیق و در محیط های جدا سازی اولیه کشت داده شد و باکتری های جدا شده از خاک ، از طریق شاخص های مورفولوژیک ، مانند شکل اسپور، ومیسلیوم رویشی شناسائی گردیدند. سپس برای جدا کردن استرپتومایسس گریزئوس از سایر استرپتومایسس ها ، تعدادی تست بیوشیمیائی انجام گردید.

با استفاده از پرایمرهای اختصاصی ژنهای aphE (که در گونه های مقاوم به استرپتومایسین یافت شده است) و StrA (یکی از ژنهای مجموعه ژنی تولید کننده استرپتومایسین) ، تعدادی PCR بر روی DNA ژنومیک استرپتوماسیس گریزئوس انجام شد. پس از تعیین شرایط مناسب ، غربالگری بر روی نمونه های خاک انجام گردید. وجود این ژنها خصوصا StrA ، نشانه توانائی باکتری در تولید استرپتومایسین می باشد.

نتایج : از ده کولونی جدا شده از خاک که مورد بررسی مولکولی قرار گرفت، شش کلونی $(F_{26}, F_5, F_4, W_5, W_3, W_1)$ باند ۷۵۰bp را نشان داد. چهار کلونی (F_{26}, F_5, W_3, W_1) باندی حدود ۹۰۰bp را هم نشان دادند. در غربالگری برای ژن aphE ، باند ۶۷۰bp در کنترل مثبت مشاهده شد ولی در ده نمونه جدا شده باند مورد نظر دیده نشد.

بحث ونتیجه گیری : با کمک روش های غیر مولکولی می توان استرپتویسس ها را تا سطح Cluster طبقه بندی کرد اما تشخیص *S. griseus* در میان گروه خود که شامل ۷۱ زیرشاخه است ممکن نمی باشد. بررسی های مولکولی نشان داد که کلونی هائی که باند ۹۰۰ bp را نشان می دادند تولید کننده های بالقوه استرپتومایسین بودند. عدم وجود باند aphE ۶۷۰bp نشانه عدم وجود ژنهای مقاومت خارج از مجموعه ژنی تولید کننده استرپتومایسین بود.

لغات کلیدی : استرپتومایسس گریزئوس ،ژنهای aphEوStrA ،غربالگری مولکولی، خاک اصفهان

In the name of God

LETTER OF APPROVAL

A Doctor of Pharmacy (Pharm. D) thesis in Biotechnology

Title:

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This thesis was confirmed with grade Excellent and rank of 20 in the session for defense dated 93/10/16 by the Board of Arbitrators consisting of the following professors:

Name and Surname	Signature
1- Dr. Mohhammad Rabbani	
2- Dr. Hamid Mir Mohammad Sadeghi	
3- Dr. Hasan Korbekandi	All the best
4- Dr. Hasan Shojaii	
5- Dr. Abas Ja'afarian Dehkordi	