Comparative Study of Garlic Extract Effect on the Expression of Genes Involved in Quorum Sensing in *Pseudomonas aeruginosa* and the Antibiotic Tobramycin

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**ABSTRACT**

**Background:** The expression of most genes involved in pathogenesis in *Pseudomonas aeruginosa* is controlled and regulated by a gene system called quorum sensing (QS). The purpose of this study was to investigate the quorum quenching of garlic extract in preventing the expression of genes involved in QS system of *P. aeruginosa*.

**Methods:** In the present cross-sectional study, 12 *P. aeruginosa* strains were collected from burn wounds, cultured on special media and then confirmed by differential tests. The PCR test was performed to detect the *lasI* and *lasR* genes. The expression level of *lasI* gene was assessed in the presence of garlic extract and tobramycin antibiotic by real-time PCR, and the active ingredient of garlic extract was analyzed by HPLC.

**Results:** Examination of 12 strains cultured on special and differential media showed that all 12 strains were *P. aeruginosa*. The PCR results indicated that all strains had 100% *lasI* and *lasR* genes. The real-time PCR results also revealed that the garlic extract was able to reduce the expression level of *lasI* gene in *P. aeruginosa*. It was found that tobramycin has a greater ability to reduce the expression level of this gene compared with garlic extract.

**Conclusion:** This study showed that the garlic extract could reduce the expression of *lasI* gene in *P. aeruginosa* isolates, and that the active ingredients of this extract could be used to treat infections as an alternative to antibiotic therapy.

**Keywords:** *Pseudomonas aeruginosa*, Garlic extract, Tobramycin, Quorum Sensing

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**Introduction**

Identifying the bacterial cell-to-cell communication led to an understanding of the fact that interdependent functions are not limited to eukaryotic organisms and can also be present in bacteria. The process of cell-to-cell coordination and communication that involves the production of extracellular and diffuse molecules, followed by the regulation of gene expression, is called quorum sensing (QS) (1). The QS is a process that has changed our view of the life cycle of bacteria, meaning that the bacteria were considered sporadic organisms in the past, and they were believed to ensure their survival.
through adaptation to environmental conditions without any communication. However, researchers have now discovered the bacterial cell-to-cell communication, signaling and interaction through the information exchange. Therefore, it is concluded that the presence of a signaling network in bacteria is a complex and necessary aspect in their lives (2).

The study of cell-to-cell communication and its effects on the transcription of the unicellular organism has a variety of practical applications. One of them is the possibility of interfering with cell-to-cell communication systems in pathogenic microbes. The QS is a way for bacterial cell-to-cell signaling and exchange information between two partners; the sender and receiver of a communication molecule benefit from the process (3). In bacteria, the QS system regulates phenotypes covering bioluminescence, the production of exoplysaccharides, virulence, conjugative transfer of plasmids, the production of antibiotics and exoenzymes, the formation of biofilms, and the inhibition of growth. Types of molecules involved in communication include acyl homoserine lactones (AHL), autoinducer2 (AI2), and modified oligopeptides (4).

**Pseudomonas aeruginosa** is an opportunistic and gram-negative human pathogen. These bacteria develop urinary tract infections, respiratory tract infections, skin inflammation and edema, soft tissue infections, bacteremia, bone and joint infections, stomach and intestinal infections, various systemic infections, especially in patients with severe burns, cancer and AIDS having experienced immunosuppression (5). Disruption of the QS system of *P. aeruginosa* has been shown to eliminate pathogenesis.

Since plants and fungi have had symbiosis with bacteria for millions of years, various mechanisms and substances may have been developed to inhibit the QS system during evolution (6). Garlic is one of the oldest plants whose medicinal effects have long been reported. Garlic has antibacterial effect due to various substances such as allin, ahoin, allicin and allistain and therefore is consumed against gram-positive and gram-negative bacteria, fungi, parasites and viruses (7).

Since medicinal plants have been used for many years and due to the importance of *P. aeruginosa* as well as the properties of garlic, the present study aimed to investigate the effect of garlic extract on the QS system of this bacterium in order to use this extract in therapeutic process if there is any inhibition of cell-to-cell communication.

**Materials and Methods**

**Sample Collection:** In the present descriptive-analytical cross-sectional study, 12 samples of *Pseudomonas* were collected from burn wounds of hospitalized patients. The samples were transferred to the laboratory, cultured on the Blood Agar, Eosin Methylene Blue (EMB) and Mueller Hinton Agar (MHA) culture media and then tested morphologically and biochemically.

**Identification of Isolates**

Colonies were confirmed using standard biochemical and microbiological tests, including gram staining, oxidase, catalase, motility, citrate, TSI, indole, Methyl Red-Voges Proskauer (MR-VP), urease, oxidation-fermentation (OF), growth at 42ºC and pigment formation on Cetrimide Agar medium, and based on the Bergey's manual of determinative bacteriology; all isolates were confirmed as *P. aeruginosa* (8).

**DNA Extraction**

The genomic DNA of the isolates was extracted using the GTP kit (Gene Transfer Pioneers, Iran). To this end, 1 to 2 mL of bacterial suspension cultured on EMB equivalent to 0.5 McFarland turbidity standard was used for extraction according to the manufacturer's instructions. The extracted DNA stocks were stored at -20ºC. The quantity and quality of the extracted DNA were examined using spectrophotometer (flow meter E6150) and agarose gel electrophoresis, respectively.

**PCR Reaction to Detect the Presence of lasI, lasR and rpoD Genes**

The PCR reaction was performed on a final volume of 20 μL containing 3 μM of DNA pattern (200 ng), 10 pmol of each primer (1 μL), 1 μL of distilled water, and 10 μM of 2x Master Mix (Amplicon). The PCR temperature program included the initial denaturation at 95ºC for 3 minutes and the 35 cycle with the denaturation at 95ºC for 30 seconds, the annealing at 55ºC for 1 minute and the extension at 72ºC for 1 minute and the final extension at 72ºC for 10 minutes. Finally, the amplified segments were observed using 1.5% agarose gel electrophoresis and etidium bromide staining. The sequence of primers used is given in Table 1. The PCR reaction was performed using a thermocycler (BioRad-USA).
Preparation of Garlic Extraction

After peeling garlic cloves, 125 g of garlic was crushed using a blender and then mixed with 2 liters of 70% formalin and shaken on the rotator for 48 hours. The resulting mixture was passed through sterile gauze and Whatman filter paper, and the resulting material was centrifuged by a refrigerated centrifuge at 5000 rpm for 30 minutes. Other waste products, such as cellulose and cell shells were removed from the extract and a clear yellowish solution was obtained, distilled for concentration, and stored at 4ºC for subsequent testing.

HPLC Test

In this study, HPLC purification was used to purify the compounds in the immunomodulator fraction of garlic extract and to establish a suitable system for further production of immunomodulators in a shorter time for clinical trials and pharmacological examinations. The fractions collected from the filtration were separated by reversed-phase HPLC on TPV10 208 Vydac C8 reversed-phase preparative column (1 * 25 cm). Purification was performed with 0.1% TFA solution in water and 0.09% TFA solution in acetonitrile and with a flow rate of 1 ml/min from 0% to 15% TFA solutions for 60 minutes. Optical density (OD) was read at the wavelengths of 215, 254 and 280 nm.

Determination of the MIC Value using Broth Dilution Method

The minimum inhibitory concentration (MIC) was determined for each of the bacteria in the exposure to the garlic extract and the tobramycin antibiotic using the standard method proposed by the Clinical and Laboratory Standards Institute (CLSI). The method used a standard 96-well microplate, each containing 100 µL of the Mueller Hinton Broth (MHB) medium. To determine the MIC value, the dilution of garlic extract was started from 2048µg/mL, and 100 µL were added to the first well and then pipetted into the lower wells, and the concentration dropped to the next wells. Next, 100 µL of bacterial suspension equivalent to 0.5 McFarland turbidity standard was poured into all wells. To determine the MIC value for the tobramycin antibiotic, the dilution was started from 32 µg/mL. In all wells, 100 µL of MHB medium was poured and subsequently the first well was added by the antibiotic stock and pipetted to the next wells. After that, 100 µL of microbial suspension equivalent to 0.5 McFarland turbidity standard was added to all wells. In addition, two rows of wells were used as positive control (medium + microbial suspension) and negative control (medium + garlic extract/antibiotic). The microplates were incubated at 37ºC for 24 hours. After this period, the first well with the observed growth was considered as the MIC value (10, 11).

RNA Extraction

In order to investigate the expression of lasI gene using rpoD reference gene, the RNA of Pseudomonas treated with garlic extract and tobramycin antibiotic according to MIC value and also untreated Pseudomonas was extracted using Cinnapa Pure-RNA kit (CinnaGen, Cat. No. PR891620, Iran). Quantus™ Fluorometer (Cat.# E6150) and Promega kit were used to quantify the RNA extracted.

Construction of cDNA

To this end, 1 µg of RNA was mixed with 1 µL of enzyme buffer, 1 µL of DNase enzyme and 0.5µl of RNase inhibitor, respectively, and then reaching final volume of 10 µL with RNase-free water. The microtubes were incubated at 37°C for 30 min and 1 µL of EDTA 0.5M was added to each. The incubation was performed for 10 min at 65°C to inactivate the DNase enzyme; 0.5 µL of random hexamer and 0.5µL of Oligo (dT) were added to the microtubes and incubated at 65°C for 5 min. After this time, the microtubes were transferred onto the ice and 13 µL of this MasterMix was added to each. The microtubes were incubated at 25°C for 5 min, at 42°C for 60 min, and at 70°C for 10 min. The resulting single-stranded cDNA was stored at -20ºC. The used MasterMix contained 1 µL of dNTP, 4 µL of RT buffer, 2 µL of RT enzyme, 0.5 µL of RNase inhibitor and 5.5 µL of RNase-free water.

Real-time PCR Process

To perform the real-time PCR process, 10 µL of PCR MasterMix, 1 µL of each of the forward and reverse primers (Table 1), 1.5 µL of constructed cDNA and 5.6 µL of water were mixed in a microtube. Samples were placed in the device with a temperature program...
including 10 minutes of initial denaturation at 94ºC, 15 seconds at 94ºC, 25 seconds at 55ºC and 35 seconds at 72ºC.

**Real-time PCR Data Analysis**

The real-time PCR data were analyzed by the software available in the one plusABI device using the $-\Delta\Delta CT$ method, and the final number ($2^{-\Delta\Delta CT}$) obtained from various repeats was statistically analyzed using the GenEX software.

**RESULTS**

**HPLC test:** Evaluation of garlic extract using HPLC method and analysis of the resulting chromatogram showed that the highest compound in the extract is allicin. This study was compared with the internal standard (Figure 1).

**MIC Determination**

After 24 hours of incubation at 37ºC, the sub-MIC value was determined to be 256µg/mL for garlic extract and 16µg/mL for tobramycin.

**Investigating the Presence of lasI, lasR and rpoD Genes**

The results of PCR molecular analysis for the lasI, lasR and rpoD genes of QS system were obtained on the basis of band observation in the mentioned region on 1% agarose gel. In the present study, all 12 (100%) samples of *P. aeruginosa* isolated from clinical samples had these genes (Figures 2 and 3).

**Analysis of Gene Expression**

The real-time PCR technique was used to determine the rate of suppression of gene expression quantitatively in the presence of garlic extract and tobramycin. The real-time PCR process was performed for the lasI gene using the rpoD reference gene, the results of which are shown in Figures 4 (in the presence of garlic extract) and 5 (in the presence of tobramycin). The results indicated a significant difference in lasI gene expression between the two groups untreated and treated with garlic extract. The fold change rate for the lasI gene was calculated to be -1.19, indicating that this gene was reduced up to 1.19 times in the group treated with garlic extract compared to the untreated group. The expression of lasI gene in the tobramycin-treated group was also significantly reduced. The fold change rate for the lasI gene was calculated to be -1.27, indicating that the gene in the tobramycin-treated group decreased up to 1.27 times compared to the untreated group. There was no significant difference between the
effects of garlic extract and tobramycin in terms of their ability to reduce lasI gene expression.

Discussion

The spread of bacterial infections and the development of their antibiotic resistance have prompted researchers to find natural antibacterial compounds. These studies are often performed on various plant compounds, their extracts, and their active compounds (12). The aim of this study was to investigate the effect of garlic extract on P. aeruginosa bacteria by evaluating the effect of garlic extract on the expression level of genes affecting bacterial QS system and in comparison with tobramycin antibiotic.

Some garlic compounds include alliin, allicin, organic acids, carbohydrates and vitamins; and the most important properties of garlic are related to allicin (13). The allicin is an essential oil with a bright yellow color and responsible for the specific aroma of garlic. The allicin is also called allicin potential or allicin yield and accounts for the antimicrobial properties of garlic. This compound plays a major part in its antibiotic properties through specific inhibition of acetylcholine A synthase; the inhibition of this enzyme inhibits lipid biosynthesis and fatty acids and ultimately disrupts the bioavailability of microorganisms (14). One of the important features of allicin is its ability to penetrate and pass through membrane phospholipids, which is why it can freely pass through the membrane and perform its functions (15). In the analysis of garlic extract obtained in the present study using HPLC technique, the allicin was the main ingredient in the extract. Therefore, its antimicrobial effects were expected to be observed on P. aeruginosa, but the main objective was to evaluate the mechanism of action of this antimicrobial agent and to investigate the expression of QS-related genes and the biofilm formation in the presence of extract.

The lasI and lasR genes are inherently present in the genome structure of most pathogenic P. aeruginosa bacteria isolated from medical centers and develop QS system, biofilm and drug resistance in these strains. In the P. aeruginosa bacteria, the QS system consists of two gene systems, LasR-LasI and RhlR-RhlI. The LasI and RhlI genes express acyl-hemoserine lactone (acyl-HSL) synthase, while the LasR and RhlR genes produce transcriptional regulatory proteins, which bind to their specific signals and activate target (virulence) genes (16). The QS system also exists in other species of Pseudomonas, but the number and sequence of their structural genes varies. Many genes in P. aeruginosa are controlled and expressed by QS, which are involved in pathogenesis, including lasI, lasR, rhlI, rhlR genes, and controlled genes called lasB, apr, and rhlAB in clinical isolates (17).

Plant compounds have been shown to inhibit biofilm formation in a variety of ways. Herbal extracts with bacteriostatic or bactericidal properties inhibit or reduce bacterial biofilm formation. However, some herbal compounds affect biofilm formation without bacteriostatic or bactericidal effects. Their advantage is the failure of bacteria to resist these compounds. For example, some plant compounds interfere with the QS system, which controls bacterial biofilm (18).

The garlic extract stops the QS system and accelerates the elimination of lung infection caused by P. aeruginosa. The garlic extract was used to treat lung infections in rats and it was found that the garlic therapy first stimulated higher levels of inflammation and significantly eliminated the pathogenic bacteria (19). Fulghesu et al. (2007) examined different QS inhibitory compounds in P. aeruginosa. They tested the inhibitory activity of three macrolide drugs and three lincosamide drugs, resveratrol, garlic extract and N-acetyl cysteine on four strains of P. aeruginosa isolated from patients with cystic fibrosis. All of the compounds tested were able to inhibit QS in the tested strains; only lincomycin and N-acetyl cysteine did not inhibit QS in one of the Pseudomonas strains (20).
In another study, the QS inhibition was investigated using a new genetic system. A set of QS inhibitor screening systems was developed that enabled them to identify a number of new natural and synthetic inhibitors. The two active inhibitors identified in this study were garlic extract and 4-nitropyridine-N-oxide (4-NPO). Transcriptome analysis based on gene arrays showed that 4-NPO and garlic extract were specific for QS-controlling virulence genes in *P. aeruginosa*. The two QS inhibitors also significantly reduced the biofilm resistance of *P. aeruginosa* compared to tobramycin (21). The QS inhibition activity was investigated by garlic extract and QS response reduction was observed in QS receptors of *Escherichia coli*, *Agrobacterium tumefaciens*, *Chromobacterium violaceum*, *Pseudomonas putida* and *Pseudomonas chlororaphis* in relation to growth inhibitory effects (22). In addition, the QS inhibition and pathogenesis attenuation of *P. aeruginosa* were investigated under the influence of garlic extract and it has been shown that oral treatment with garlic extract significantly reduces the density of pulmonary bacteria. In vitro experiments also showed that the virulence factors and the formation of QS signals by the bacterium *P. aeruginosa* decreased in the presence of fresh garlic extract (23).

Accordingly, the effect of garlic extract on the QS system of *P. aeruginosa* has been proven frequently, but none of the studies mentioned have expressed the mechanism of this effect and examined the expression of QS genes. In the present study, the expression level of *lasI* gene as one of the important genes in bacterial QS system was determined by real-time PCR technique, the results of which showed that the garlic extract at a concentration of 256 μg/mL (as MIC value) decreases the expression of *lasI* gene. In order to compare decreasing effect of garlic extract on the expression of these genes with the effect of tobramycin, the expression of *lasI* gene in the presence of this antibiotic was also investigated, which showed a greater decrease compared to the garlic extract, but not significant. Therefore, the promising results are that the active ingredients of garlic can be extracted and used as adjunctive antibiotic therapy or as an independent treatment in infections. This active ingredient was found to be allicin in the present study. It is recommended that the extracted and purified allicin in future research should be applied as an anti-QS and therefore antibacterial agent for the treatment of *Pseudomonas aeruginosa* infections.

**Conflict of Interest**

The authors declared no conflict of interests
بررسی مقایسه‌ای بین اثر عصاره سر در بیان زن‌های دخیل در کروم سنسنیگ در باکتری‌های مورد سودوموانس آتروپینو و آنی‌بیوتیک‌های تورامایسین

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مقدمه

شناختی اربابیت بین سلولی در میان باکتری‌ها، منجر به فهم ایم، یافته‌شده که عامل‌های همانندی با یکدیگر منحنی به ارگانیسم‌های یونکس و در باکتری‌ها نیز وجود دارد. در فرآیند همانگی و اربابیت بین سلولی که شامل تبادل مولکول‌های جاری سلولی (QS) و قابل انتشار در بین پی ظنیم زن‌های است، می‌توان سنسیبیت اطلاق می‌گردد (۱). کروم سنسنیگ فراگین ارگانیسم‌ها است که دید ما را نسبت به نحوه مورد حاصل ایمکان داده‌های شکارکردگی‌ها ارگانیسم‌های کاربردهای عمی متنوعی دارد. می‌یک از آنهای امکان داخل با سبیل‌های اربابیت بین سلولی در میکروب‌های پانزون است. راهی برای سیگنال‌های بین سلولی با کاتریپلات است و یک مطالعه اربابیت بین دو شرکت است، یعنی فستونک و گرنده یک مولکول اربابیت، از قریند سود میرند (۲). مقاله میکروب‌شناسی پزشکی ایران Majallah-i mikrub/shinâs-i pizzishkî-i Tiran.
فوتیپهایی را تنظیم می‌کند که شامل پیوسته‌سازی، تولید اگزولی ساکاریدها و پرولاز، انتقال پلاسمید کنژوگه، تولید آنتی‌بیوتیک و اگزولز، تشکیل بیوپلاستوم و مامات از رشد است. انواع مولکول‌های ارتباطی شامل آسیل مواد لاکتون (AHL) مولکول‌های تغییر اپی‌گسپتید 4 (AI2) پایه این تغییر و اپی‌گسپتید 4 (AI2) از این پیوسته‌سازی می‌باشد. باکتری‌های نرم، باکتری‌های آتیکسی، انتقال پلاستک و تولید پیوست در این باکتری‌ها وجود دارد.

تاپید هوازی‌ها

پیوسته‌سازی DNA استخراج DNA، از نظر اولیه با استفاده از الگو GTP توسط انتقال زن‌بی‌آناتومی انجام گرفته که با نظیر دانه‌ای که می‌تواند در بیماری‌های باکتری‌ای که به‌طور نمک‌پردازی کشف شده می‌باشد، در حیطه باکتری‌ها و باکتری‌های انتقال پلاستک است. نمک‌پردازی که با نظیر نمک‌پردازی کشف شده می‌باشد، در حیطه باکتری‌ها و باکتری‌های انتقال پلاستک است. نمک‌پردازی که با نظیر نمک‌پردازی کشف شده می‌باشد، در حیطه باکتری‌ها و باکتری‌های انتقال پلاستک است.
عصاره‌گی، سیر

پس از چندایزی پوست از جبههای سیر، 125 گرم سیر با استفاده از مخلوط گن خردشده و سپس با یک لیتر فرمول 20 درصد مخلوط و به مدت 28 ساعت رو چاله‌دار، شیک‌شده مخلوط حاصل از تنظیم استرولی و صفحه و انگشت عبور داده شد و ماده حلال در سانتی‌متر یک‌دایار و با دو ppm ۵۰۰۰ به مدت ۳۰ دقیقه سانتی‌متری فیل‌زد و در با این ترتیب های سیر و ناخواسته از جمله مواد آنتی‌بیوتیک، مواد سلولی، مواد در عصاره خشک گردید و محولی شفاف و زرد رنگ به دست آمد که به منظور تغییر شدن، تقطیر شده و برای استفاده بعدی در دمای ۴ درجه سلسیوس نگهداری شد.

HPLC

در این تحقیق به تحلیل به روش HPLC به هدف خالص‌سازی ترکیبات موجود در فراکشین‌های سیر و ایجاد میکروینی مناسب جهت تولید بیشتر ماده آنتی‌بیوتیک در زمان کوتاه به بهتر کارآمدی‌های دانشجوی در سایر کشورها و در تحقیقات بیشتر و جدید بهره‌برداری، HPLC مطرح می‌شود. 

Azmoun

آزمون

در این تحقیق از تحلیل به روش HPLC به هدف خالص‌سازی ترکیبات موجود در فراکشین‌های سیر و ایجاد میکروینی مناسب جهت تولید بیشتر ماده آنتی‌بیوتیک در زمان کوتاه به بهتر کارآمدی‌های دانشجوی در سایر کشورها و در تحقیقات بیشتر و جدید بهره‌برداری، HPLC مطرح می‌شود.

۱۳۹۹ شماره ۱، بهمن

پرونده

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میکروتیوپ‌ها اضافه شد و به مدت ۵ دقیقه در دمای ۴۵ درجه سلسوس قرار گرفتند. سپس از گذشت این زمان، میکروتیوپ‌ها به روش یک مخلوط شده و به هر کیلو لیتر ۱۲ میکرونتریکس اضافه شد. میکروتیوپ‌ها ۵ دقیقه در دمای سلسوس، ۲۰ دقیقه در ۲۴ درجه سلسوس و ۱۰ دقیقه در ۷۰ درجه سلسوس قرار گرفتند. نتیجه‌گیری شد که میکروتیوپ‌ها برای اولین بار نگه‌داری گردیدند. درمان نگهداری در دمای ۴۵ درجه سلسوس و ۵ درجه سلسوس، ۲۵ دقیقه در دمای ۴۴ درجه سلسوس، ۲۰ دقیقه در دمای ۷۵ درجه سلسوس و ۲۰ دقیقه در دمای ۷۰ درجه سلسوس قرار گرفتند.

آنانالیز داده‌های Real-Time PCR

منظور انجام واکنش Real time PCR مقایسه ۱۰۰۰ میکروتیوپ، جرم‌پراکنده و ۱۰۰ میکروتیوپ، جرم‌پراکنده در یک هیک از پرایمرهای فوروارد و بروز دیگر از میکروتیوپ محیط شدند. نمونه‌ها در دستگاه با برنامه دمایی شامل ۲۰ دقیقه دانلودیسون و در دمای ۹۴ درجه سلسوس، ۲۵ دقیقه دانلودیسون و در دمای ۹۲ درجه سلسوس، ۲۵ دقیقه دانلودیسون و در دمای ۷۰ درجه سلسوس، ۲۵ دقیقه دانلودیسون و در دمای ۶۵ درجه سلسوس و ۲۰ دقیقه دانلودیسون و در دمای ۶۰ درجه سلسوس قرار داده شدند.

آزمون HPLC

از واکنش Real Time PCR، همه کمک‌ها فراورده در دستگاه یک روش Real Time PCR به روش دادهشه به روش (۲ΔΔCT) حاصل 

بحث‌های افزایش

ارزیابی عضایه تهیه شده از سیب با استفاده از روش HPLC و برشی کروماتوگرام حاصل، نشان داده که بیشترین تکیه موجود در عصاره آلبیسین است. این بررسی با مقایسه با استاندارد داخلی صورت گرفت (شکل ۱).

نرم‌افزارهای جریانی

پردازش نتایج آزمایشات PCR برای ژن‌های lasI و rpoD با استفاده از آزمایشات PCR برای ژن‌های lasI و rpoD در دستگاه genEX تجزیه و تحلیل آماری شدند.

این نکات

از واکنش PCR برای ژن‌های lasI و rpoD در دستگاه genEX تجزیه و تحلیل آماری شدند. 

شکل ۱. کروماتوگرام HPLC مربوط به آلبیسین (IS) و استاندارد داخلی (IS)
بحث
گروه آنلاین‌های باکتریایی و ایجاد مقاومت آنتی‌بیوتیکی در آنها، موجب شدن تپش باری ترکیبات ضدبakteریایی طبیعی، موضوعی جدای برای یوزه‌های باکتریایی. این مطالعات اغلب روی ترکیبات گاهی مختلف، عصاره‌های آنها و ترکیبات مورد عدالت در آنها صورت می‌گیرد. در تحقیق حاضر، با تأثیر عصاره سپر روی یک باکتری سودوموناس آنتزوئوژن مورد بررسی قرار گرفت. این بایستی از طریق ارزیابی اثر عصاره سپر بر میزان بیان سپر میوه با کسانماتیک باکتری و مقایسه آن با آنتی‌بیوتیک تورامایسین صورت گرفت.

از ترکیبات سپر آنلاین، آسیپین، اسیده‌های آمین، کروپهیدایت و ویتامین‌ها می‌توان نام برده که مهم‌ترین خواص سپر مربوط به آلاین است (14). آسیپین یک ترکیب رنگی با رنگ ژرد روش است که عطر خاص سپر را به وجود آورده. به علاوه، رنگ سپر نیز که متفاوت می‌باشد، ترکیبی است که به وسیله‌ی آنتی‌بیوتیک تورامایسین صورت گرفت. این یکی از عصاره‌هایی است که می‌تواند به‌طور کمکی در حفظ میکرو‌کوزم ملایم به‌صورت خاص در حضور آنتی‌بیوتیک تورامایسین و اثرات مربوط به آن استفاده کند. این مطالعه به‌طور کلی نشان می‌دهد که نتایج این تحقیق به بیان‌گری این اثبات که باعث افزایش استحکام بی‌شمار و تعیین آنتی‌بیوتیکی می‌باشد.

![شکل 4: نمودار ΔΔCt در حضور عصاره سپر](image1)

![شکل 5: نمودار تغییرات ΔΔCt در حضور آنتی‌بیوتیک تورامایسین](image2)

بررسی بیان زن: برای تعیین میزان سرور بیان زن در حضور عصاره سپر و آنتی‌بیوتیک تورامایسین به‌صورت گمی، از تکنیک Real-Time PCR استفاده شد. واکنش Real-Time PCR و با استفاده از زن مرجع rpoD و lastl براز انجام شد. نتایج این واکنش در شکل‌های 4 و 5 حضور عصاره سپر و(lastl) مشاهده شدند. در اینجا نمودار و(lastl) به‌عنوان عصاره سپر و(lastl) برای زن(lastl) محاسبه شد که بیانگر این است که این در حضور ترکیب شده با عصاره سپر نسبت به گروه غیر ترکیب شده با عصاره سپر کاهش یافته است. بنابراین واکنش رابطه با کاهش fold change 1/19 برابر 19 در حضور ترکیب شده با تورامایسین نیز به‌طور قابل توجه است. میزان اثر عصاره سپر و آنتی‌بیوتیک تورامایسین از نظر نتایج آنها در کاهش بیان نمودار مساعد شد.
در مدل موشی اختصاری دارد، همچنین این دو ماه‌کننده کروم سنتی‌سینگ مواد آپیلینات سودوموناس آتروپنیوز/.

در حال حاضر، برای زنده سازی و در مورد دارویانی با تشکیل وزنی، دانشمندی در سودوموناس آتروپنیوز/ توسعه کروم سنتی‌سینگ کنترل پیمان می‌شود که در داروی‌های زیادی در سودوموناس آتروپنیوز/ استفاده شده. به طور کلی، در این پژوهش در مورد سودوموناس آتروپنیوز/، ارتباط بین سودوموناس پنیک و سودوموناس کلروفیوز در ارتباط با اثرات

مهم‌کننده رشد، مشاهده شده است (23). همچنین مهار کروم سنتی‌سینگ و تشخیص نارنجی سودوموناس آتروپنیوز/ تحت تأثیر سیر بررسی و نشان داده شد است در میان خراکی با سیر مقدار باکتری‌هایی روی یک طرح قابل توجه کاهش می‌شود. از اشارات

نیز نشان داد که فاکتورهای ویروس و تولید سیگنال‌های کروم سنتی‌سینگ باکتری سودوموناس آتروپنیوز/ در حضور عصاره سیر تازه کاهش بیدا می‌کند (24).

سری کروم سنتی‌سینگ مصرف شده و حذف عقون ریوی سودوموناس آتروپنیوز/ را سرعت می‌بخشد. از جمله اعمال برای درمان عقون ریوی موش استفاده کرده و نشان دادند که درمان با سیر ایما در جات التهاب بالاتری را تحریک می‌کند و به طور قابل توجهی باکتری‌های عقون می‌باشد (20).

در طی مطالعات دیگر، مهار کروم سنتی‌سینگ با استفاده از یک سیستم نتایجی ارزشی دارد برای پیش‌بینی در سودوموناس آتروپنیوز/ اگر این کروم سنتی‌سینگ ایجاد شود که آنها قادر می‌گردند سری کروم سنتی‌سینگ و سنتی‌سینگ/ استفاده در تولید نشان می‌دهند که در این مطالعه مشخص شدند، عصاره سیر و 4-نیتریپروپیمیدین (N-4-PMO) بودند. آنالیز ترکیب‌های براساس آنزیم نتیجه نشان داد که عصاره سیر 4-
The study assessed the role of compound 

laS in the quorum sensing system of Pseudomonas aeruginosa. In a recent publication, the authors describe the role of the lasI gene in the regulation of biofilm formation and virulence factors. They observed a significant decrease in biofilm formation and increase in antimicrobial susceptibility when lasI was knocked out.

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