Antagonistic activity of Streptomyces (DS3) and Nocardia (DN5) isolates against selected bacteria isolated from cream samples

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Abstract

Food-borne infectious bacterial infections are highly present in the world especially in countries with low-food-biosafety policies. Controlling such infections requires finding effective and safe tools that minimize the detrimental influence of those bacteria on consumers. The present work was built up to identify antagonistic activities of river water and sedimental isolated Streptomyces (DS3) and Nocardia (DN5) against selected bacteria isolated from samples of local cream cheese from Al-Diwaniyah City, Iraq. DS3, DN5, and cream cheese bacteria (CCB) were isolated using regular and selective cultivating media, and the microorganism identities were confirmed using certain biochemical tests. The identity of the DS3 and DN5 was confirmed by using a polymerase chain reaction (PCR) method targeted 16S rRNA gene. The anti-bacterial activity (ABA) of DS3 and DN5 against selected CCB (E. coli, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Streptococcus pyogenes, and Pseudomonas aeruginosa) were tested using Müller-Hinton method. The findings showed high ABA of DS3 and DN5 against all isolates of CCB. However, DS3 revealed higher effects on CCB than those detected from DN5 although DN5 declared higher ABA against K. pneumonia than that recognized from DS3. In conclusion, DS3 and DN5 may have natural anti-bacterial activity that may safely eliminate infectious agent presence in local cream cheese.

Keywords: Anti-bacterial, antibiotic resistance, anti-microbial, Nocardia (DN5), Streptomyces (DS3).

DOI: http://doi.org/10.36295/ASRO.2021.24557

Page: 367-372

Volume/Issue: Volume: 24 Issue: 05

Introduction

Most of the medical, veterinary, and agricultural utilized antibiotics come from Streptomyces bacteria. Molecular analyses showed that each isolate can produce dozens of these metabolites, and a large number of relevant specific gene groups were found in the genetic materials of those bacteria. Such species are thus increasingly being examined in anticipation of contributing substantially to the production of novel medicinal products in order to fight the world development of antibiotic resistance in pathogens, and the availability of medical uses for those biologically active substances. Moreover, Streptomyces play a major role in processing fungal and plant cellular wall some species have formed close relationships with pests and crops, and others have become infectious species. Streptomyces are uncommon for bacteria that develop into a matrix of fungal mycelium-like mat as from which aerial branches are sustained by spore chains.

In the last 40 years, global attempts to find new natural substances have used sea microorganisms for recognizing those materials and utilizing them for medical or food production purposes. Actinobacteria organisms are unique sources of new molecules with commonly specific compositions and medicinal potential uses. Actinomycetes are of a widespread bacteria in both human-made and natural ecosystems and are strongly recognized as a great source of antimicrobial and biologically active compounds, essential in the biomedical field. One of the most significant origins of biological active substances is nocardial organisms which were identified as antimicrobial producers that inhibit the growth of a wide range of pathogenic bacteria such as Vibrio and Nocardia species.

Nocardia is the genus of Actinomycetes belonging to the family Nocardiaceae. Trevisan (1889) initially identified the group and called it in recognition of Edmond Nocard who presented the very first species in 1888. Nocardia is a slightly
acid fast, Gram positive and filamentous bacilli with aerobic characteristics. About 86 different species are included in this genus [7-8]. Food-borne infectious bacterial infections are highly present in the world especially in countries with low-food-biosafety policies. Controlling such infections requires finding effective and safe tools that minimize the detrimental influence of those bacteria on consumers. The present work was built up to identify antagonistic activities of river sedimental isolated Streptomyces (DS3) and Nocardia (DN5) against selected bacteria isolated from samples of local cream cheese from Al-Diwaniyah City, Iraq.

Materials and methods
Bacterial isolates of Streptomyces and Nocardia from sediment and water
All the samples were exposed to various processing techniques. In the beginning, gravel and debris were removed from the samples. Then, they were, after air-dried, completely mixed and 2-mm-sieve-filtered. Dilutions, using sterile distilled water, were generated up to $10^{-5}$. For the water samples, $1\text{ml}$ of the sample was added to saline at $9\text{ml}$ with thorough up mixing. Dilutions at $10^{-2}$ to $10^{-5}$ were prepared. From each diluted sample, $0.1\text{ml}$ was spread over the selective media such as glycerol yeast extract agar, actinomycetes isolation agar, and trypticase soy agar provided with $6\%$ NaCl. Moreover, nalidixic acid at $75\mu g/ml$ and cycloheximide at $80\mu g/ml$ were used to inhibit the growth of non-required fungi and bacteria. The sample cultivation was made in triplicates that were incubated for 1-2 weeks at $28\pm 2°C$. For obtaining high rates of purity, sub-cultivation of the powdery and pigmented colonies was performed in actinomycete isolation agar. The resulted pure colonies of Actinomycetes were preserved in $10\%$ glycerol at $-80°C$ and monthly transferred on newly prepared media for examination purposes [9]. The growth characteristics and biochemical tests such as salt (1%-12%), pH (2-12), and temperature (10, 15, 20, 25, 30, 40, and 45°C) tolerance tests (generated on SYE at 30°C for 2-3 weeks) were performed using methods from [10].

Amplification of 16S rRNA gene
DS3 and DN516S rRNA gene was targeted using the primers, F: 5′- GT AGA T TGA TCC TGG CTC AG-3′ and R: 5′- GGT TAC CTT GTT ACG ACT T-3′, [11]. Ten per cent of the extracted DNA was included in a total volume of the PCR reaction of 50µl, which also contained each primer at $0.4\mu M$, each dNTP at $0.2Mm$, BSA at $0.2\mu l$, DNA polymerase (Promega) at 1U. The PCR thermocycler conditions were 5min of denaturation at 94°C, 30 cycles of (30s of main denaturation at 95°C, 30s annealing at 60°C, and 2min of main extension at 72°C), and 10min final extension at 72°C. One-per-cent-agarose-gel electrophoresis pretreated with ethidium bromide was made.

Isolation of bacteria from cream cheese
Bacteria were isolated from cream cheese samples purchased from local stores in Al-Diwaniyah City, Iraq, by homogenizing $10gm$ the cream samples in $90ml$ saline. Then, certain dilutions were cultivated on NA and TSA media agars (Biokar Diagnostics, Beauvais, France), and incubated for 48hrs at 30°C. Morphology analyses and Gram-staining were performed, and biochemical tests such as catalase and the profile of sugar fermentation, using API20, tests were generated.

Antibacterial activity analysis
The ABA of DS3 and DN5 against selected CCB were tested using Müller-Hinton method. The supernatant of each sample broth was inoculated in each well, and the media were incubated for 24hrs at 37°C. The diameter of inhibitory zones (mm) was followed to determine the ABA against E. coli, S. aureus, B. subtilis, K. pneumonia, S. pyrogenes, and P. aeruginosa that were isolated from cream cheese.

Results
The following figure, 1, shows the Gram positive staining of DS3 and DN5.
Figure 1: Gram positive staining of *Streptomyces* and *Nocardia*.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Growth Characteristics</th>
<th>SYE</th>
<th>SYE Db₂O</th>
<th>MA</th>
<th>NA</th>
<th>PDA</th>
<th>ISP1</th>
<th>ISP2</th>
<th>ISP3</th>
<th>ISP4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptomyces sp. DS1</strong></td>
<td>Growth</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Aerial Mycelium</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Gray</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Grey</td>
<td>White</td>
</tr>
<tr>
<td></td>
<td>Substrate Mycelium</td>
<td>White</td>
<td>White</td>
<td>Pale Yellow</td>
<td>White</td>
<td>Pale Yellow</td>
<td>White</td>
<td>Beige</td>
<td>Beige</td>
<td>Pale Yellow</td>
</tr>
<tr>
<td></td>
<td>Diffusible Pigment</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Brown</td>
<td>Brown</td>
<td>Brown</td>
<td>Black</td>
<td>Brown</td>
</tr>
<tr>
<td><strong>Nocardia DN5</strong></td>
<td>Growth</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Aerial Mycelium</td>
<td>White</td>
<td>Grey</td>
<td>White</td>
<td>White</td>
<td>White</td>
<td>Grey</td>
<td>Grey</td>
<td>White</td>
<td>Beige</td>
</tr>
<tr>
<td></td>
<td>Substrate Mycelium</td>
<td>Brown</td>
<td>White</td>
<td>Brown</td>
<td>Pale Yellow</td>
<td>Pale Yellow</td>
<td>Beige</td>
<td>Beige</td>
<td>Grey</td>
<td>Brown</td>
</tr>
</tbody>
</table>

Table 1: reveals the growth features of DS3 and DN5 in various cultivating media.

**Growth characteristics of Streptomyces and Nocardia**

The table, 1, reveals the growth features of DS3 and DN5 in various cultivating media.

**NaCl tolerance test**

The following table, 2, shows the effects of NaCl concentrations on the sporulation activities of the DS3 and DN5.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>2%</th>
<th>3%</th>
<th>4%</th>
<th>5%</th>
<th>6%</th>
<th>7%</th>
<th>8%</th>
<th>9%</th>
<th>10%</th>
<th>11%</th>
<th>12%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces sp. DS3</td>
<td>Growth</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td></td>
<td>Sporulation</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
<tr>
<td>DN5</td>
<td>growth</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
</tr>
</tbody>
</table>

Ng: no growth;  + Poor growth;  ++ Moderate growth;  +++ Good growth
**PH tolerance test**

Table 3: displays the findings of pH tolerance tests on the activities of DS3 and DN5.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>$pH$ 2</th>
<th>$pH$ 3</th>
<th>$pH$ 4</th>
<th>$pH$ 5</th>
<th>$pH$ 6</th>
<th>$pH$ 7</th>
<th>$pH$ 8</th>
<th>$pH$ 9</th>
<th>$pH$ 10</th>
<th>$pH$ 11</th>
<th>$pH$ 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces sp. DS3</td>
<td>NG</td>
<td>NG</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>NG</td>
<td>NG</td>
<td></td>
</tr>
<tr>
<td>Nocardia DN5</td>
<td>NG</td>
<td>NG</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>NG</td>
<td>NG</td>
<td></td>
</tr>
</tbody>
</table>

Ng: no growth; + Poor growth; ++ Moderate growth; +++ Good growth

**Temperature tolerance test**

Table 4: Demonstrates the outcomes of the temperature tolerance test on the activities of DS3 and DN5.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>10°C</th>
<th>15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>28°C</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomyces sp. DS3</td>
<td>NG</td>
<td>NG</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Nocardia DN5</td>
<td>NG</td>
<td>NG</td>
<td>NG</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

Ng: no growth; + Poor growth; ++ Moderate growth; +++ Good growth

**PCR amplification of the 16s rRNA gene**

The identity of the DS3 and DN5 was confirmed by using the PCR that targeted 16S rRNA gene. The amplification was at 1500bp.

![Figure 2: Agarose gel electrophoresis of the 16s rRNA gene of Streptomyces and Nocardia. M: DNA ladder (1kb). Lane 1: PCR](image-url)
Anti-bacterial activity of Streptomyces or Nocardia on the cream cheese bacteria

The findings showed high ABA of DS3 and DN5 against all isolates of CCB. However, DS3 revealed higher effects on CCB than those detected from DN5 although DN5 declared higher ABA against K. pneumonia than that recognized from DS3, table 5.

Table 5: Anti-bacterial activity of Streptomyces or Nocardia on the cream cheese bacteria.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>E. coli</th>
<th>S. aureus</th>
<th>B. subtilis</th>
<th>K. pneumonia</th>
<th>S. pyrogenes</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>DS3</td>
<td>12±1</td>
<td>22±1</td>
<td>15±1</td>
<td>17±1</td>
<td>18±1</td>
<td>-</td>
</tr>
<tr>
<td>DN5</td>
<td>8±2</td>
<td>9±2</td>
<td>10±1</td>
<td>21±1</td>
<td>13±2</td>
<td>9±1</td>
</tr>
</tbody>
</table>

Discussion

A growing global health and economic challenge is the resistance of clinically important pathogenic microorganisms to antibiotics commonly being consumed. The acquired of non-susceptibility to antimicrobial agents is caused by either a mutation in a gene or by plasmid-based transported resistance from a bacterium to another. Bacteria employ various molecular resistance processes such as inactivity of antibiotics, receptor structural alteration or active cell removal by efflux pumps of an antibiotic. These resistant bacteria may be circulated in the environment via various tools; however, food represents one of the main sources for such foodborne pathogens [12]. This huge health problem requires the intervention for finding novel bacterial metabolites that have unique anti-microbial effects against those resistance bacterial species. DS3 and DN5 represent major sources for those molecules [1-6].

The findings revealed successful antibacterial effects of the DS3 on the growth activities of the selected cream cheese bacteria. This agrees with Sripreecsak and Athipornchai [13] who identified 13 Streptomyces isolates from soil in Thailand that generated potential anti-microbial metabolites that inhibited the growth activities of a wide range of fungi and, somehow, a similar bacterial group of bacteria as studied in the current investigation. However, their isolates were not able to inhibit the growth of E. coli and P. aeruginosa [13]. Moreover, Kibret et al [14] detected 416 isolates of Streptomyces with certain bacterial members, such as their designated Go-475 isolate, with strong bioactive cross-product substances, some of them are newly identified, that decreased the livability of some fungi and Gram positive pathogenic bacteria. In addition, they found that the DNA gnomic materials have the ability to code for antimicrobial metabolites [14].

The findings revealed successful antibacterial effects of the DN5 on the growth activities of the selected cream cheese bacteria. Sharma et al [15] have recognized bacterial isolates of Streptomyces from forest Soil designated as PB-52 and found that the ethyl-acetate broth extract of PB-52 revealed the least minimum inhibitory concentration (MIC) tested on K. pneumoniae. Furthermore, significant destruction of the integrity and cell shape was seen after using the extract as revealed by electron microscopic analysis [15]. In addition, GC-MS analysis performed on the extract by the authors, Sharma et al [15], demonstrated some chemical substances with bioactive effects such as (Z)-3-tetradecene, dodecyl acrylate, 2,4-di-t-butyl-6-nitrophenol, hexahydro-pyrrolo[1,2-$d$]-pyrrole, 1,4-dione, and (E)-5-ecosene. In conclusion, DS3 and DN5 may have natural anti-bacterial activity that may safely eliminate infectious agent presence in local cream cheese.

References

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