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Biochemical and Molecular Characterization of Gram-Negative Strain 37A Isolate from Spring Water Fountains

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Abstract

In this study, water samples from thirteen spring water fountains were collected in Burdur, Turkey. A gram-negative bacterial strain was isolated from cultures of water samples on Bunt and Rovira (BR) and 0.1 X Nutrient agar media. The isolated strain was characterized for cell wall structure, colony morphology, catalase expression, antibiotic sensitivity and 16S ribosomal RNA (rRNA) gene sequence. Results showed that the strain is gram-negative, catalase positive and rod shaped with pink colony morphology. The disc diffusion test for the strain revealed its sensitivity to cefoxitin, ofloxacin, tetracyclin, telithromycin, tobramycin, and sulfamethoxazole-trimethoprim antibiotics. Blast search for 16S rRNA gene sequence indicated that the strain is *Erwinia persicina* which is pathogenic strain for plants.

Keywords: spring water; contamination; Erwinia persicina; pathogen.

1. Introduction

Water naturally discharged from underground to surface is called spring water. While spring water is fed by rainfalls and snow seeping into the soil, it can also receive the carried environmental contaminants by the same means [1, 2]. Remnants of livestock and sewage nearby can pollute spring water and change its qualities determining its safety and drinkability [3]. Even though municipal water distribution is widely accessible to residences and stands as a safer resource, spring water fountains are still a common water supply for the local community in Burdur, Turkey.

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In this study, water samples obtained from spring water fountains in Burdur province and villages were monitored for bacterial content and a gram-negative, pigmented bacteria strain was isolated and characterized.

2. Materials and Methods

2.1. Sampling

Water samples was taken from thirteen spring water fountains in Burdur province. Samples were collected into sterile tubes under aseptic conditions for bacterial content analysis. At the same time, the pH, conductivity, temperature, and salinity of water from each source were evaluated.

2.2. Isolation and Phenotypic Characterization of Bacterial Strains

Samples brought to the lab were inoculated in Bunt and Rovira (BR) medium [4] and 0.1 X Nutrient agar. Agar plates were incubated at 20 ^{0}C for 24h. Growing colonies after 24h were reinoculated on BR agar to obtain pure colonies. Isolated bacterial samples were subjected to Gram staining, catalase test and cultured for growth at 2 ^{0}C, 10 ^{0}C, 12 ^{0}C, 18 ^{0}C and 37 ^{0}C.

2.3. DNA Extraction and Sequence Analysis

DNA isolation was performed from pure bacterial isolate of gram-negative, pigment-producing strain 37A by using PureLink Genomic DNA isolation kit (Invitrogen) according to the manufacturer's protocol. DNA was quantified and 16S ribosomal RNA gene region was PCR amplified by using universal 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-CGGTTACCTTGTTACGACTT-3') primers. Amplification products were separated by agarose gel electrophoresis and gel images were captured.

For species identification, PCR products were sequenced using the same PCR primers and raw sequencing data was processed by FinchTV and a Blast search was conducted in GenBank.

2.4. Antibiotic Sensitivity Test

0.5 McFarland suspension of bacteria in 0.9% NaCl was spread on Mueller-Hinton agar plates and antibiotic disks were placed. Agar plates were incubated at 30 ^{0}C for 24h and zone diameters around discs were measured.

2.5. Biofilm Formation Assay

Bacterial isolate was cultured in 96-well plate containing 50% TSB, 50% TSB plus 1.25 mM $CaCl_2$, BR medium and BR medium prepared with tap water. After 24h incubation, unattached bacteria were removed by washing the wells with 0.9% NaCl solution. Later, samples in the wells were fixed with methanol and stained with crystal violet. Sample absorbance was recorded at 590 nm. Results were evaluated according to Clinical Laboratory Standards Institute.

3. Results

A gram-negative, catalase positive, rod-shaped, pink-colored colony forming bacteria under different temperatures were isolated (Figure 1). Sequence analysis of approximately 1.5 kb PCR product for 16S rRNA gene region has shown match with Erwinia persicina (Figure 1).

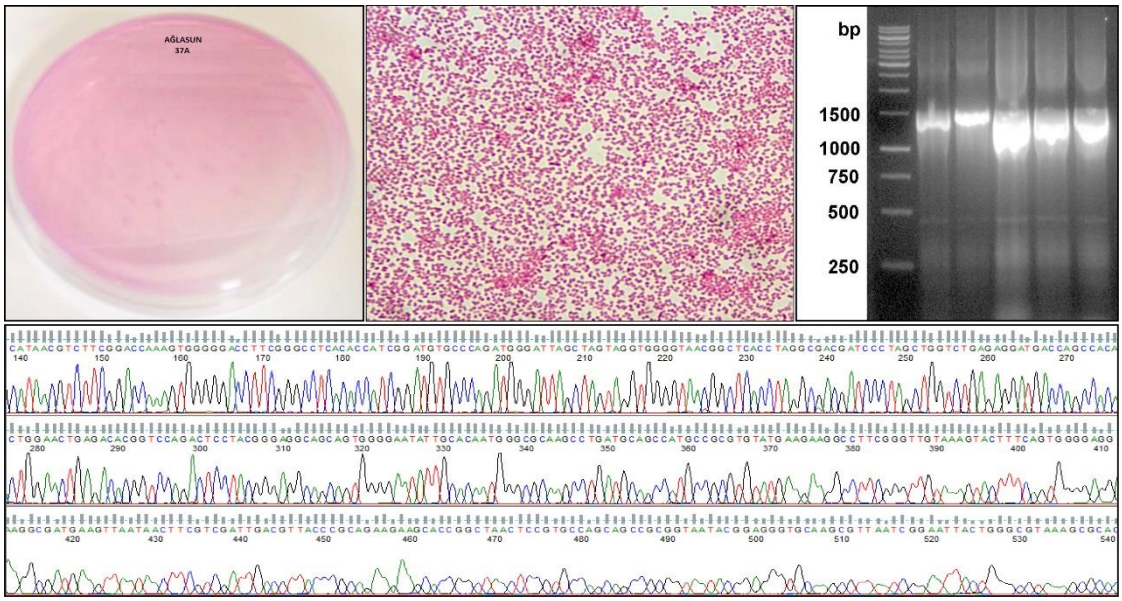

Figure 1: Colony morphology and sequencing data for 16S rRNA gene PCR product of gram-negative strain 37A. Pink-colored, rod-shaped colonies were grown and 16S rRNA region was amplified by PCR. A 1480 bp PCR product was obtained and sequenced. Blast search of this sequence detected a 99% match with *Erwinia persicina* sequence (Genbank ID: AB681774.1)

Isolates were sensitive to cefoxitin, ofloxacin, tetracyclin, telithromycin, tobramycin, and sulfamethoxazole-trimethoprim (Figure 2). Biofilm production ability for the strain was not detectable.

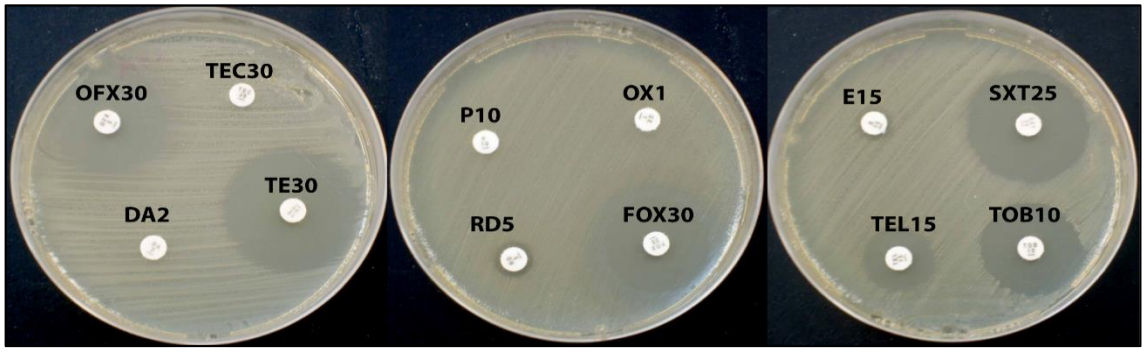

Figure 2: Antibiotic sensitivity of gram-negative strain 37A. Bacteria spread on Mueller-Hinton agar plates show sensitivity to cefoxitin (FOX,29 mm ZOI), ofloxacin (OFX, 25 mm ZOI), tetracyclin (TE, 28 mm ZOI), telithromycin (TEL, 16 mm ZOI), tobramycin (TOB, 23 mm ZOI), and sulfamethoxazole-trimethoprim (SXT, 31 mm ZOI). ZOI: zone of inhibition.

4. Discussion

In this study, bacteria isolated from spring water fountains were identified as *Erwinia persicina*, a pathogenic strain for plants. It was first isolated from tomatoes but later characterized by Hao et. al [5]. *E. persicina* can spread through contaminated seeds and soil and infects various plants including soybean, pea, common bean, lettuce and bananas [6]. Even though other *Erwinia* species such as *E. amylovora* [7, 8], *E. carotovora* [9] and *E. chrysanthemi* [10, 11] have previously been detected in natural water, the presence of *E. persicina* in spring water has first been detected by this study. As shown with *E. amylovora* isolated from natural water which preserves both its viability and pathogenicity [7, 8], *E. persicina* from spring water can also stay viable and form colonies on solid media. High endurance of this species under diverse temperature, salinity and pH conditions [12] might have increased its survival chance in spring water. However, whether the pathogenicity of the isolate is maintained still needs to be determined by infection experiments on plants.

Even though *E. persicina* was detected on biofilm developed on palaeolithic rock paintings [13] and *E. persicina*-derived extracellular polysaccharides (EPS), a biofilm component, were deeply analyzed, in the current study biofilm production by the isolated strain was undetectable in tested culture conditions. In line with previous reports indicating that *E. persicina* is β- lactamase positive [14], *E. persicina* identified in this study were resistant to penicillin (P) and oxacillin (OX). Besides, teicoplanin (TEC), clindamycin (DA), rifampicin (RD) and erythromycin (E) failed to constraint the *E. persicina* growth. However, the isolate was sensitive to cefoxitin, ofloxacin, tetracyclin, telithromycin, tobramycin, and sulfamethoxazole-trimethoprim. In addition to biochemical and genetic characterization, the persistence of the strain in the study areas needs to be determined by taking water samples at different times of the year. The risk of bacterial spread by water becomes higher when the bacteria-contaminated water is used for irrigation of plants or consumed by animals. Survey of *E. persicina* infections in agricultural fields close to sampling location will also help in risk assessment for water-mediated transmission of the pathogen.

Despite the increasing number of studies reporting *E. persicina* infection on plants, there are a few studies on *E. persicina* infection in animals. In an experimental infection model with sheep, the animals were monitored after receiving a subcutaneously injection of *E. persicina* [15]. The animals developed signs of acute infection which did not persist after 48h [15]. In contrast, in experimental infection model of mice, increased proinflammatory cytokine levels along with necrotic lesions in multiple organs including liver, intestine, kidney and lungs were recorded [16]. These provided the clue that animals might also be the host for *E. persicina*. In addition to animal studies, only one group has reported *E. persicina* in a patient's urine sample with urinary tract infection [17]. With this limited information, further investigation is necessary to address the potential of *E. persicina* to cause infection in humans.

5. Conclusion

The results show that the spring water sources chosen for sampling in this study contained a plant pathogen, *Erwinia persicina*. This species is highly resilient to adverse environmental conditions such as diverse temperature, pH, salinity and can spread via soil, infected seeds [12]. Unlike other *Erwinia* species such as *E.*

amylovora and *E. pyrifoliae* causing disease in the members of Rosaceae family [18], *E. persicina* causes necrosis and wilting in seasonal crops such as legumes and tomatoes and infection of perennial, woody plants by this strain has not been detected [12]. *E. persicina* is closely related with *E. amylovora* based on over 90% similarity for 16S rDNA and 41% similarity for overall mean genome [19]. This high sequence similarity suggests that a comparative analysis of these two strains for virulence factors and determinants of host range help to determine the potential of *E. persicina* to infect trees [20]. Besides its phytopathogenic properties, limited experimental data indicate that *E. persicina* have the ability to cause infection in animals [15, 16]. Detection of *E. persicina* from spring water samples should be reevaluated in terms of health risks after performing comprehensive pathogenicity studies with plants, human and animal cells.

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