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CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF SERRATIA SP. FROM COMPOST BY THE USE OF 16SRIBSOMAL RNA GENE SEQUENCE

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ABSTRACT

Serratia bacterium was isolated from the compost samples and it was identified as Serratia sp., based on microscopic and biochemical characteristics. For molecular identification using specific PCR, the chromosomal DNA was extracted from the purified isolate and the DNA was subjected to amplification of the 16S r RNA gene. The PCR product was about 1200bp, the obtained amplicone was sequenced. The sequence analysis revealed that the isolate belongs to S. marcescens isolates with similarity 95%. The DNA sequence was deposited in gene bank under accession number of KP 797836 and the strain was labeled as SerratiamarcescensFek 323. The phylogentic analysis showed that our isolate is more closely related to chinease S. marcescens strain.

Key words: *Microbial biosurfactant- Emulsification – Identification of Bacilli isolates.*

INTRODUCTION

Serratiamarcescens is gram-negative non-spore-forming bacterium belonging to the genus *Serratia*and family *Enterobacteriaceae* [1] host strains of *Serratia*are motile with peritrichous flagella and produce a red diffusible pigment called prodigiosin. In the soil *Serratiamarcescens*might play a role in the biological cycle of metals by mineralizing organic iron and dissolving gold and copper [2].

Different plant associated roles have been put forward for *Serratiamarcescens*, including that of a herbicide degradation bacterium [3] a plant growth promoting *rhizobacterium* [4]. Although *S. marcescens*is a conditional pathogenic bacterium that is capable of causing disease in diverse organisms, including humans [5] corals [6], insects [7], and plants [8], it is also a very important industrial strain which has been applied in fermentation for the production of various enzymes [9,10, 11]. Recently,

Abdel-Wehab [12] used *Serratiamarcescens* in enriching compost to express its ripening. This work aims to isolate and characterize of *Serratiamarcescens*Fek 323 isolated from compost samples.

MATERIALS AND METHODS

Bacterial isolation

Different compost samples were collected from heaps done at BeniSwef Governorate from which a composite compost sample was released for screening purpose. Serial dilutions of soil samples were performed and plated on Nutrient agar medium for isolating the bacteria [13].

Morphological, biochemical and enzymatic characterization

Morphology was observed by gram staining and hanging drop method [14] and moreover. Biochemical characterization was done with different tests using the Biolog^{TM} GEN III MicroPlate identification System [15], such as indole, methyl red, vogesproskeaur and citrate. The carbohydrate fermentation tests such as, fructose, glucose, by lactose and sucrose were carried out to check for acid and gas production. Various enzymatic tests such as gelatinase, protease and Dnase (Chitenase) were performed to find out potential of the organism. Antibiotic sensitivity test for different antibiotics was done to check for the sensitivity of organism by disc diffusion method. Biolog is a commercial tool for identify unknown bacterium. It analyzes microorganism in 94 phenotypic tests: 71 carbon source utilization assays and 23 chemical sensitivity assays. The test panel provides a "Phenotypic Fingerprint" of the microorganism, which can then be used to identify up to species level.

Molecular characterization by 16s rRNA

Bacterial DNA extraction

The bacterial genomic DNA was extracted using wizard genomic DNA purification kit QIAGENE DNA purification kit (Germany) according to the manufacture procedures.

The 16S r DNA amplification using specific PCR

The full length (1550-bp) of the 16S rRNA gene was amplified by the polymerase chain reaction (PCR) as described by Sambrook*et al.* [16]. The universal primers used for 16S region in Eubacteria amplification are: Start (forward) primer Bac8F: 5' AGA GTT TGA TCC TGG CTC AG3' and the End (backward) primer 1392 R: 5' GGT TAC CTT GTT ACG ACT T 3'.

The PCR reaction was performed as follow; a total volume of 50 µl containing 5 µl 10 x buffer, 4 µl 25 mM $MgCl_2$, 4 µl 2.5 mMdNTPs, 2 µl 10 pmol forward primer, 2 µl 10 pmolbackward primer, 2 µl 50 ng of bacterial genomic DNA and 0.4 µl (5 units/µl) Taq DNA polymerase (Promega, Germany). PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed for one cycle at 95°C for 5 min followed by 30 cycles each with 45 s at 95°C for denaturation, 1 min at 50°C for annealing and 2 min at 72°C for elongation. Reaction mixture was then incubated at 72°C for 10 min for final extension.

DNA sequencing for the amplified 16S rRNA gene

The DNA sequence was performed using automated DNA sequence and terminator dye (Macrogen Company, Korea).The sequenced sample was analyzed for nucleotide matching from gene bank by the nucleotide blast, where the sequence was pasted in the FASTA format and analyzed.

Sequence alignment and phylogenetic analysis

Pairwise and multiple DNA sequence alignment were carried out using CLUSTALW multiple sequence alignment program version 1.82 (http: //www -.ebi.ac.uk/ clustalw), [17]. Bootstrap neighbor joining tree was generated using MEGA version 3.1 [18] from CLUSTALW alignment. Comparison with sequences in the Gene Bank database was achieved in BLASTN searches at the National Centre for Biotechnology Information site (http: //ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Colony morphology

The colonies of *Serratia* isolate were observed on nutrient agar medium plates after 24 hrs of incubation at 37^{o}C. Colony morphology on nutrient agar showed smooth, circular entire and pigmented colonies (Figure 1a,b). Growth conditions were optimized by testing growth on Nutrient agar. Maximum growth was obtained at 32°C with pH 7 in Nutrient broth. Red color diffusible pigment prodigiosin production was observed after 24hr of incubation **[**19].

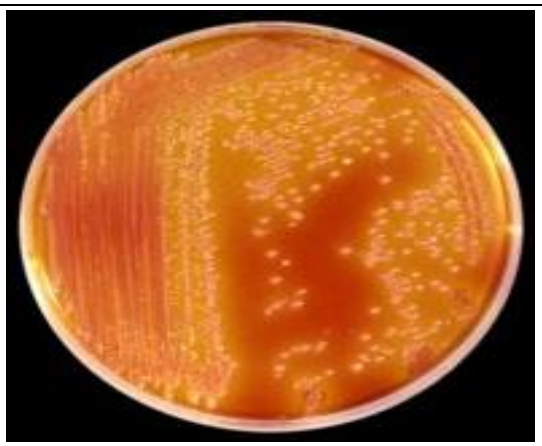

Figure 1a: *Serratiamercescenes* grown on nutrient agar medium.

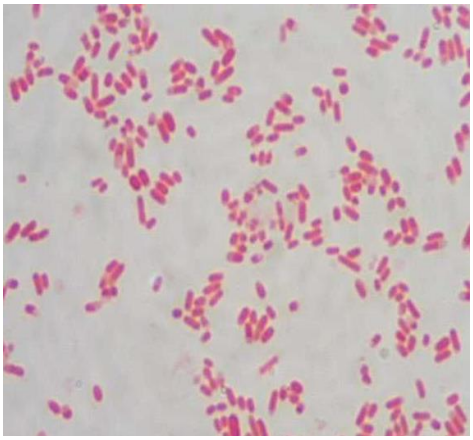

Figure 1b: *Serratiamercescenes* (1000 x).

Microscopic, Biochemical and Enzymatic analysis

The cells stained Gram negative upon gram staining. They were rod shaped and arranged singly Grimont [20]. Motility of the strain was observed under wet mount which indicates the presence of flagella. Various biochemical tests were performed to characterize the strain and the isolate showed negative for indole test, negative for methyl red, positive for vogesprosker and positive for citrate utilization test. These observations were in accordance with the reported biochemical characters of *Serratia*.

Later tests were performed to study the enzymes like gelatinase, protease &dnase which are important in virulence character expression by *Serratia*. Gelatin liquefaction was observed on gelatin agar, which confirms the presence of gelatinase enzyme. When inoculated on to a DNA agar medium, zone of clearance was observed around the colonies when flooded with 2 N HCl

which showed the presence of an extracellular dnase enzyme. Interestingly this isolate was identified to produce big zone of clearance on Dnase agar test medium. Protease production was observed on skim milk agar plate as clear zones around the colonies after 24 hr incubation.

Different sugar fermentation tests were carried out further to analyze the biochemical reactions in the organism. This isolate was able to ferment sucrose, glucose and fructose while it could not ferment lactose (Table 1). These results match with the reported literature on *Serratia*.

Table 1: Phenotypic characters for *Serratia* sp.

Test	Result	Test	Result
Gram staining	Gram negative rods arranged singly	Dnase activity	+ve
Pigment production	+ve	Casein hydrolysis	+ve
Motility	+ve	Catalase	+ve
Indole	--ve	Glucose fermentation	+ve
Methyl red	--ve	Lactose fermentation	+ve
Vogesproskeur	+ve	Sucrose fermentation	+ve
Citrate	+ve	Fructose fermentation	+ve
Gelatin liquefaction	+ve		

In spite of tentative biochemical identification of the isolate as Serratia, a detailed molecular characterization was undertaken in order to identify the position of the isolate among various Serratiaspecies reported so far.

Identification of bacterial strain by 16S rRNA Gene

The 1500 bp PCR product of the 16S rRNA gene was subjected to DNA sequencing. The PCR product gave an amplicone with right molecular weight (1500bp) (Figure 2).

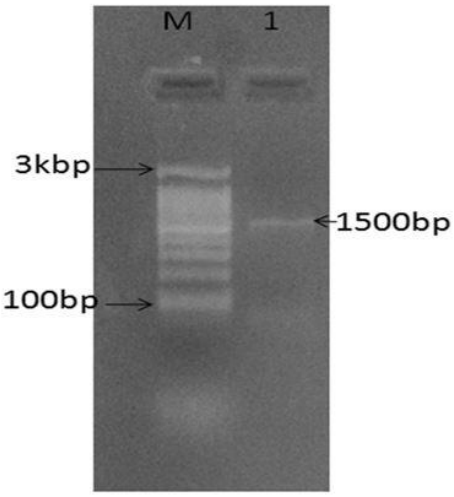

Figure 2: PCR amplification of the 16S rRNA gene from the bacterial isolate. Lanes; M: 3Kbp DNA molecular marker. Lane 1: The PCR amplicone of the 16S rRNA gene (1500bp).

DNA sequencing for the amplified (1500bp) 16SrRNA gene

The PCRamplicone of the 16S rRNA was subjected to DNA sequencing and the sequence analysis revealed that, the nucleotides DNA sequence was closely similar to Serratiamarcescens.

Alignment and Phylogenetic analysis

Pair wise and multiple DNA sequence alignment for the DNA nucleotides sequence of the isolate was carried out with the other *Serratiamarcescens* in gene bank (Figure 3). The obtained sequence was submitted to BLASTN in order to find homology with other bacterial 16S rRNA sequences. Alignment of 16S rRNA sequences of bacterial isolate investigated in this study revealed that the tree of the bacterial isolate was divided into four Clusters. Cluster 1 included eight isolates, Cluster 2 included four isolates and Cluster 3 included only one isolate. The phylogenetic analysis indicated that the bacterial isolate (Egyptian isolate) was placed in the cluster four and closely related to the bacterial isolates with accession numbers (gi685167698, gi645904451and 648109706), which are all Indian isolates.

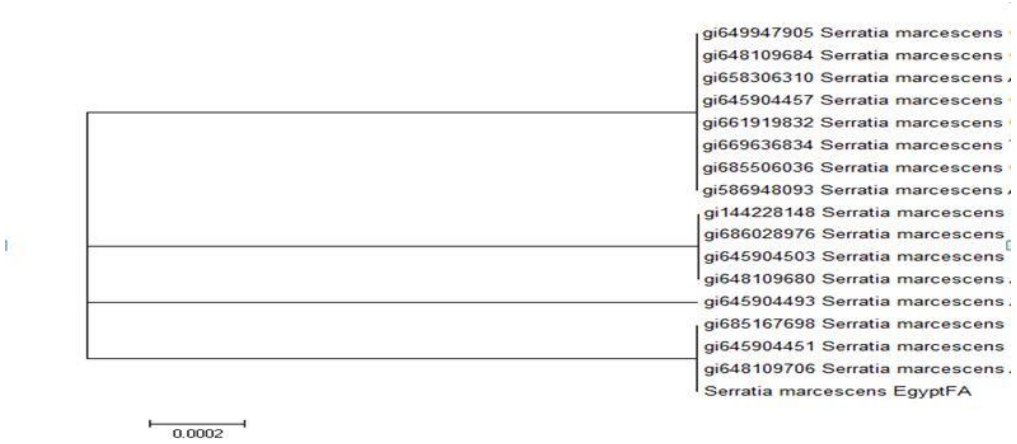

Figure 3: Phylogeny tree for the 16S rRNA gene of the Egyptian isolate compared with the other *Serratiamarcescens* strains listed in Gene Bank.

CONCLOSION

The bacterium isolated from compost samples of our work was identified by using different microscopic, biochemical, enzymatic tests as a member of Enterobacteriaceae according to Bergeys Manual of Systemic Bacteriology. The red pigment prodigiosin production confirmed it to be a member of genus *Serratia*. As this isolate has the potential of producing extracellular Dnase activity, molecular characterization and phylogenetic analysis was carried. Phylogenetic analysis proved the *Serratiamarcescens*Fek 323 isolate to be to be *Serratiamarcences*with nucleotide sequence accession number of KP 797836 edited by the Gene Bank at USA in Febrauary, 2015.

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