Identification of Novel Bacteria by Using 16S rRNA from Clinical and Soil Samples

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Abstract

The 16S rRNA gene is a useful tool and molecular indication for identifying novel bacterial species from different sources. It is widespread for members of this field and thanks to the continuous expansion of information sequence databases. We isolated Gram-negative and Gram-positive bacilli from the respiratory infections and soil respectively, and phenotypical and molecular identifications were performed. Genetic analysis indicated that the isolated bacteria were a new species belonging to *Bacterium* strain *and Proteus sp. Bacterium* strain JND-RSIb-21A with Accession number MW013547.1 which closely related (99.8%) with *Bacillus subtilis strain JND-RSIb-21A* for its Transversion point mutation (G instead of C) at the position 445 bp. *Proteus sp.* strain AMJ131 with Accession number MW015095.1 the isolate related closely (99.8%) to *Proteus mirabilis* strain AMJ131for two-point mutations Transition of (G instead of T) and Transversion of (T instead of A) at position 9bp and 10bp respectively.

Keyword: *Bacterium* strain, *Proteus sp.,* 16S rRNA gene, soil isolate, Gram-positive bacilli

Introduction

The genus *Proteus,* apart of the *Enterobacteriaceae* family of rod-shaped bacilli, Gram-negative bacteria [1], facultative anaerobic, swarming motility by flagella, ability to self-elongate and secrete a polysaccharide when in contact with solid surfaces; this allows for attachment and easy motility along surfaces. Its ability to form biofilms and is suggested to contribute to resistance to host defenses and certain antibiotics [2]. Widely spread in the environment mainly in water, soil, humans, and animals[3]. It is an opportunistic pathogen that is implicated in various human diseases of the respiratory tract, eye, ear, skin[4], and urinary tract [5]. Its hardy, adaptable, and potentially pathogenic residents of the human gastrointestinal tract and have been underappreciated as a cause of the gastrointestinal disease [6]. Soil microbiology emerged as a distinct branch of soil science [7]. Soil contains a wide range of microorganisms described as a 'black box' [8]. Soils are one of the world's hotspots for biodiversity [9] and it is a natural habitat in which microbes live, multiply and die. Interest in microbial diversity has grown rapidly in the scientific community. Increasing attention is being drawn to microorganisms because the fertility of soil depends not only on its chemical composition but also on the qualitative and quantitative nature of microorganisms inhabiting it [10]. The activity of microorganisms in soil is important for the robust functioning of soil and related ecosystem services.[11]. Most of the antibiotic producers used today are soil microbes [12]. One of the strategies to reduce the time for microbial identification is the use of molecular biology techniques which may also be supplemented with numerous molecular fingerprinting techniques[13]. Fast detection and identification of microorganisms is a challenging and significant feature from industry to medicine. Standard approaches are known to be very time-consuming and labor-intensive (e.g., culture media and biochemical tests). Conversely, screening techniques demand a quick and low-cost grouping of bacterial isolates, and current analysis call for broad reports of microorganisms, involving the application of molecular techniques (e.g., 16S ribosomal RNA gene sequencing-based on polymerase chain reaction) [14].

Materials and Methods

Sample collection

Sputum samples were collected from patients with respiratory infections in the respiratory and chest disease center in Basra city. Soil samples were collected from the pharmacy college garden/Basrah university and prepared by paraffin baiting technique [15].

Isolation and purification of bacterial isolates

Sterile dry swabs were used to streak sputum samples and soil samples onto sterile Petri dishes containing Sabouraud dextrose agar for 3 weeks at 37°C [16]. The antifungal agent cycloheximide (actidione) at 50 µg /ml was added to the sterilized media at 46°C [17]. Single colonies were obtained using sterile inoculation needles. Colonies were then stained with Gram staining [18]. Conventional and specific biochemical tests were used for identification [19].

DNA Extraction from Bacteria

The bacterial isolates were transported to an eppendroff tube (1.5) ml. The extraction of genomic DNA was achieved by Geneaid bacterial DNA extraction kit(GEE150), and the extraction steps were performed according to the instructions of the kits supplied company. then DNA was detected by gel of 1% agarose containing 0.5 µl ethidium bromide and electrophoresed at 60 volts for 1.5 hours.

PCR for 16S Ribosomal DNA

Primers 27-forward 5'-AGAGTTTGA TCCTGGC-3' and 1492-reverse 5'-GGTTACCTTGT TACGACTT-3'[20]. were applied to amplify 16SrDNA genes in eppendroff tube (20 µl) mixture (Intron, Korea) consisting of 5 µl Mastermix, 10 pmol primers (1µl) for each bacterium sample, 1µl DNA template, and 34.5 µl nuclease-free water. PCR program was 92 ºC for 2 min, 35 cycles of 94 ºC denaturation for 30 sec., 51.8 ºC annealing for 45 sec. and 72 ºC extension for 1.5 min., finally, 72 ºC for 5 min. The bands of 1500 bp were observed by adding 5 µl of PCR product in 2% agarose gel with 0.5 µl ethidium bromide and electrophoresed with 5 µl of 1 kbp DNA ladder (Bioneer) [20].

Sequencing and Identification of 16S rDNA gene

The PCR 16SrDNA gene was purified from gel using MEGAquick-spin™ Total Fragment DNA Purification Kit (Intron) Korea and then sequenced according to Macrogen Company conditions using an automated DNA sequencer. And by "BLAST" The alignment was identified for each bacteria, from the website http://blast.ncbi.nlm.nih.gov, and "CLUSTAL Omega" http://www.ebi.ac.uk /Tools /msa /clustalo/. were used for comparing all sequences [20].

Phylogenetic Tree

The 16SrDNA sequences data for each identified bacterial isolates were aligned for the concatenated of different lengths for isolates ranging from (537-706) bp and phylogenetic trees were inferred by using the Molecular Evolutionary Genetics Analysis" MEGA7" software.

Results

Identification by 16Sr DNA Gene

Sequencing 16SrDNA gene from 5 bacterial isolates was observed on agarose gel at a suitable size (1500 bp) in comparison with the DNA ladder as in Figure (1). Only 4 were identified by 16S rDNA gene sequencing and compared with their type strains. The bacterial isolates belong to, *Bacillus sonorensis*, *Bacillus subtilis*, *Bacterium* strain, *and Proteus sp.*

Phylogenetic Tree of Bacterial Species

The phylogenetic tree (Figure 2) shows the distribution and phylogenetic relationships among the studied bacterial species, the bacterial strains showed closely related isolates (0.1549, 0.0140) for branch value. While for others identical were 0 and scale value=0.050). While the Phylogenetic tree for newly recorded isolate *Bacterium* strain JND-RSlb-21A illustrates closely related isolates (0.0027, 0.0018 and for others identical were 0 and scale 0.0005), and for *Proteus sp* strain AMJ131 the phylogenetic tree shows closely related isolates with (0) for identical isolates, and scale= 0.001. (Figure 3and 4)

Identification of New Global Bacterial Strains

Two bacterial isolates were identified as new strains

showing differences with their type strains in some numbers and placements of the bases isolates. *Bacterium* strain JND-RSIb-21A with Accession number MW013547.1 which closely related (99.8%) with *Bacillus subtilis strain JND-RSIb-21A* for its Transversion point mutation (G instead of C) at the position 445 bp (Figure 5). *Proteus sp.* strain AMJ131 with Accession number MW015095.1 the isolate related closely (99.8%) to *Proteus mirabilis* strain AMJ131for two-point mutations Transition of (G instead of T) and Transversion of (T instead of A) at position 9bp and 10bp respectively as shown in (Figure 6).

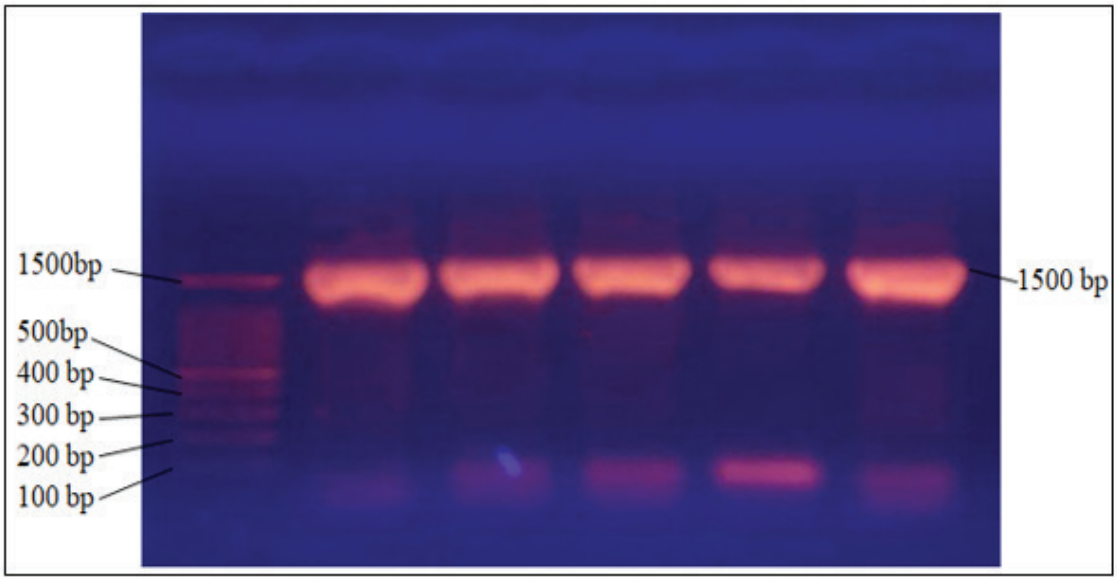

Figure 1: the Agarose gel electrophoresis of the 16SrDNA gene for each bacterial isolate.

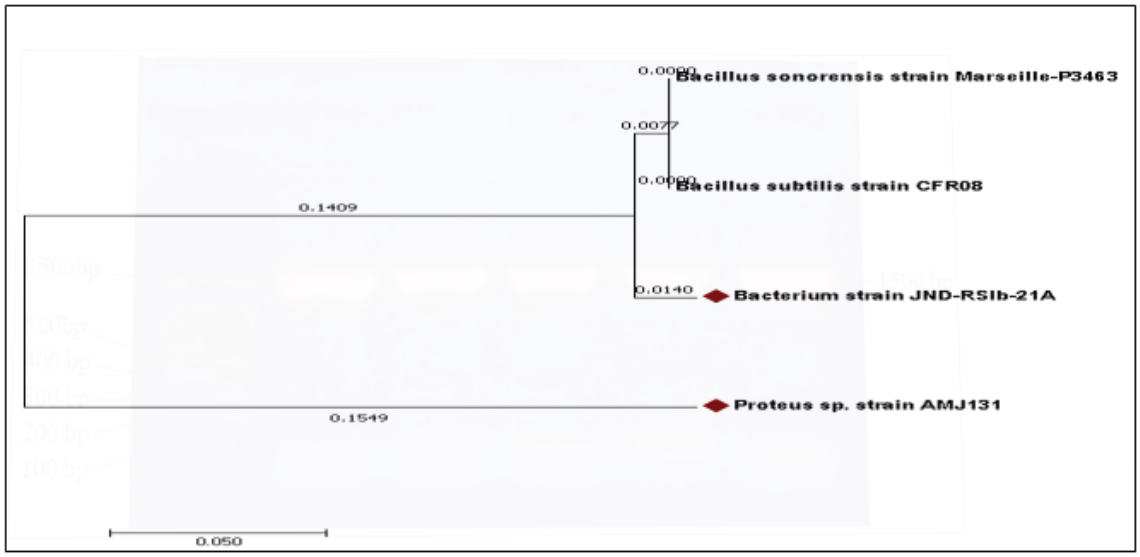

Figure 2. The phylogenetic tree for the bacterial isolates, newly recorded isolates

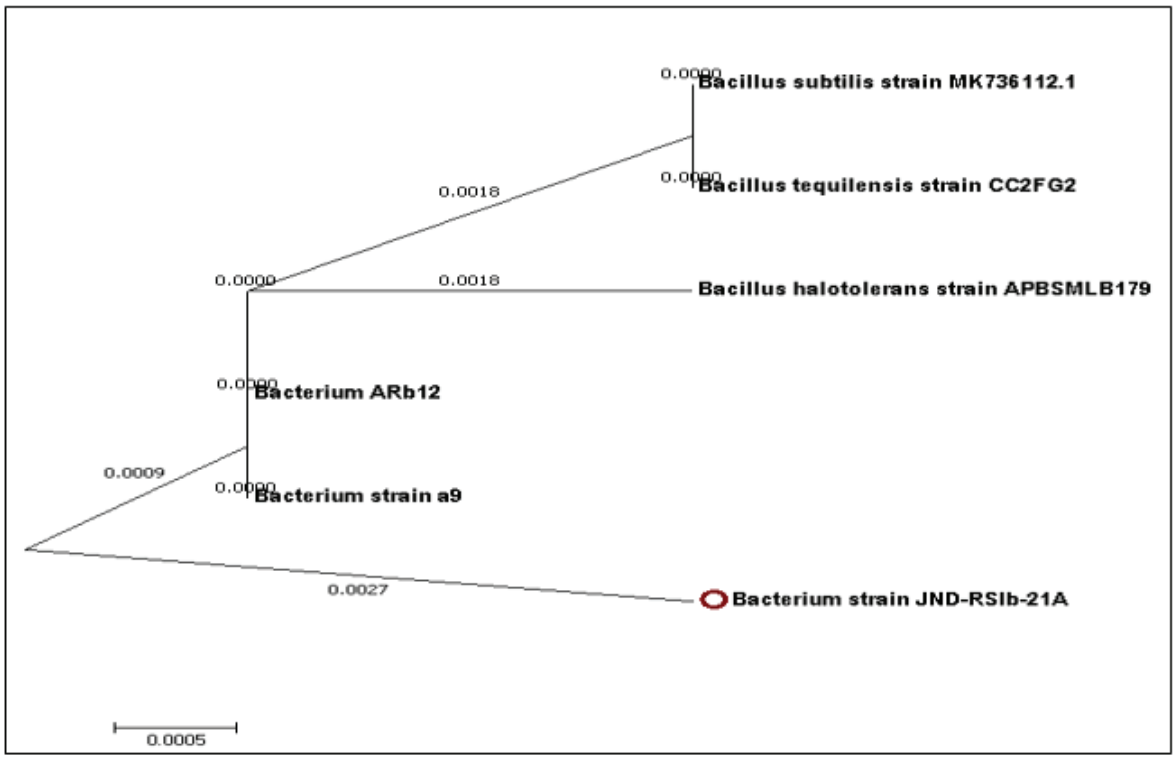

Figure 3: Phylogenetic tree for newly recorded isolate Bacterium strain JND-RSlb-21A

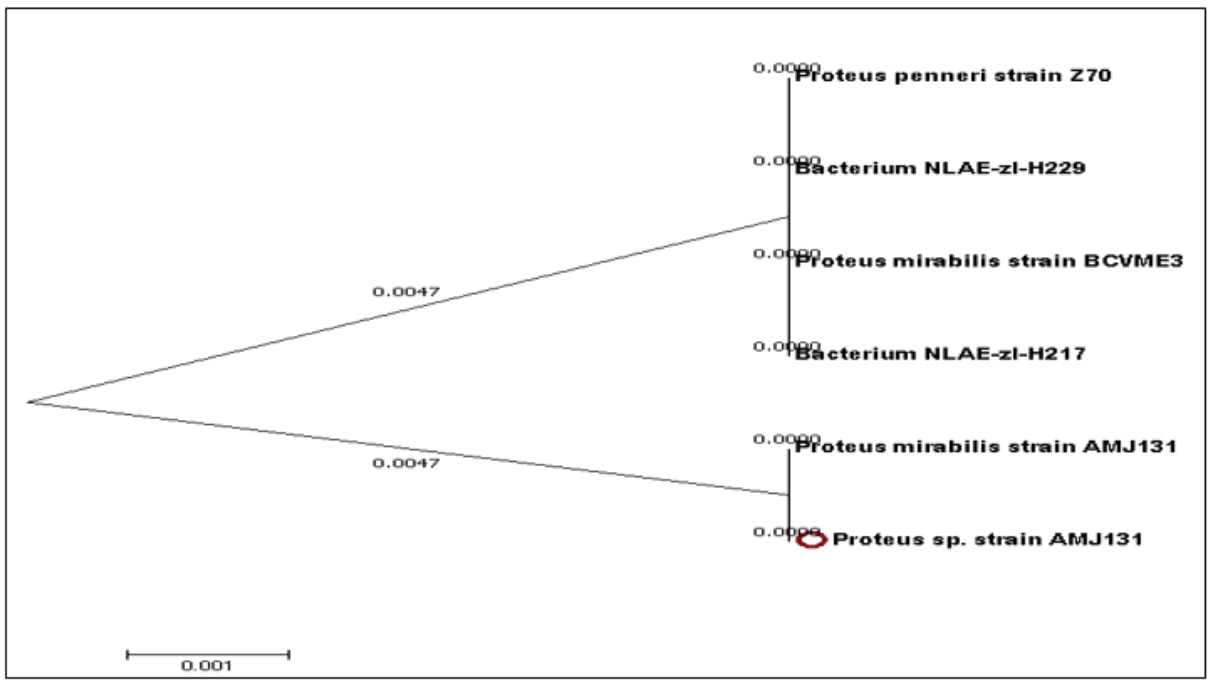

Figure 4: Phylogenetic tree for newly recorded isolate Proteus sp. strain AMJ131

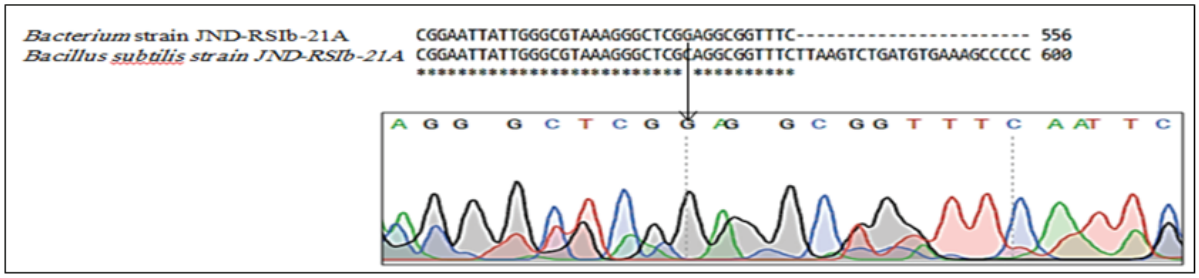

Figure 5:. Comparison of 16S rDNA nucleotide sequences (556 bp) for the isolate *Bacterium strain* JND-RSIb-21A from present study and *Bacillus subtilis* strain JND-RSIb-21A. A gene or point mutation type Transversion (G instead of C) at the position 445 bp.

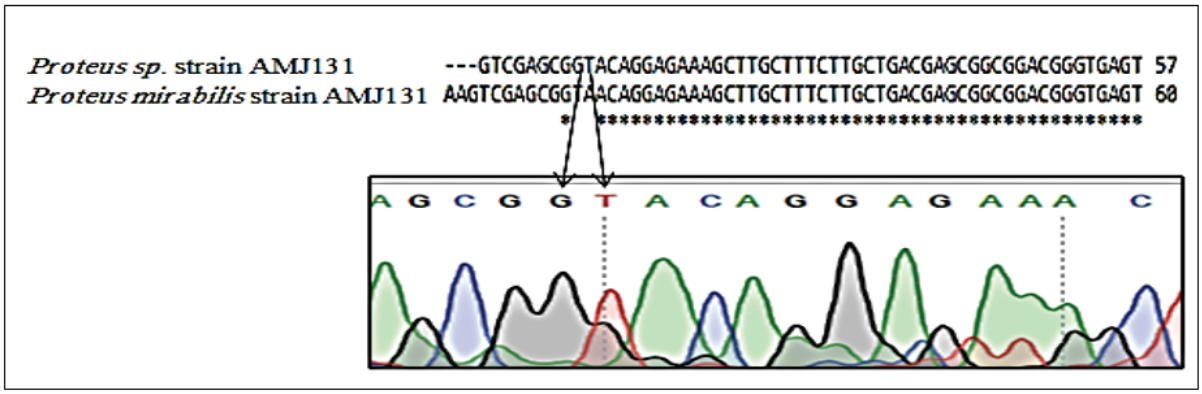

Figure 6: Comparison of 16S rDNA nucleotide sequences (539 bp) for the isolate *Proteus sp.* strain AMJ131 from the present study and *Proteus mirabilis strain AMJ131* gene or two-point mutation type Transition (G instead of T) and Transversion (T instead of A) at the position 9 bp and 10 bp respectively.

Discussion

Proteus belongs to the family Enterobacteriaceae and the tribe of Proteeae [21]. In the late 19th century, Hauser was reported characterized the first isolates of Proteus [22]. Proteus was isolated from sputum cultures according to the morphological and microscopical features, biochemical and API 20 E tests confirm [23]. The microorganisms in soil are very important to the planet in our lives, according to their role in the nutrient cycles [24]. Therefore, soils rich in nutrients are considered a fertile environment for a wide range of microorganisms, but the number of bacteria in the soil exceeds the total of other populations of microorganisms in number and type. [25]. *Enterobacter* spp., *Pseudomonas* spp., *Ralstonia* spp., *Proteus* spp., *Aeromonas* spp., *Burkholderia* spp., *Pantoea* spp., *Raoultella* spp., *Achromobacter* spp., *Escherichia coli,* and *Leclercia* spp. respectively isolated from the soils of Iraq [26]. Rapid replication of DNA from less starting material by PCR makes it a more sensitive technique for the detection of bacterial species. PCR-based identification of bacterial DNA and sequencing of the 16S rRNA gene has become a standard molecular method and highly specific to each bacterial species. PCR-based methods are used for the identification of bacteria that are difficult to grow in laboratory conditions [27]. The 16S rRNA gene is commonly used for the identification and classification of microbes from environmental samples. In this study, we use this gene for the identification of different isolates from soil and the human respiratory system. The 16S rRNA gene could be used as a phylogenetic marker because of its functional constancy and the presence of conserved and variable sequence regions evolving at very different rates [28]. A phylogenetic tree is a diagram that refers to evolutionary relationships among different species based on similarities and differences in their genetic characteristics. The phylogenetic tree was designed according to the bacterial isolates which showed 99%

or 100% similarity with the type strains, giving two bacterial isolates as new strains, in this study (Figure 2) for the phylogenetic tree of the isolates illustrate. The *Bacillus sonorensis* strain Marseille-P3463 is closely related to *Bacillus subtilis* strain CFR08 but differs from the newly recorded strain *Bacterium* strain JND-RSlb-21A and *Proteus sp.* strain AMJ131 because of the changing in 16s rRNA gene as a result of mutation [20]. To explain the relationship between newly recoded isolates with other strain we draw two phylogenetic trees (Figure 3) shows the newly recorded strain *Bacterium* strain JND-RSlb-21A differ from other strain selected from GenBank (*Bacillus subtilis* strain MK736112.1, *Bacillus tequilensis* strain CC2FG2, *Bacillus halotolerans* strain APBSMLB179, *Bacterium* ARb12, *Bacterium* strain a9) which were isolated from the various sources, and that may be for transversion point mutation (G instead of C) at the position 445 bp as mention above in (Figure 5). The newly recorded strain *Proteus sp.* strain AMJ131 is closely related to *Proteus mirabilis* strain AMJ131 as illustrate in the phylogenetic tree (Figure 4) but it differs from other strain from different sources selected from GenBank (*Proteus penneri* strain Z70, Bacterium *NLAE-zl-H229*, *Proteus mirabilis* strain BCVME3, *Bacterium* NLAE-zl-H217) and that difference explain according to two-point mutations Transition of (G instead of T) and Transversion of (T instead of A) at position 9bp and 10bp as in (Figure 6) [20,29]. A mutation is a permanent alteration in the sequence of the nitrogen base of the DNA that is generally may change the end product of the specific gene, mutations lead to change in the genes that are very important in bacterial evolution and that make a difference in the distribution of isolates in the phylogenetic tree. Mutations may occur by exposure of the bacteria to certain environmental factors such as radiation and chemical mutagens. Also, overuse of broad-spectrum antibiotics leads to a mutation and emergence of a new strain such as in bacteria isolated from humans such as the urinary tract [20].

Conclusion

Respiratory infections are the most prevalent and a chronic health problem in humans all over the world. Different bacterial species have the important role in the environment especially in soil. In this study, new bacterial strains were isolated from the soil samples of pharmacy college garden and patients with respiratory infections belonging to *Bacterium* strain JND-RSIb-21A *and Proteus sp.* strain AMJ131 respectively. Result of 16S rRNA analysis showed that *Bacterium* strain JND-RSIb-21A with Accession number MW013547.1 which closely related (99.8%) with *Bacillus subtilis strain JND-RSIb-21A and Proteus sp.* strain AMJ131 with Accession number MW015095.1 which closely related (99.8%) to *Proteus mirabilis* strain AMJ131.

Conflict of Interest: we declare that there is conflict of interest

Ethical Approval: the research approved by scientific and ethical committee at our department

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