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Relationship of nanobacterium Cupriavidus gilardii with formation of kidney stones

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Abstract:

Fifty five kidney stones were gathered from 50 patients in Al-Sader clinical city in Al-Najaf territories. Kidney stone killed by (ESWL), (PCNL) or open an operation .The normal size of stones was under 0.5 cm and with normal load of 0.7 g . The PCR and sequencing results showed that the nanobacteria we refined is 80% like Nanobacterium sp. nano P 16S ribosomal RNA quality. This is a pioneer study,the first review in IRAQ that is secluded nanobacteria from kidney stone and show the connection among nanobacteria and kidney stone infection.

Keyword: nanobacterium, cupriavidus gilardii, kidney stones.

Introduction:

Nanobacteria are the littlest cell-walled microscopic organisms, found in human and cow blood and in business cell culture serum. metabolic speeds of Nanobacteria are extraordinarily lazy, they can make carbonate apatite on their cell envelope mineralizing rapidly a large portion of the accessible calcium and phosphate (National Research Council ., 1999) .
A few reports on clinical preliminary and serological recognition of Nanobacteria in neurotic material, principally the calcified tissues (aneurysms, carotid plaques, femoral blood vessel plaques, and cardiovascular valves) related with atherosclerosis. There are a few signs that ultrasmall microorganisms can cause or go with urinary contamination, periodontosis, and even malignant growth advancement (Miller et al., 2004 ; Laskin et al .,2005)

A few speculations have been advanced to clarify the etiology of nephrolithiasis however none has had the option to respond to completely the inquiries concerning the instrument of renal calculi arrangement. the known component of stone arrangement is the ensuing strategies like pee supersaturation, gem nucleation and collection, achieving maintenance of gems (nidi) and proceeded with development on the held crystals(Jeong et al., 2007)

The development of kidney stones could be prompted after intrarenal infusion or contamination with Nanobacteria (Ansari et al., 2017) . It has as such been suggested that the biogenic apatite layer present on the cell surface may go presumably as a nidus moving the course of crystallization and improvement of calcified stores (Hu delist et al., 2004).

Bio mineralization alludes to the cycles by which organic entities structure minerals, additionally depict as portrays the testimony of mineral inside or outside the cells of living creatures (Boskey ., 2003).

It field that ranges both the inorganic and the natural world. Albeit by far most of living beings don't frame mineralized stores, the wonder is still very wide spread , All five realms contain

individuals that mineralize. These organic entities are fit for framing approximately 60 distinct minerals , calcium is the cation of decision for most living beings.

The calciumbearing minerals include around half of known biominerals (Lowenstam and Weiner, 1989). Kidney stones are mineral stores in the renal calyces and pelvis that are discovered free or associated with the renal papillae . They contain clear and normal parts , Stone improvement is significantly normal with speeds of up to 14.8% and growing over the span of late years. , and a recurrent speed of up to half inside the underlying 5 years of the basic stone scene (Khan et al., 2016).

The aim of study:

Isolation and identification of Nanobacteria (cupriavidus gilarrdii)from kidney stones.

Materials and methods:

Materials

Polymerase chain reaction materials :

1: PCR master mix :According to Maxime PCR PreMix kit (i-Taq).

2: Molecular weight DNA marker :According to KAPA Universal Ladder kits .

3:DNA extraction from Nanobacteria :According to Protocol of G- spin DNA extraction .

4. :Agarose gel electrophoresis of DNA .

Preparation of the Agarose gel :According to Sambrook et al (1989) .

The primer used in the study :

The primer was investigated by IDT (Integrated DNA Technologies company, Canada).

Forward: 5'- AGAGTTTGATCCTGGCTCAG- 3'

Reverse: 5'- GGTTACCTTGTTACGACTT- 3’

Product size
1485 base pair

Molecular detection of NB using PCR

PCR PreMix Kit (Table 3-1) is the item what is blended each part: I-Taq DNA Polymerase, dNTP combination, response cradle (Table 3-2) . Do PCR simply add a layout DNA, groundwork set, and D.W (Table 3-3). The subsequent explanation is that it has Gel stacking cradle to do electrophoresis, so we can do gel stacking with practically no treatment.

Table(3-1): The Components of the Maxime PCR PreMix kit (i-Taq)

Material	Concetration
5U/ µl	i-Taq DNA Polymerase
2.5mM	DNTPs
1X	Reaction buffer (10X) Gel loading buffer

Table(3-2): Mixture of the specific interaction for diagnosis gene

Components	Concentration
Taq PCR PreMix	5µL
Forward primer	1.5 µL (10 picomols/ µL)
Reverse primer	1.5µL (10 picomols/ µL)
DNA	5 µL
Distill water	UP TO 20 µL

Table(3- 3): The optimum condition of detection gene

No.	Phase	Tm (°C)	Time	No. of cycle
1 cycle	3 min	95◦C	Initial Denaturation	1
40 cycle	45sec	95◦C	Denaturation - 2	2
	45sec	52◦C	Annealing	3
	1.5min	72◦C	Extension-1	4
1 cycle	10 min.	72◦C	Final Extension	5

DNA Sequencing and Sequence Alignmen

Sequencing of gene was performed by national instrumentation center for environmental management (nicem) online at (http://nicem.snu.ac.kr/main/?en_skin=index.html), biotechnology lab, machine is DNA sequencer 3730XL, Applied Biosystem), Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www.ncbi.nlm.nih.gov) and BioEdit program.

RESULTS:-

55 kidney stones were gathered from 50 patients in Al-Sader clinical city in Al-Najaf areas. Kidney stone eliminated by extracorporeal shockwave lithotripsy (ESWL), Percutaneous

nephron lithotomy (P C N L) or open an medical procedure .The normal size of stones was under 0.5 cm and with normal load of 0.7 g .

The PCR and sequencing results showed that the band of nanobacteria is show up on 1485 bp

Initial step to guarantee that DNA isn't divided .we use electrophoresis and the outcome allude to that DNA was complet and not divided . To guarantee of the presence of DNA in examples , after extraction we tried it by Biophotometer and the aftereffect of focus between 130 ug\ml to 378 ug\ml , while the purity is between 1.59 to 1.86 (OD 260\280).

Figure (4-1): Gel electrophoresis of genomic DNA extraction from *Nanobacteria*, 1% agarose gel at 5 vol /cm for 1:15 hour

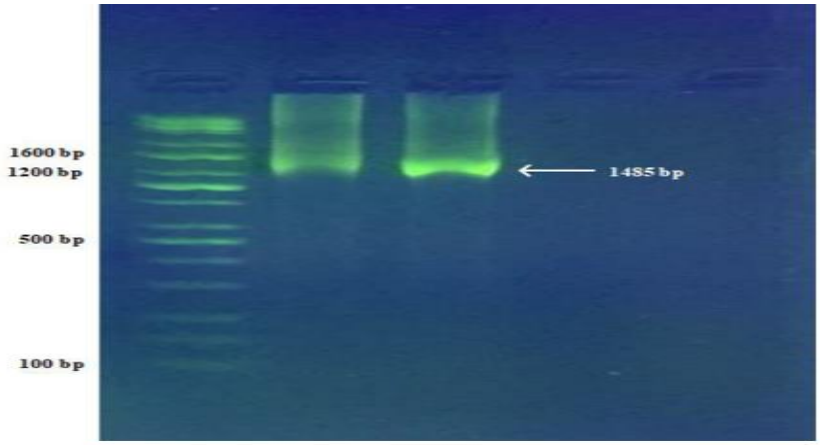

Figure (4-2): PCR product the band size 1485 bp. The product was electrophoresis on 2% agarose at 5 volt/cm. 1x TBE buffer for 1:30 hours. M: DNA ladder (100-10000).

Sequencing

The sequences producing significant alignments: 80 % identical with nanobacterium sp. Nano p 16 s rRNA **(Figure 4-4 and 4-5).**

The partial seguencing is :

AACGAAGGCGGCTGCAGGCTTAACACATGCAGTCGAACGGCCCACCAGGGGGTTGCAGACGGGTT
GGTAAGTGGGGGAAAGATAGCCTAAGCTCCGAATGTGCGCGTGCGAGATCGATAACTCCGGGAAA
CTGCAATTCATACCGCATACGAGCTACGGGGGAGAGACTGGGACCTCGGGGACTAGGATATGACCA
TGGGTTGGATTAGCTAGTTCGTGATGTTAAGGCCTACCAAAGCCACGATCCATATCTGTTCTGAGAG

GATGATGAGCCACTTGTGGAACTGAAACTCGGTCCAAACCCCTACGGGAGGCGGCGGTGGGGAAG
ATTGGAAAATGGGGGCATGAGCCTAATCCAGCCATACCGCTTGCGTGATTAAGGTCATAGGGTTGT
GAAGCTCTTTACATCGTGAGAAGATAATGAGGAATTCGGAGAAGAGGACCAGGCTAACTTGGTGCC
ATCAGCCGTGGAAATAGAACGGGGCTAGCGTTGTTCGGAATTTCTGGGCGTAAGCGCACGTAGGTG
GATATTTAAGTGAGGGTAAAGGTTCCAGAGCTTAACTCTGGAACACCATTGAATTACTGGGTATCTT
GGGTATGGAAAAGGTAAGTGGAATTCCGAGTTTAGGGGTGGAATCCGGGATATCCGGGGGGCATA
ACTACCAGGGGCGAAGCGGCTTACTGGGGATTGCAATTTACTCGTGACACATTTATGAGGTGCGTTA
AAGGGGGGGGAACAAACAGGATTAGATATCGTTGTAGTTCCCCCCCCTAAACGATGAATTTTTCCTT
CGGGCAGTTTACTGTTGGGGCGCAGCAGGCATTAAACCTCCCCCCGGGGGAGTACCATCCAAAATA
AAAACTCAAAGGAATTGACGGGGGTCCGCACCAGGGGTGGAGAATGTTGTTTAATTCTAAGCAACG
CGCAGAAACTTACCAGCTCTTTACATTCGGGTTATGCGCGGGTGGAGAACGATGTCCTTTCATTAGG
CTGTCCACAGAACAGGTGCTGCATGGCGGTCGTCAGCTCCTGTCATTAGATTTTAGGTTAAGTCCCGC
AACGACCGCCCCCCCCCCTTAGTTACCCGCGTTGAGTTGAAGGCACTTTAACGCGACGTTTTTTTTGC
GGCCGGTGATACACCCGCCCAGAAGATGGGGGGGATGTCGTCAATTTCTCCTGGCCCCACTTACAAT
TGTTTTGCTAGGCTACAACGAGACGTGTTAATCTATGGTGATTACAGAGGAAGCGAGACTGCGCTGT
CGAGCTAACTCTCCAAAAGCAATCTCAGATCGAATTGCGCTCTGCAACACAAGTGCATGAGAGTTCG
AATCGCTAGTTACCGCAATCAGCATGGTGAGGTGAATCCCTTCCCGGGCCCTCTGCACACCGCACAT
CATACCAGGGGAGTCGGTTTTAACCCGAAGGTAGTGCGCTAAACGCAAGGAGGAAGCTAACCGCCA
CGGGTAGGGGCAGCGACTGAGGTG

Length = 1485 base pairs
Molecular Weight = 449932.00 Daltons, single stranded
Molecular Weight = 903269.00 Daltons, double stranded
G+C content = 51.99%
A+T content = 48.01%
Nucleotide compositions of *nanobacterium* as showed in (figure 4-28).

Nucleotide	**Number**	**Mol%**
A	378	25.45
C	330	22.22
G	442	29.76
T	335	22.56

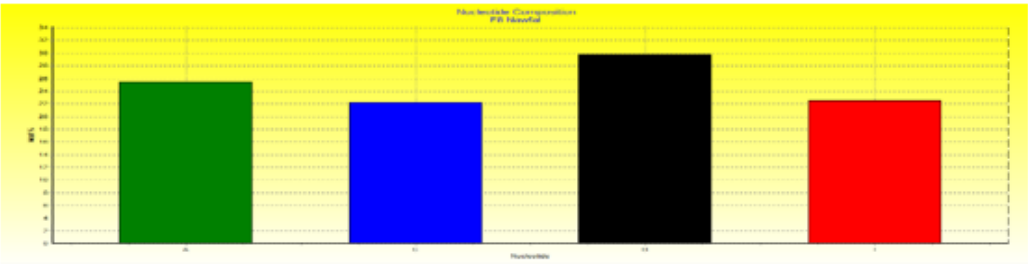

Figure (4-3): Nucleotide compositions of *nanobacterium* ,which is show that : A 378 , C 330 , G 442 and T 335

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☑	Nanobacterium sp. nanoP 16S ribosomal RNA gene, partial sequence	1207	1207	100%	0.0	80%	JN029830.1
☐	Agrobacterium tumefaciens strain S-188E 16S ribosomal RNA gene, partial sequence	1198	1198	100%	0.0	79%	JF513176.1
☐	Agrobacterium sp. strain CIP 107444 16S ribosomal RNA gene, partial sequence	1195	1195	100%	0.0	79%	MF443190.1
☐	Uncultured bacterium clone OTU48 16S ribosomal RNA gene, partial sequence	1195	1195	100%	0.0	79%	KP975304.1

figure (4-4): the sequences producing significant alignments: 80% Identical with nanobacterium sp. Nano p 16 s rRNA.

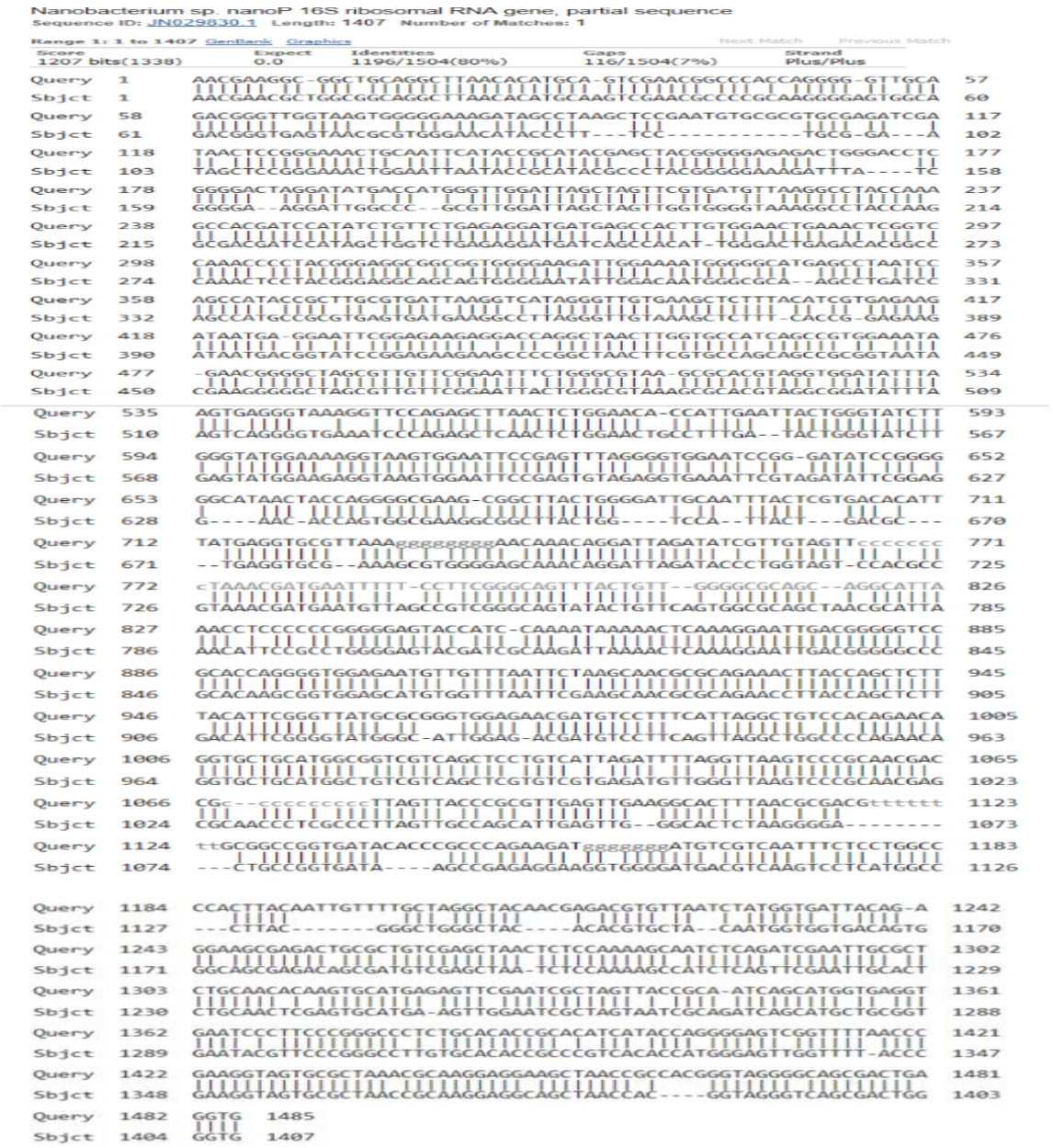

Figure (4-5): the partial nucleotide sequence of 16 sRNA NB .Which show 80% similarity with *nanobactreium sp* .

Discussion:

PCR results show the 1485 bp portion . in this review we have severe strategies of PCR. Nothing could be found in the negative benchmark group and there was no microscopic organisms. We support the aftereffects of PCR. Subsequently, the expansion in exact new 16S rRNA groupings and the advancement of elective gees for sub-atomic recognizable proof of certain taxa ought to additionally work on the handiness of sub-atomic ID of NB. The 16S rRNA sequencing gives unambiguous information even to uncommon separates, which are reproducible in and between labs.

16S rRNA arrangements homology investigation upholds the view that biomineralization was presence of NB distinctive strain in the diverse tissue .While most writers discovered NB in kidney stones, Drancourt neglected to separate NB in refined material from 10 models, though recognized nanoparticles in material separated by SEM (Drancourt et al., 2003). This blunder between results gained by SEM direct examination of renal stones and culture motivation is an enchanting one. When in doubt, most reports have shown that examination by SEM is more capable than culture to perceive NB . One request rises out of this finding: in the event that NB are precursors of renal stones, as affirmed by various specialists, why culture is a less useful area methodology? A single possibility is that for start creating NB it would be fundamental a base beginning number of particles which would not occur in all stones (Simonetti et al., 2012),Kumon found NB in around 60%of the urinary stone models among Japanese and Paraguayan patients(Kumon et al., 2011) .

Nucleic corrosive examination on NB has numerous issues, e.g., nucleic corrosive extraction is troublesome because of apatite and separated DNA-like material has hindered the intensification of exogenous bacterial DNA in PCR techniques. More exertion ought to be made for the portrayal of NB(Kajander et al., 2003).Conclusion: -All type of kidney stone contine nanobacteria.

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