IMPORTANCE OF THE BIOINFORMATICS TOOL IN THE CONFIRMATION OF BACTERIAL SPECIES ISOLATED FROM RAW MILK : CASE OF *Stenotrophomonas spp.*

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Résumé

La présente étude a concerné la caractérisation biomoléculaire et bioinformatique des bactéries psychrotrophes résistantes à lacéphazoline, en particulier *Stenotrophomonasspp.*Les échantillons de laits crus (N=64) de 30 vaches ont été récoltés à Biskra, au Sud-est d'Algérie. L'ensemble des caractères testés a permis l'identification présomptive de *Stenotrophomonasspp.* La séquence de l'ARNr 16S a été utilisée par l'outil bioinformatiqueBlastN du portail NCBI. Les résultats de l'alignement avec les séquences ARN 16S de GenBank ont confirmé l'espèce : il s'agit de *Stenotrophomonasmaltophilia*.

Mots clés :*Stenotrophomonasmaltophilia, BlastN, ARN 16S.*

Abstract

The present study concerned the biomolecular and bioinformatic characterization of psychrotrophic bacteria resistant to cephazolin, in particular *Stenotrophomonas spp*.The raw milk samples (N = 64) of 30 cows were collected in Biskra, in the South-East of Algeria. All the characteristics tested allowed the presumptive identification of *Stenotrophomonas spp*. The sequence of the 16S rRNA was used on the BlastN bioinformatics tool of the NCBI portal. The results of the alignment with GenBank 16S RNA sequences confirmed the species: it is *Stenotrophomonasmaltophilia*.

Keywords:*Stenotrophomonasmaltophilia, BlastN, 16S RNA.*

ملخص

خصت هذه الدراسة التشخيص البيولوجي الجزيئي و المعلوماتي لبعض السلالات البكتيرية التي تنتمي إلى النوع Stenotrophomonas المحبة للبرودة و المقاومة للمضاد الحيوي cephazolin . قمنا بجمع 64 عينة حليب من 30 بقرة في ولاية بسكرة بالجنوب الشرقي للجزائر. سمحت كل الصفات المختبرة بتحديد أولي مزعوم لبكتيريا *Stenotrophomonasssp* . وقد قارنا سلاسل 16S RNA مع السلاسل المتواجدة في قاعدة المعطيات GenBank التي تأويها بوابة NCBI .بعد استعمال البرنامج المعلوملتيBlastN ، أكدت نتائج مقارنة السلاسل اليوكليوتيديةRNA 16S أن البكتيريا المعزولة هي *Stenotrophomonasmaltophilia* .

الكلمات المفتاحية :*Stenotrophomonasmaltophilia, BlastN, 16S RNA*

Bacterial identification is a very sensitive step in microbiological investigations. The simple search for germs on common culture media does not allowaccurate and reliable identification; sobimolecularmethods are used which are more precise. Indeed, molecular biology has become an extremely large and complex field due to the advancement and expansion of genetic engineering techniques and the amount of gene information contained in the genomes of the different microorganisms.

The simple phenotypic identification, based on simple morphological tests (appearance of colonies, cellular forms, etc.), biochemical (presence of enzymes, carbohydrate metabolism, amino acids, etc.) and physiological (respiratory type, pH, growth temperatures, tolerance of NaCl, etc.), is no longer sufficient to confirm the belonging of any germ to a given species. This is due to the fact that the similarities of the phenotypic results and their interpretation can be confusing and lead to a false confirmation of the species [4].

Nowadays, the bioinformatics tool takes a considerable place in the analysis of the results and especially in the prediction of the structures and the gene functions. Among the bioinformatics techniques used for the identification and classification of bacterial species, the alignment of nucleic (and / or protein) sequences is of paramount importance because it makes it possible to compare sequences by relying on reliable computer algorithms and A considerable source of information represented by biological databases.

Concerning our case, we aligned the 16S RNA sequence of our strain with those of GenBank using the BlastN (Basic Local Alignment Search Tool) of the NCBI portal (http: //blast..ncbi.nlm .nih.gov /). This type of comparison saves a lot of time and manipulation and leads to more precise and therefore more reliable results because the only procedures are those of PCR amplification and sequencing, as opposed to phenotypic identification methods in which a wide range of morphological, biochemical and physiological tests are used, resulting in a considerable number of microbiological tests without the certainty of the systematic place occupied by the bacteria to be identified.

MATERIALS AND METHODS

Geographical location: The areas affected by the sampling are located in the Wilaya of Biskra (Table 1).

Table 1: Geographical location of dairy farms.

Location	Altitude (m)	Latitude	Longitude
Doucen	200	34°30’	5°3’
El Hadjeb	139	34°47’	5°36’
Sidi Okba	55	34°45’	5°54’

In all the farms visited, the feed of the animal was mainly made of silage which is reinforced by the food supplements. The cement lining of the soil and walls is often absent, and the cows are exposed directly to the soil where silage and fecal matter are mixed. Milking is done mechanically using milking machines to facilitate and speed up the process.

At each sampling, the first jets are eliminated and 25 mL (Official Journal of the Democratic and Popular Republic of Algeria) of raw milk are collected directly from the four udders of each cow and transferred to sterile bottles. Samples are transported to the laboratory at + 4 °C in a cooler. The number of samples taken is not constant at each sampling, as sick and pregnant cows have not been taken.

Media for the isolation and identification of bacteria. The media used are:

1. Nutritional agar supplemented with 5% of horse-baked blood: for reseeding and purification of strains. The blood is added when the agar medium is super cooled. The flasks are then taken to a water bath at 75-80 ° C for 10 minutes. We remark at this point that the chocolate agar could have been used; Except that we did not have pure hemoglobin to incorporate it in Mueller-Hinton medium.
2. Nutrient agar supplemented with 5% fresh horse blood: to test the β-hemolysis of strains *Listeria spp*. The addition of horse blood occurred at about 45-50 ° C.
3. Baked blood nutritive agar with added cefazolin at a concentration of 20mg/L: to promote the isolation of *Listeria spp*. Cefazolin is an antibiotic belonging to the family of cephalosporins of the first generation.
4. A series of so-called standard media has been used for biochemical and physiological characterization of isolated bacteria: TSI medium, Simmons citrate, nitrate broth, Esculin agar, Urea-Indole medium, 10-volume hydrogen peroxide (catalase), Medium Clark and Lubs, Mannitol-Mobility.

Volumes of 0.1 mL of raw milk (stock solution) and dilutions 1/2 and 1/4 were spread on the surface on the culture media:

− Nutrient agar without the addition of antibiotics for the search for total germs before preservation of the cold samples (4 °C),
− The nutritive agar with the blood of horse cooked with Cefazolin added,

The cultures were incubated for 48 h at 37 °C. All the isolates were subcultured for purification on a baked blood agar not supplemented with Cefazolin and then incubated for 48 h at 37 °C. After incubation, a series of phenotypic and genotypic identification tests were performed.

The phenotypic identification of the isolates was carried out using morphological, cultural and physio-biochemical

criteria. The tests and phenotypic identification tests performed were:

1. Examination of the macroscopic appearance of colonies
2. Gram staining
3.Mobility at 25 and 37 °C
4.Presence of catalase
5.Presence of oxidase
6. Methyl red (RM test)
7. Voges-Proskauer reaction (VP test)
8. Production of indole
9.Hydrolysis of esculin
10.Presence of urease
11.Reduction of nitrates
12. Production of H2S (TSI test)
13. Hemolytic activity
14.Antibiogram: Fusidic Acid, Tetracycline, Vancomycin, Gentamycin, Colistin, Erythromycin, Penicillin and Cefazolin

Molecular Study

The main steps required specific products.

A- Extraction of the genomic DNA: The bi-distilled water was used for thermal shocks.
B- Amplification of the 16S rRNA by PCR: Mixed solution containing the reagents mentioned in the following table:

Table 2: Composition of the mixed solution for the PCR.

Products	Volumes (1 tube)	Volumes (6 tubes)
H_20 bi-distilledsterile	4,6 µl	27,6 µl
Tampon taq x 5 fois	4 µl	24 µl
dNTP (1mM)	4 µl	24 µl
Mg Cl_2 (25mM)	1,2 µl	7,2 µl
fD1 (10µM)	2 µl	12 µl
Rs16 (10µM)	2 µl	12 µl
Taqpolymérase (5U /µl)	0,2 µl	1,2 µl
Genomic DNA	2 µl	12 µl
Final volume	20µl /tube	120µl / 6 tubes

The primers used for amplification correspond to the standard, that is to say a size comprised between 18 and 26 base pairs, a% GC not exceeding 60%, and they contain (at least, One of them) 3 A or T in the last five nucleotides:

–Forward primer (fD1): 20 bp,% GC = 50, **5'AGAGTTTGATCCTGGCTCAG3'**

– Reverse primer (Rs16): 22 bp,% GC = 45.5,**5'TACGGCTACCTTGTTACGACTT3'**

C- Electrophoresis of the DNA fragments: 1% agarose gel, loading solution (1ml containing: 2.5 mg of bromophenol blue, 300 µl of glycerol, qs 700 µl of sterile distilled water).

D- Sequencing Reaction: PCR products, dideoxynucleoside triphosphates (ddNTP) labeled with fluorochromes, polyacrylamide gel.

E-Purification of the sequencing products: Magnesil Green solution, 90% ethanol, Formamide, Magnesil Yellow solution, Magnesil Green, water-free nuclease solution.

F- Bioinformatic analysis of the sequences: the material used is a computer tool free of rights available on several portals and websites, in particular on NCBI. It offers the ability to compare (align) an unknown sequence against known sequences on the NCBI database. This database is called GenBank, the most widely consulted worldwide.

Sequencing is performed on previously purified PCR products. It is realized according to the automated technique of Sanger. This method consists of performing a specific amplification (PCR) in which dideoxynucleoside triphosphates (ddNTP) have been used which are generally labeled with fluorochromes. The incorporation of ddNTP by the DNA polymerase blocks the elongation of the DNA molecule being copied. We thus obtain a family of DNA fragments synthesized with different lengths.

These nucleic fragments are separated by electrophoresis according to their length. The smaller migrate faster. The discriminating power of the polyacrylamide gel is such that it makes it possible to differentiate strands of DNA which differ in length only by a single nucleotide.

The sequence of the amplified DNA is then read by an automatic scan which makes it possible to distinguish, thanks to different fluorochromes, the four bases A, T, C or G. The use of computer software makes it possible to provide an electrophoretic trace with different colors for each elementary base.

Bioinformatic analysis

The bioinformatic analysis of the 16S RNA sequences was carried out on the NCBI site in order to align our 16S RNA sequences with those of the nucleic library (GenBank in this case). These are the sequences of strains grown on agar supplemented with Cefazolin.

BlastN, a local alignment tool of the NCBI, is a royalty-free online program to search for similar or even identical sequences in a sequence library from a query nucleic sequence. The researchers use molecular markers such as 16S RNA sequences, or microsatellites to confirm the identification and classification of their species [4].

After aligning each of our 16S rRNA sequences with the BlastN tool, only the GenBank sequences having a similarity (with our sequence) greater than or equal to 99% and a zero or almost zero E-value were chosen because the molecular definition of the genus Stipulates that the homologies of the 16S rDNA sequences must be greater

than or equal to 97%. A homology greater than or equal to 99% translates the belonging to the same species. Whereas a homology score of less than 97% does not allow identification. In the case where several sequences are proposed by the bank, and which have the same E-value and the same percentage of identity, we have decided for the sequence having presented the best alignment score with our query sequence.

RESULTS AND DISCUSSION

The macroscopic appearance of the colonies of *Stenotrophomonas spp*. on nutrient agar showed orange yellow colonies, large (3 to 4 mm), round with regular contours, flat, viscous and glossy. Gram staining revealed isolated bacterial cells of bacillary form. The results of the tests used for the pre-identification are shown in Table 3.

Table 3: Preliminary identification tests for *Stenotrophomonas spp.*

Morphology	Mobility	-
	Gram	+
Physiology	Oxidase	+
	Catalase	+
Biochemistry	Lactose	+
	Saccharose	-
	Mannitol	+
	Gaz	-
	H_2S	-
	Indole	-

Stenotrophomonasspp was found with an incidence of 2/64 or a frequency of 2.94% (Table 4). The remainder of the bacterial flora resistant to cefazolin consists of *Acinetobacter spp., Alcaligenes spp., Enterococcus spp., Flavobacterium spp., Pseudomonas spp., Staphylococcus spp., Bacillus spp., Yersinia spp. and Listeria spp.*

Table 4: Frequencies of 68 samples of raw milk

Bacteria	Numbers	%
Stenotrophomonas	2	2,94
Acinetobacter	6	8,82
Alcaligenes	1	1,47
Enterococcus	11	16,18
Flavobacterium	1	1,47
Pseudomonas	2	2,94
Staphylococcus	6	8,82
Bacillus	2	2,94
Yersinia	1	1,47
Listeria	3	4,41

We note a high incidence of *Enterococcus spp.* compared to the rest of the bacteria. *Stenotrophomonas spp.* was found in two cases out of 68 samples analyzed.A distribution of 2.94%. The presence of suspected pathogens such as *Listeria* and *Staphylococcus* in raw milk from cows has been reported by many authors [1, 2, 3]. The on-site milking procedure could induce contamination and the lack of hygiene that we have noted can increase the microbial contamination of the milk. Microorganisms, especially pathogens, can contaminate milk by dirty udders, suckers, and equipment used for milking [5, 6]. Other authors associate the presence of pathogenic germs with the nature of the geographical site and the season [7, 9].

16S RNA sequencing involved strains of *Enterococcus spp., Acinetobacter spp., Pseudomonas spp., Bacillus spp. and Stenotrophomonas spp.*

The sequences obtained are aligned one by one with the BlastN tool to compare them with the nucleic sequences already present on GenBank. The result of the alignments led to the following bacterial identifications (Table 5):

Table 5: 16S DNA Sequences

>***Enterococcus 682 pb***
TTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGT
GTACAAGGCCCGGGAACGTATTCACCGCGGCGTGCTGA
TCCGCGATTACTAGCGATTCCGGCTTCATGCAGGCGAGT
TGCAGCCTGCAATCCGAACTGAGAGAAGCTTTAAGAGA
TTTGCATGACCTCGCGGTCTAGCGACTCGTTGTACTTCCC
ATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATG
ATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACC
GGCAGTCTCGCTAGAGTGCCCAACTAAATGATGGCAACT
AACAATAAGGGTTGCGCTCGTTGCGGGACTTAACCCAAC
ATCTCACGACACGAGCTGACGACAACCATGCACCACCT
GTCACTTTGTCCCCGAAGGGAAAGCTCTATCTCTAGAGT
GGTCAAAGGATGTCAAGACCTGGTAAGGTTCTTCGCGTT
GCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGC
CCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACT
CCCCAGGCGGAGTGCTTAGTGCGTTTGCTGCAGCACTGA
AGGGCGGAACCCTCCAACACTTAGCACTCATCGTTTACG
GCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCA
CGCTTTCGAGCCTCAGCGTC

>***Acinetobacter 617 pb***
GACWCCTACTTCTGGTGCAACAAACTCCCATGGTGTGA
CGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGC
GGCATTCTGATCCGCGATTACTAGCGATTCCGACTTCAT
GGAGTCGAGTTGCAGACTCCAATCCGGACTACGATCGG
CTTTTTGAGATTAGCATCCTATCGCTAGGTAGCAACCCT
TTGTACCGACCATTGTAGCACGTGTGTAGCCCTGGCCGT
AAGGGCCATGATGACTTGACGTCGTCCCCGCCTTCCTCC
AGTTTGTCACTGGCAGTATCCTTAAAGTTCCCGACATTA
CTCGCTGGCAAATAAGGAAAAGGGTTGCGCTCGTTGCG
GGACTTAACCCAACATCTCACGACACGAGCTGACGACA
GCCATGCAGCACCTGTATGTAAGTTCCCGAAGGCACCAA
TCCATCTCTGGAAAGTTCTTACTATGTCAAGGCCAGGTA
AGGTTCTTCGCGTTGCATCGAATTAAACCACATGCTCCA
CCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTTTAGT
CTTGCGACCGTACTCCCCAGGCGGTCTACTTATCGCGTT
AGCTGCGCCACTAAAGCCTCAAAGGCCCCAACGGCT

>***Pseudomonas 624 pb***
GGWGAAGGGAGCTTGCTCCTGGATTCGCGGCGGACGGG
TGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGATA
ACGTCCGGAAACGGGCGCTAATACCGCATACGTCCTGA

GGGAGAAAGTGGGGGATCTTCGGACCTCACGCTATCAG
ATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAAAG
GCCTACCAAGGCGACGATCCGTAACTGGTCTGAGAGGA
TGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCC
TACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCG
AAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGT
CTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGC
AGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGA
ATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAAT
ACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTA
AAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGTGAAAT
CCCCGGGCTCAACCTGGGAACTGCATCCAAAACTACTGA
GCTAGAGTACGGTAGAGGGTGGTGGAATTTCCTGTGTAG
CGGTGAAATGC

>***Bacillus 66 pb***

GGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGT
AACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCC
GGGAAACCGGGGCTAATACCGGATGCTTGTTTGAACCG
CATGGTTCAAACATAAAAGGTGGCTTCGGCTACCACTTA
CAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTA
A**Y**GGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGA
GGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGA
CTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG
GACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGA
AGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAAC
AAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTA
ACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCG
GTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGG
CGTAAAGGGCTCGCAGGCGGTTCCTTAAGTCTGATGTGA
AAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTG
GGGAACTTGAGTGCAGAAGAGGA

>***Stenotrophomonas 789 pb***

CTGATCTGCGATTACTAGCGATTCCGACTTCATGGAGTC
GAGTTGCAGACTCCAATCCGGAGTGAGATAGGGTTTCTG
GGATTGGCTTGCCCTCGCGGGTTTGCAGCCCTCTGTCCC
TACCATTGTAGTACGTGTGTAGCCCTGGTCGTAAGGGCC
ATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTC
ACCGGCGGTCTCCTTAGAGTTCCCACCATTACGTGCTGG
CAACTAAGGACAAGGGTTGCGCTCGTTGCGGGACTTAA
CCCAACATCTCACGACACGAGCTGACGACAGCCATGCA
GCACCTGTGTTCGAGTTCCCGAAGGCACCAATCCATCTC
TGGAAAGTTCTCGACATGTCAAGACCAGGTAAGGTTCTT
CGCGTTGCATCGAATTAAACCACATACTCCACCGCTTGT
GCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGA
CCGTACTCCCCAGGCGGCGAACTTAACGCGTTAGCTTCG
ATACTGCGTGCCAAATTGCACCCAACATCCAGTTCGCAT
CGTTTAGGGCGTGGACTACCAGGGTATCTAATCCTGTTT
GCTCCCCACGCTTTCGTGCCTCAGTGTCAGTGTTGGTCC
AGGTAGCTGCCTTCGCCATGGATGTTCCTCCCGATCTCT
ACGCATTTCACTGCTACACCGGGAATTCCACTACCCTCT
ACCACACTCTAGTCGCCCAGTATCCACTGCAATTCCCAG
GTTGAGCCCAGGGCTTTCACAACAGACTTAAACAACCAC
CTACGCACGC

W= A ou T, Y= T ou C

The score of each alignment represents the number of times that there is match of the nucleotides (Match) between our sequence and that of GenBank. The higher the value of the score, the higher the identity (% identity) of the two aligned sequences and in some cases reaching 100%. For our case, the five strains were identified as a result of BlastN's alignment and the percentages of identity are all greater than 97%; which allows us to confirm the species proposed by the result of BlastN. The use of the bioinformatics tool made it possible to complete the phenotypic identification which was not then complete given the number of characters analyzed (n = 10). This number remains very insufficient to pronounce on any identification or classification even at the taxonomic level of the genus. On the other hand, the BlastN tool, based on a comparative alignment of the 16S RNA sequences, confirmed the species. It is even possible to construct a phylogenetic construction according to a phylogram to represent the genetic kinship ties between species identified using construction algorithms such as Neighbor Joining [8].

The construction of the phylogram passes through a multiple alignment of the sequences and the computation of the matrix of the distances (Table 6). The phylogram of the five strains is constructed according to the Neighbor Joining method (Figure 1).

Table 6: Results of BlastN alignments with GenBank sequences

	Score	E-value	identity
Enterococcusfaecalis strain JCM 5803	1223	0	99%
Acinetobactercalcoaceticus NCCB 22016	1131	0	100%
Pseudomonas aeruginosa strain DSM 50071	1074	0	98%
Bacillus mojavensis strain IFO15718	1105	0	99%
Stenotrophomonasmaltophilia	1452	0	99%

The matrix revealed that the smallest distance was observed between *StenotrophomonasMaltophilia* and *Acinetobactercalcoaceticus* (Table 7). There was a 12.3% difference between the nucleotides of the two 16S RNAs. This value would express the rate of mutations between the two DNA sequences leading to two different but similar or even homologous 16S RNAs.

Table 7: Distance matrix according to the multiple sequence alignment

	E. faecalis	*A. calcoaceticus*	*P. aeruginosa*	*B. mojavensis*
A. calcoaceticus	0,188			
P. aeruginosa	0,577	0,585		
B.mojavensis	0,601	0,593	0,270	
S. maltophilia	0,187	0,123	0,579	0,597

However, the most distant germs, compared to the primary structure of their 16S RNAs, are *Enterococcus faecalis* and *Bacillus mojavensis* (60.1%). The topology of the phylogenetic tree confirms the strong similarity between the 16S RNAs of A. *Calcoaceticus* and *S. Maltophilia*, because the clade formed by these two bacteria returns in 99% of the cases (bootstrap value); which would express the existence of a homology between these two sequences.

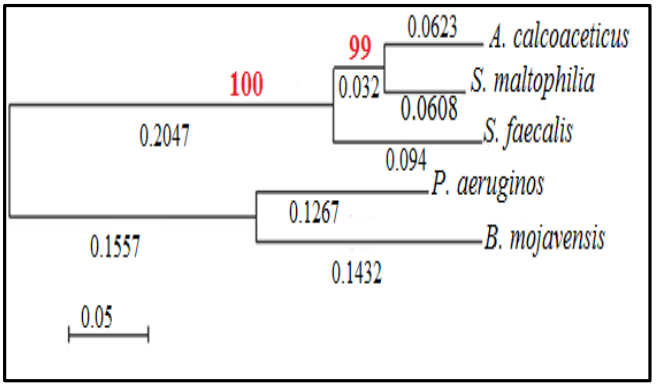

Figure 1: Phylogram of the five species according to the Neighbor Joining algorithm.

CONCLUSION

We have been able to highlight the importance of bioinformatics tools in the identification and classification of bacteria. Bimolecular methods, including 16S RNA sequencing, provide more accurate systematic information compared to conventional bacterial identification methods that rely on mostly imprecise phenotypic tests, leading to a misinterpretation of the results resulting in an erroneous identification.

The identification of bacteria such as *StenotrophomonasMaltophilia* requires from the microbiologist various and specific crop media and a greater number of microbiological tests. Using the 16S sequence of this bacterium allowed us to confirm its species by simple alignment with the GenBank sequences using the BlastN tool.

The bioinformatics tool allows for more intensive and interesting analyzes such as gene annotation and the search for consensus sequences. The latter is a bioinformatic step necessary to confirm a common molecular signature within a set of nucleic sequences. It would be better to use these methods for better bacterial identification.

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