Molecular Identification of *Geobacillus* WCH 70 Isolate according to Nitrate reductase gene sequence

R. K. Mohammed
College of Sciences/ University of Baghdad

Abstract

This study aimed to provide the molecular identity of *Geobacillus* WCH70 according to Nitrate reductase gene. DNA was extracted the purity was 1.8 ng\ µl, then Nitrate reductase (NR) gene was amplified by a specific primer pairs, the results showed that the size of NR gene was 1626 bp after use a 1% gel electrophoresis. Then Nitrate reductase gene was sequenced according to the chain termination method. The sequence results were showed the nucleotide similarity 100% percentage of identical nitrate reductase *Geobacillus* WCH70, on the other hand the sequence similarity was ranged between 77% and 88% with sequence of other microorganisms.

Key Words: Nitrate reductases NR, *Geobacillus*WCH 70
e-mail: phd_rana@yahoo.com

التحديد الجزيئي للعزلة المحلية *Geobacillus* WCH 70 بالاعتماد على التتابع الجيني لجين النايتريت ريدكتيز

رنا كاظم محمد
كلية العلوم/ جامعة بغداد

الخلاصة

هدفت هذه الدراسة الى التشخيص الوراثي للعزلة المحلية *Geobacillus* WCH70 بالاعتماد على وجود جين النايتريتريت ريديكتيز. استخلص الدنا بنقاوة 1.8 نانوغرام/ مايكروليتر، ثم تمت مضاعفة الجين المسؤول عن انتاج النايتريت ريديكتيز باستخدام زوج من البوادىء المتخصصة، لوحظ من النتائج ان حجم جين النايتريتريت ريدكتيز كان 1626 bp بعد الترحيل الكهربائي على هلام الاكاروز تركيز 1% بعدها تم تحديد التتابع الجيني لجين النايتريتريدكتيز باستخدام طريقة ايقاف السلسلة اظهرت نتائج تحديد التشابه للقواعد النتروجينية للنموذج قيد الدراسة متطابق مع التتابع لجيني لجين النايتريتريت ريدكتيز للعزلة WCH70 بنسبة، 100% من ناحية اخرى كانت نسبة التشابه للنموذج قيد الدراسة مع الانواع البكتيرية الاخرى من حيت التشابه بالمحتوى الوراثي تتراوح بين 77-88%.

الكلمات المفتاحية: النايتريتريت ريدكتيز NR جيوباسلس WCH70.

Introduction

Nitrogen is a basic element for life because it is a component of the two preeminent biological macromolecules: proteins and nucleic acids. Nitrate reduction plays a key role in the nitrogen cycle and has important agricultural, environmental, and public health implications (1). As assimilatory nitrate reduction, performed by bacteria, fungi, algae, and higher plants (2, 3, 4). Some organisms have more than one type of nitrate reductase for example *E.coli* consists of three types of NRs. The availability of more than one type of NRs indicates the preference for anaerobic respiration. Thermophilic bacteria are described as aerobes although the low availability of oxygen in high temperature environments (5, 6). Nitrate reductase primary reaction is catalyzing nitrate to nitrite. Based on the electron donors the nitrate reductases are classed into three groups (7). Thermophilic organisms have a potential to produce thermostable enzymes with higher stability and longer life (8, 9). Using thermostable bacteria in industrial processes reduces the probability of contamination, increase diffusion rate and reduces

the cost of external cooling (10). Nitrite reductase is the key enzyme in the dissimilatoryde nitrification process. The reduction of nitrite to NO can be catalyzed by the products of two different nitrite reductase genes: one product contains copper (the *nir*K product), and the other contains cytochrome cd1 (the *nir*S product). The two genes seem to occur mutually exclusively in a given strain, but both types have been found in different strains of the same species (11, 12). Although structurally different, both enzyme types are functionally and physiologically equivalent. nirS is more widely distributed; nirK is found in only 30% of the denitrifiers studied so far. However, *nir*K is found in a wider range of physiological groups (13,14). Eukaryotic and prokaryotic assimilatory nitrate reductase share no sequence similarity and have little in common beyond their physiological function (15, 16). The aim of this study, we report on the application of new primer pairs for nitrite reductase genes to determine the NR gene of denitrifying *Geobacillus*WCH70 by amplifying and sequences NR fragments successfully, then detect the similarity and identity of query with other different populations of denitrifying bacteria.

Material and Methods

- **Bacterial Strain**: *Geobacillus* WCH 70 Isolate was provide by the Biotechnology department\ Collage of Science\ Baghdad University.
- **DNA Extraction**: *Geobacillus*WCH 70 chromosomal DNA was extracted and purified using a spin column kit (Clontech, Mountain View, CA, USA)(17).
- **Primer's**: Two PCR primers were designed and used to amplify Nitrate reductases of *Geobacillus* WCH 70 supplied by Integrated DNA Technology(IDT\ Canada). To obtained DNA fragment was 1626bp.

FW: 5'-ATGGCATACGAAAAAGTATGG-3'
RW: 5'-TTATGCTACCGTGCTGAC-3'

- **Amplify Nitrate reductases gene**: Amplification was preformed using automated thermal cycler (Amplitron II; Thermolyne' Dubuque' IA), using 0.5 µl pfu DNA polymerase (Stratagene), 1 µL 20 µM primer pairs of forward/reversward primers were added into PCR mixture, 1.5 µL of *Geobacillus* WCH70 chromosomal DNA was used as a template, 5 µL of 10 mMdeoxynucleotide triphosphates (dNTPs) Mix, 5 µL of 10X PCR reaction buffer, and 36 µL of sterile distilled water was added to complete the final volume to 50 µL. PCR cycle was programmed as follows: One cycle of 95 ºC for 3 min; 35 cycles of 95 ºC for 30 sec, 52 ºC for 30 sec, 72 ºC for 3 min; and final extension at 72 ºC for 5 min. The PCR products were detected by electrophoresis on a 0.8% agarose electrophoresis containing ethidium bromid run at a constant voltage (100 V) for 30 min, and visualized by using a Gel documentation systems (USA). Then the PCR product was purified by StrataPrep® PCR purification kit (Stratagene, Dedar Creek, TX, USA). (17,18).
- **Sequencing of Nitrate reductases gene**: Nitrate reductases gene was amplified from *Geobacillus*WCH70 and detected on agarose gel, then the complete sequenced was performed in the center of applied genomics institute\ Canada. (19) Sequence similarity of *Geobacillus*WCH70 to other Nitrate reductases from other organisms was detected using bioinformatic database in the National center for bioformation (NCBI\BLAST) on website WWW.NCBI.net. Where the accession number in NCBI CP001638.1

Results and Discussion

- **Amplified of Nitrate reductases gene**: Genomic DNA was extracted from G. WCH70 production by using DNeasy® tissue Kit (Qiagen, Valencia,CA, USA). Specific primers were used for amplification. Results indicated in Fig. (1) showed the amplified nitrate reduction gene fragment with a molecular size of about 1626

bp. while in the other studies the nitrate reduction from other bacteria such as *Thiosphaera pantotropha* consisted of cluster genes *napEDABC* encoding the periplasmic nitrate reductase, all five open reading frames have a codon usage and GC bias at the third position similar to that found in *P. denitrificans* genes (9, 20, 21), analysis of the 500 bp 3' to *napC* did not identify any possible open reading frame in this region, thus appears likely that the periplasmic nitrate reductase operon of *T. pantotropha* comprises the five genes *nap*EDABC. (22,23)

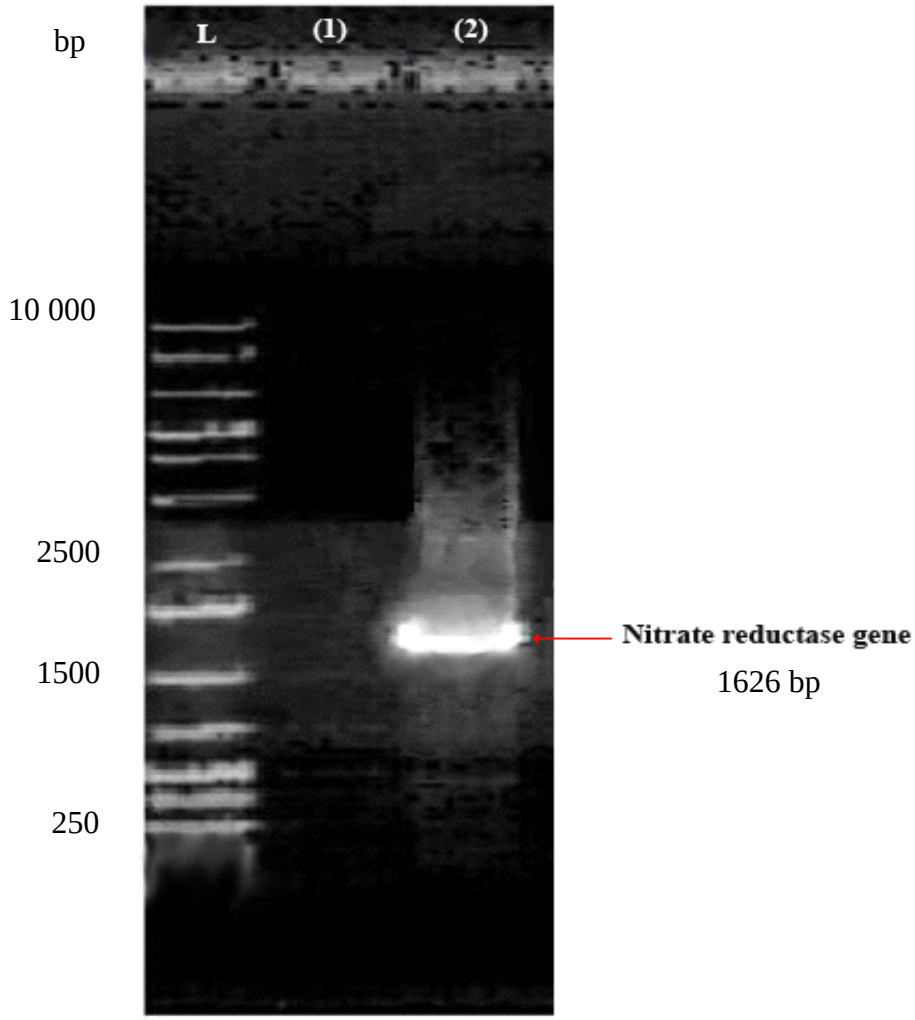

Fig. (1) *Geobacillus* WCH70 Nitrate reductases gene. Electrophoretically analyzed on agarose gel (0.8%) for 1 hour
Lane (1): Landmarker ladder (10000bp).
Lane (2): Nitrate reductases gene fragment.
This fragment was eluted, and sequencing.

- **Sequencing of nitrate reduction gene**: To determine *Geobacillus* sp. WCH70 Nitrate reductase gene sequence identity to sequences of other microorganism nitrate reductase, nitrate reductase gene was firstly sequenced and analysed to determine ORF. Results indicated in Fig. (2) showed the complete *G.* WCH 70 nitrate reductase gene of 1626 bp in length, rich in GC 44.7% codes for 541 amino acids, was detected in the center of applied genomics institute/ Canada. Sequencing was achieved according to chain termination method(22), query results of nitrate reductase gene was 100% identical to the nucleotide sequence and deduced amino acids of *Geobacillus* sp. WCH70 nitrate reductase gene covering 100% DNA region of gene as mentioned in Fig. (3). From the results it was found that the degree of similarity between the nucleotide sequence of nitrate reductase gene (query) to the *Geobacillus* WCH70 was 100% identical.

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1    atggcatacgaaaaagtatgggcagataatccgaaattaaacaaaatggaattaaaaaag  60
      M  A  Y  E  K  V  W  A  D  N  P  K  L  N  K  M  E  L  K  K
61   cttaaaaaagatggactaaaaatttttgaagatattccttattatgcaaagcatggattc 120
     L  K  K  D  G  L  K  I  F  E  D  I  P  Y  Y  A  K  H  G  F
121  tcttcgattccgcaagaagaatgggattatttcaaatgggcagggctttacctgcaacgc 180
     S  S  I  P  Q  E  E  W  D  Y  F  K  W  A  G  L  Y  L  Q  R
181  ccaaaagaagatggctattttatgatgcgcgtttgtgttccttccggaattttatctaac 240
     P  K  E  D  G  Y  F  M  M  R  V  C  V  P  S  G  I  L  S  N
241  gagcagctcgaaacgttagcaggcatcgcccgtgattacggacgcggtttgtttgacatt 300
     E  Q  L  E  T  L  A  G  I  A  R  D  Y  G  R  G  L  F  D  I
301  acaacccgtcaatctgttcaattccattggctgcgaatcgaacaaattcctgatattttt 360
     T  T  R  Q  S  V  Q  F  H  W  L  R  I  E  Q  I  P  D  I  F
361  gatcgtttaaaacgggttggattatctaccgctggagcttgcggcgatatcacccgcaac 420
     D  R  L  K  R  V  G  L  S  T  A  G  A  C  G  D  I  T  R  N
421  atcgtcggcaacccgcttgctggtatcgatccggacgaactgtttgacacgagagaaatc 480
     I  V  G  N  P  L  A  G  I  D  P  D  E  L  F  D  T  R  E  I
481  Gttcgtgaagtatatgagtttttccaacataacgaagacttctcgaacttgccgcgcaaa 540
     V  R  E  V  Y  E  F  F  Q  H  N  E  D  F  S  N  L  P  R  K
541  tataaaatatcgatcagtgccaacgtctacaacacggttaacgcagaaatccattgcttg 600
     Y  K  I  S  I  S  A  N  V  Y  N  T  V  N  A  E  I  H  C  L
601  tcgtttacaccggcgaaaaaagtgatcgacggcaaagaagtgctcggattccatgtgaaa 660
     S  F  T  P  A  K  K  V  I  D  G  K  E  V  L  G  F  H  V  K
661  Gtcggcggcggtttatcgtctgctccatatcttgcccaaacactggatgtgttcgtgacg 720
       V  G  GG  L  S  S  A  P  Y  L  A  Q  T  L  D  V  F  V  T
721  ccagatcgcgtgaaagacgtcgctgttgctgtcacaacgattttccgcgattacggctat 780
     P  D  R  V  K  D  V  A  V  A  V  T  T  I  F  R  D  Y  G  Y
781  Cgcgaaaaacgccatcgcgcccgtctcaaattccttgtcgctgactggggcgttgataaa 840
     R  E  K  R  H  R  A  R  L  K  F  L  V  A  D  W  G  V  D  K
841   tttaaagaaaaattaattgagctcacggggcctctcccatccaaaggggaagacgtacaa 900
     F  K  E  K  L  I  E  L  T  G  P  L  P  S  K  G  E  D  V  Q
901   aagggctggaaccctggttacttctacggcgtccatcgccaaaaacaagaaggattaaat 960
     K  G  W  N  P  G  Y  F  Y  G  V  H  R  Q  K  Q  E  G  L  N
961  ttcatcggctttaatgtgccagttggccgcttgagcgcggatgaagtattcgaaatcgcg 1020
     F  I  G  F  N  V  P  V  G  R  L  S  A  D  E  V  F  E  I  A
1021 cgcatagcccgccaatacggcaacggcgaagtccgcacatgcaattcacaaaacctaatt 1080
     R  I  A  R  Q  Y  G  N  G  E  V  R  T  C  N  S  Q  N  L  I
1081 attccaaatattccagataaatatgttgacgctgtattacaggaaaaagtgtttgaacgg 1140
     I  P  N  I  P  D  K  Y  V  D  A  V  L  Q  E  K  V  F  E  R
1141 atttctatccgtccgaacacattcaccggctattctgtttcttgtacaggcaccgagttt 1200
     I  S  I  R  P  N  T  F  T  G  Y  S  V  S  C  T  G  T  E  F
1201 tgcaatttggcgctcgtcgagacgaaagaacggatgcggcgcgtcgctgaatatttagat 1260
     C  N  L  A  L  V  E  T  K  E  R  M  R  R  V  A  E  Y  L  D
1261 cagcatttagaactggacgttccggtacgcattcatatggttggctgtccaaactcatgc 1320
     Q  H  L  E  L  D  V  P  V  R  I  H  M  V  G  C  P  N  S  C
1321 ggccaacggcaaattggagatatcggcctgcaaggaatcaaaacaaaaacaaaagacaga 1380
     G  Q  R  Q  I  G  D  I  G  L  Q  G  I  K  T  K  T  K  D  R
1381 ggcatgattgaagcgtttgaaatttacgtcggcggcacgctctacaacggcggcaaatac 1440
     G  M  I  E  A  F  E  I  Y  V  G  G  T  L  Y  N  G  G  K  Y
1441 aatcaaaaattaaaaggaaaagtagatgcggaacgaatttccgaagtgcttttgcagcta 1500
     N  Q  K  L  K  G  K  V  D  A  E  R  I  S  E  V  L  L  Q  L
1501 ttaacctatttcaaagaaacaaaattgccgggcgaaacattcttagcttacttagatcgc 1560
     L  T  Y  F  K  E  T  K  L  P  G  E  T  F  L  A  Y  L  D  R
1561 gttggaatagaagcgttgcaaacagaactagacagcattttagcgcaagtcagcacggta 1620
     V  G  I  E  A  L  Q  T  E  L  D  S  I  L  A  Q  V  S  T  V
1621 gcataa                                                      1626
      A  -
```

Fig. (2) Nucleotide sequence of open reading fram (ORF)Nitrate reductases gene from *Geobacillus* WCH70.

On the other hand results mentioned in Table (3) showed the G. WCH70 nitrate reductase gene sequence was less identical (77-88%) to other *Geobacillus* sp. (*Geobacillus thermoglucosidasius* strain DSM 2542, complete genome, *Geobacillus thermoglucosidasius* C56-YS93, complete genome, *Geobacillus* sp. Y4.1MC1, complete genome, *Anoxybacillus* sp. B7M1, complete genome).

Table (3)Nucleotide sequence alignment of *G.* WCH70 nitrate gene with related sequences of different microorganisms. NCBI / Blast (WWW.NCBI.net).

Description	Max identity	Query coverage
Geobacillus sp. WCH70, complete genome	100%	100%
Geobacillus thermoglucosidasius strain DSM 2542, complete genome	88%	100%
Geobacillus thermoglucosidasius C56-YS93, complete genome	88%	100%
Geobacillus sp. Y4.1MC1, complete genome	88%	100%
Anoxybacillus sp. B7M1, complete genome	77%	97%

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