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Research article

16S rRNA gene sequence of local isolate of new strain of *Bacillus* that produces alkaline phosphatase

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ABSTRACT

Twenty one bacterial isolates were obtained from different sources, 9, 9 and 3 isolates of *Bacillus* were isolated from different kinds of food, soil and water, respectively. They were identified according to morphological of their colonies and biochemical tests. The results showed that all of them were belong to the genus *Bacillus*. *Bacillus* FH4 isolate (isolated from potato) was determined as the highest producer of alkaline phosphatase (1.433 U/ml). *Bacillus* FH4 isolate was identified by 16s rRNA, the phylogenetic tree was done by MEGA5 program. The isolate was closet to *Bacillus lichenifrmis*, the bacterial strain was submitted to NCBI and specified as *Bacillus licheniformis* FH4. IRQ with accession number KF531930.

Keywords: Alkaline phosphatase, *Bacillus*, 16S rRNA .

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INTRODUCTION

Alkaline phosphatase (E.C.3.1.3.1) belongs to the class of hydrolases and acts on phosphate groups. This enzyme catalyzes the hydrolysis of almost every phosphomonoester to give inorganic phosphate and the corresponding alcohol, phenol or sugar, and also catalyzes transphosphorylation in presence of large concentration of phosphate acceptor [1]. In nature, alkaline phosphatases are found in many organisms, both prokaryotes and eukaryotes. The enzymes are produced by bacteria, fungi, are relatively abundant in fish and mammals although they are absent from higher plants [2]. Alkaline phosphatase found in different mi-

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microorganism such as; *Pseudomonas* [3], *Aerobacter* [4] and *Bacillus* species [5]. The bacterial alkaline phosphatase are found in periplasmic space, external to the cell membrane [6] and induced under low phosphate concentration that indicates the bacterial alkaline phosphatase is also involves in phosphate metabolism [7]. *B. lechiniformis* alkaline phosphatase represented as extracellular enzyme that lead to get pure enzyme with fewer steps than with intracellular or membrane bound form [8].

MATERIALS AND METHODS

Samples Collection

Samples were collected from 3 sources: soil (17 samples), water (7 samples) and foods (22 samples). One gram of soil and food was suspended in 9 ml of serial distilled water. 10 ml of water samples was taken; all samples were subjected to water bath (80ºC for 5 min) then 0.1 ml was inoculated onto nutrient agar plates. The growing bacteria were isolated according to the morphological characters of colony (color, shape, margin, etc..) and microscopic features (Gram stain, shape, spore forming) each type was inoculated in another nutrient agar plate to get pure isolated colony

Alkaline phosphatase (ALP) production medium

This medium was referred as PB ALP production medium (PB indicated Pandy and Banik), it consists from glucose (1 gm), peptone (0.5 gm), yeast extract (0.1 gm), $MgSO_4.7H_2O$ (0.02 gm), KH_2PO_4 (0.002 gm), NaCl (0.5 gm) and $CoCl_2$ (0024 gm). All components were dissolved in 90 ml distilled water. pH was adjusted to 8.5 and then the volume was completed to 100 ml by distilled water. The mixture was sterilized by autoclaving at 121˚ C for 15 min [9].

DNA extraction and preparation for polymerase chain reaction (PCR)

DNA extracted by fast DNA spin kit for soil (mpbio –lot 18413). The manufacture's instructions were followed. The extracted DNA was run into agarose gel electrophoresis. PCR primers was used according to previous standadrd study [10], 27f , AGA GTT TGG ATC NTG GCT CAG and 1492R, CGG TTA CCT TGT TAC GAC TT. Then the mixture was shaken for 10 seconds and transferred into the thermocycler. Concentration of PCR product was checked by NanoVue spectrophotometer. The concentration was adjusted to 20-80 ng/µl. 5µl of sample DNA was mixed with 5 µl of forward primer (5 picogram/µl) then labeled with bar code and sent to GATC Company in Germany for DNA sequencing.

RESULTS AND DISCUSSION

Isolation of *Bacillus*

Different samples of food, soil, and water were collected to isolate the bacteria (*Bacillus*), Nine isolates of *Bacillus* (40.9%) were obtained from food samples, 9 isolates from soil samples (52.9%) and 3 from water samples (42.9%). All isolates were identified initially by morphological and biochemical tests, such as Gram stain, bacterial and colony shape, spore forming, growth at 50ºC, aerobic respiration, motility, production of catalase and gelatinase (**Table 1**).

All isolates characterized as Gram positive rod, negative result to indole test, citrate utilization test and VP test were observed. Positive results were seen to motility, gelatinase and catalase tests. These results with the preheated sample considered that all isolates were *Bacillus* [11].

Screening of alkaline phosphatase producing isolates

The bacterial isolates were examined for alkaline phosphatase production in PB ALP medium, results of alkaline phosphatase was checked by specific (**Fig. 1**).

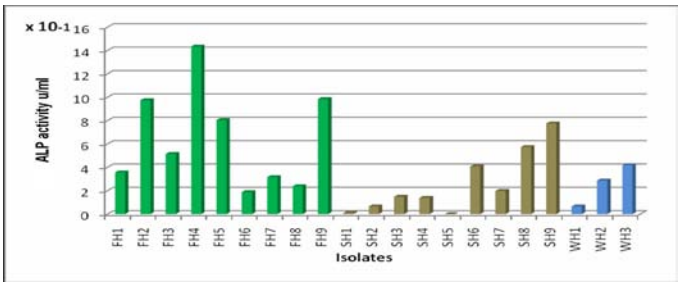

Fig 1. Alkaline phoasphatase activity of different isolates of *Bacillus* (extra cellular enzyme) FH, food Isolates; SH, soil Isolates; WH, Water Isolates.

The *Bacillus* that isolated from food samples produced alkaline phosphatase higher than the isolates that isolated from soil and water samples. FH4 isolate had the highest rate of activity of alkaline phosphatase (1.433 U/ml), this isolate was obtained from potato. A wide variety of foods including starchy foods such as potato, pasta and cheese products have been implicated with *B. cereus*, *B. subtilis* and *B. licheniformis* [12]. These species were known as alkaline phosphatase producers [5,6,13].

Analysis of DNA and 16s rRNA of *Bacillus* FH4

Fig 2a shows the whole genomic DNA of *Bacillus* FH4. The length of this DNA was more than 10000 bp. While, the length of polymerate 16s rRNA gene was around 1500 b (**Fig 2b**) this value is nearby the standard product of 16s rRNA. 16S ribosomal RNA (or 16s rRNA) is a component of the 30S small subunit of prokaryotic ribosomes. It is 1.542 kb in length. The genes coding for it are referred to 16S rDNA and are used in reconstructing phylogenies, multiple sequences of 16s rRNA could exist within a single bacterium [14]. 16s rRNA has a structural role, acting as a scaffold defining the positions of the ribosomal proteins. The 3' end contains the anti-Shine-Dalgarno sequence, which binds upstream to the AUG start codon on the mRNA. The 3'-end of 16s

rRNA binds to the proteins S1 and S21 known to be involved in initiation of protein synthesis [15]. 16S rRNA gene sequences contain hyper-variable regions that can provide species-specific sequences useful for bacterial

Table 1. morphological and biochemichal characters of isolates of *Bacillus*.

Isolates	Gm	shape	spor	Indo	MR	VP	Citra	Gel	Motil	Cata	Glu	Suc	Mal	Fru
WH1	+	**rod**	+	-	+	-	+/-	+	+	+	+	+	+	+
WH2	+	**rod**	-	-	+	-	-	+	+	+	+	+	+	+
WH3	+	**rod**	-	-	+	-	-	+	+	+	+	+	+	+
SH1	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
SH2	+	**rod**	+	-	+	-	+	+	+	+	-/+	+	+	+
SH3	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
SH4	+	**rod**	+	-	+	-	+	+	+	+	+	+	-	+
SH5	+	**rod**	+	-	+	-	-	+	+	+	+	+	-	+
SH6	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
SH7	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
SH8	+	**rod**	-	-	+	-	-	+	+	+	+	+	+	+
SH9	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
FH1	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
FH2	+	**rod**	+	-	+	-	+/-	+	+	+	+	+	+	+
FH3	+	**rod**	+	-	+	-	-	+	+	+	+	+	-	+
FH4	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
FH5	+	**rod**	+	-	+	-	+/-	+	+	+	+	+	-	+
FH6	+	**rod**	+	-	+	-	-	+	+	+	+	+	-	+
FH7	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
FH8	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+
FH9	+	**rod**	+	-	+	-	-	+	+	+	+	+	+	+

Gm: Gram stain, Indo: indole test, MR: methyl red, VP: vogas proskoures, Citra:Citrate utilization test, Gel: gelatinase production, Motil: motility test, Cata: catalase test, Glu: glucose , Suc: sucrose, Mal: maltose, Fru: fructose**.**

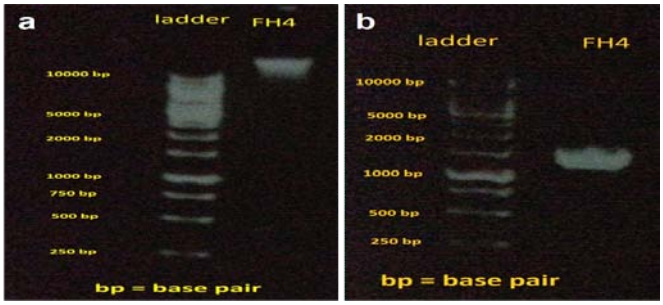

Fig 2. *Bacillus* FH4 DNA and 16s rRNA gene run through agarose gel. A,) extracted DNA (b) polymerate 16s rRNA gene by PCR.

identification [16,17]. 16s rRNA gene sequencing has become prevalent in medical microbiology as a rapid and cheap alternative to phenotypic methods of bacterial identification [18]. It was originally used to identify bacteria. 16S sequencing was subsequently found to be capable of reclassifying bacteria into completely new species, or even genera [19,20]. It has also been used to describe new species that have never been successfully cultured [21,22]. The 16S rRNA gene is used for phylogenetic studies as it is highly conserved among different species of bacteria and archaea [18]. Carl Woese pioneered this use of 16S rRNA [23] .The most common primer pair was devised by Weisburg and co workers and was currently referred to as 27F and 1492R; however, for some applications shorter amplicons may be necessary for example for 454 sequencing with titanium chemistry [18].

Sequencing and phylogenetic tree of FH4 isolate

Sequencing result of 16s rRNA for *Bacillus* FH4 isolate is explained in **fig 3**. The sequence submitted to the National Center for Biotechnology Information (NCBI) and released at the electronic site of NCBI at 1st of October 2013. The sequence was comparing with sequence of *Bacillus* strains in the GeneBank, 16s rRNA of *Bacillus* FH4 was aligned with sequences of 170 *Bacillus* strains and with *Alicyclobacillus acidocaldarius* as a comparative genus. MEGA5 program was used for statistical analysis. Phylogenetic tree obtained (**Fig 4**).

5'-GCGGAC—AGATGGGAGCTTGCTCCCTGAT—
GTCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACT
GGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCG
CATGGTTCAATTATAAAAGGTGGCTTTTAGCTACCACTTACAGATGGACC
CGCGGCGCATTAGCTAGTTGGTG—
AGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTG
ATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCG
CGTGAGTGATGAAGGTTTTCGGATCGTAAAACTCTGTTGTTAGGGAAGA
ACAA--
GTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCA
CGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTT
GTCCGGAATTATTGGGCGTAAAGCGCGCGCA-GGCGGTTT-
CTTAAGTCTGATGTGAA--
AGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAG
TGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGA
GATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGA
CGCTGAGGCGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGT
AGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCT
TTAGTG---3'

Fig 3. Nucleotides sequence of 16s rRNA gene of *Bacillus* FH4 isolate.

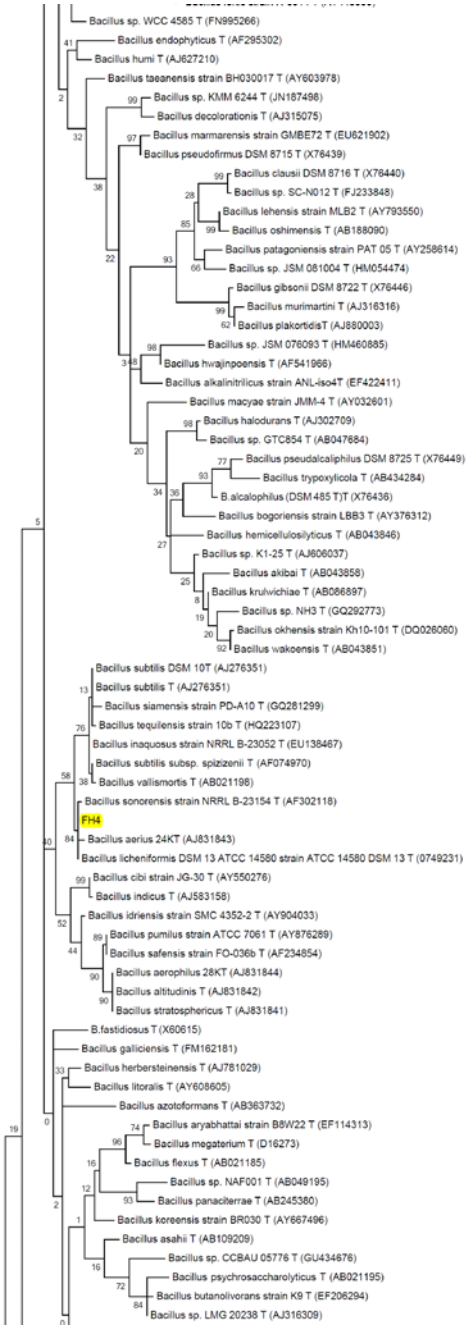

Fig. 4 Molecular phylogenetic analysis by Maximum Likelihood method The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (24). The tree with the highest log likelihood (-10755.9626) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pair wise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 170 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 634 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (25).

From the phylogenetic tree, *Bacillus* FH4 isolate seems to be more closer to *B. licheniformis*, *B. sonorensis* and *B. aerius*. The microscopic examination of *Bacillus* FH4 isolate showed that it is long and thin bacilli, Gram positive and may be arrange some times as a strep bacilli. All results of biochemical test for *Bacillus* FH4 isolate were identical with the results for *B. licheniformis*.

A robust and portable typing scheme for *B. licheniformis* was established by Madslien and co-workers, based on six house-keeping genes separated the species into two distinct lineages. These two lineages seem to have evolved differently. The MLST scheme developed by Madslien and coworkers could be used for further studying of evolution and population genetics of *B. licheniformis* [26]. The isolate that studied in current study was named by Gene Bank of National Center for Biotechnology Information (NCBI) as *B. licheniformis* FH4-IRQ strain and the accession number is KF531930 (http://www.ncbi.nlm.nih.gov).

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

1. **Millan JL.** (2006) Alkaline phosphatase: structure, substrate specificity and functional relatedness to other members of a large super family of enzymes. *Purinergic Signal.* **2**:335-41.
2. **Fernley HN.** (1973) Thiophosphorylation of Alkaline Phosphatase. **Nature** *new biol* 241:110-111.
3. **Friedberg I, Avigad G.** (1967) Some properties of alkaline phosphatase of *P. flourescens*. *Eur J Biochem* **1**: 193-198.
4. **Wolfenden R, Spence G.** (1967) Depression of phosphomonoesterase and phosphodiesterase activities in *Aerobacter aerogenes*. *J Biochim Biophys Acta* **46**: 296-298.
5. **Takeda K, Tsugita A.** (1967) Phosphoesterase of *B. subtilis*. II crystallizationand properties of alkaline phosphatase. *J Biochem* **61**: 231-241.
6. **Hulett FM, Campbell L.** (1971) Purification and properties of alkaline phosphatase of *B. licheniformis. J Biochem* **10**: 1364-1371.
7. **Comb RB, Bowers GN, Posen S.** (1979) Alkaline phosphatase. Plenum. Press, New York.
8. **McNicholas JM, Hulett FM.** (1977) Electron microscope histoch-emical localization of alkaline phosphatase(s) in *Bacillus licheniformis*. *J Bacteriol* **129**:501-15.
9. **Pandey praying SK, Banik RM.** (2010) Optimization of process parameters for alkaline phosphatase production by *Bacillus licheniformis* using Response Surface Methodology. *J Agri Technology* **6**: 721-732.
10. **Jiang H, Dong H, Zhang G, Yu B, Chapman LR, Fields MW.** (2006) Microbial Diversity in Water and Sediment of Lake Chaka, an Athalassohaline Lake in Northwestern China. *Appl Environm Microbiol* **72**: 3832–3845.
11. **Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST.** (1994) Group 17: gram-positive cocci, p. 527–558. In W. R. Hensyl (ed.), Bergey's manual of determinative bacteriology, 9th ed. Williams and Wilkins, Baltimore, Md.
12. **Stenfors ALP, Fagerlund A, Granum PE.** (2008) From soil to gut:*Bacillus cereus* and its poisoning toxins . *FEMS Microbiol Rev* **32**:579-606.
13. **Kostadinova S, Marhova M.** (2010) Purification and Properties of Alkaline Phosphatase From *Bacillus cereus*. Diagnosis Press ISSN 1310-2818 .24. **2**:602-606.
14. **Case RJ, Boucher Y, Dahllöf I, Holmström C, Doolittle WF, Kjelleberg S.** (2007) Use of 16S rRNA and rpoB Genes as Molecular Markers for Microbial Ecology Studies. *Appl Environ Microbiol* **73**: 278–88.
15. **Shine J, Dalgarno L.** (1974) The 3'-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. *Proc Natl Acad Sci* **71**:1342-1346.

16. **Kolbert CP, Persing DH.** (1999) Ribosomal DNA sequencing as a tool for identification of bacterial pathogens. *Curr opin microbial* **2**: 299–305.
17. **Pereira F, Carneiro J, Matthiesen R, van Asch B, Pinto N, Gusmao L, Amorim A.** (2010) Identification of species by multiplex analysis of variable-length sequences. *Nucleic Acids Res* **38**: 203–203.
18. **Clarridge JE III.** (2004) Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev* **17**: 840–862.
19. **Weisburg WG, Barns SM, Pelletier DA, Lane DJ.** (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**: 697–703.
20. **Brett PJ, DeShazer D, Woods DE.** (1998) *Burkholderia thailandensis* sp. nov., a *Burkholderia pseudomallei*-like species. *Int J Syst Bacteriol* **48**: 317–320.
21. **Schmidt TM, Relman DA.** (1994) Phylogenetic identification of uncultured pathogens using ribosomal RNA sequences. *Methods Enzymol* **235**: 205–22.
22. **Gray JP, Herwig RP.** (1996) Phylogenetic analysis of the bacterial communities in marine sediments. *Appl Environ Microbiol* **62**:4049–4059.
23. **Woese CR, Fox GE.** (1977) Phylogenetic structure of the prokaryotic domain: The primary kingdoms. *Proc Natl Acad Sci* **74**: 5088–5090.
24. **Tamura K, Nei M.** (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* **10**:512-526.
25. **Tamura K, Peterson DM, Peterson N, Stecher G, Nei M, Kumar S.** (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Mol Biol Evol* **28**: 2731-2739.
26. **Madslien EH, Olsen JS, Granum PE, Blatny JM.** (2012) Genotyping of *B. licheniformis* based on a novel multi-locus sequence typing (MLST) scheme. *BMC Microbiol* **12**:230.

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