Analysis of 16S rRNA for identification of new bacteria

Nurhayati
Biologi, Diponegoro University, Semarang, Indonesia
nur_setyahayati @yahoo.com

The major problem associated with bacteria classification, identification and identification is due to a lack of data and methods. Correct data is crucial for the nomenclature of bacteria. Bacteria in this regard, molecular approach is advantageous because it reveals the identitiy of bacteria. The 16S rRNA gene as molecular characteristic have been developed to be a useful marker molecular of procaryote for systematics. Identification of bacteria by 16S rRNA gene sequencing is considered to be more accurate than phenotypic characteristics. In conclusion, the sequence analysis of 16S rRNA proved to be a useful molecular data for identification new strain of pathogenic bacteria. The isolate was identifiedas *Bacillus cereus* strain C4 after 16S rRNA gene sequencing and alignment by BLAST.

Keywords : NA, 16S rRNA, *Bacillus cereus*, phylogenetic study

Introduction

Aristotle to the mid-19th century divided organisms into two kingdom : Plantae and Animalia, ernest Haeckel proposed thrid kingdom protista, in 1937 Edouard chatton suggested term *procariotique* and *eucariotique*, in 1969, R.H. Wittahaker proposed five kingdom, Fungi. In the late 1970,s Carl Woese had sequenced the complete genome *Methanococcus jannaschii* and divide procaryote into : Bacteria and archaea, biolgists now classify organisms in three superkingdom or domains : Archaea, Bacteria and Eukarya (Solomon *et al.,* 2011). Closely related species are assigend to the same genus, and closely related genera are grouped in a single family. Families are grouped into orders, orders into classes, classes into phyla, phyla into kingdoms, some kingdms into domains (Solomon e*t al.,* 2011).

Microbes are perform specialized in the earth. Microbes impart many patogenic in environment. According to World Health organization Statistics of 2011, infectious diseases remain in top five causes of mortality worldwide (Srinivisan *et al.,* 2015). Systematics studies of an unknown is highly essential to reveal its identity, diversity and relationship among other organisms in its functional aspects.

Characterization is mandatory before deducing the novel identitiy. Based upon characteristics isolated bacteria can assigned to specific genera. Characteristics using morphological, physiological and biochemical characteristics are time consuming and unaccurate characteristics. By using the 16S rRNA gene as house-keeping gene sequence for phylogenetic studies, domains of Bacteria has been developed in contrast to the tradisional classification of organisms i.e. prokaryotes and eukaryotes (Das *et al.,* 2014). The 16S rRNA gene as molecular data has changed drasticaly the definition of bacteria as the house keeping gene sequence have been developed in contrast to the morphological, physiological and biochemical characteristics.

The 16S rRNA as marker molecular provide the needed information for identification of microbes including bacteria. The advantage of marker molecular to analyse molecular characterization is the sequence of molecular marker without any previous phenotypic, morphological, biochemical and physiological on the target spesies. From comparative RNA sequencing, three phylogenetically distinct cellular lineages have been revealed, The lineages called domains, are the Bacteria and Archaea (both consisting of prokaryotic cells) and the Eukarya (eukaryotes) (Madigan *et al.*, 2012).

Materials and methods

Bacterial isolate and phenotypic identification methods

The patogenic bacteria were screened as previously studied by plating the serially diluted inoculum on nutrient agar. Single colony of isolate was subcultured and tested for purity and then subjected to Characterization.

Genomic isolation

Total genomic DNA was extracted from selected bacterium isolate C4 using commercial kit (Roche).

The16S rRNA gene amplification and sequencing

The 16S rRNA gene was amplified using the universal primer forward 27F (5'-AGAGTTTGATCMTGGCTCAAG-3') and reverse 1492R (5'-ACCTTGTTACGACTTCAC-3'). The 16S rRNA amplification were done in thermocycler.

PCR solution contained 2,5 µL of template DNA; 0,4 µM of primer 27 F and 0,4 µM of primer 1492 R; 18,5 µL PCR-grade water, 2,5 µL 2x KAPA Taq extra hotstart ready mix with dye. PCR reactions were carried out in an thermocycler programmed for the initial denaturation at 95^{o}C for 3 min and 29 cycles as follows : denaturation 95^{o}C for 1 min, annealing at 55^{o}C for 1 min, extention 72^{o}C for 1 min and final extention 72^{o}C for 7 min. Identification to the species level was defined as a 16S rDNA sequence similarity of > 99 % with that of the type strain sequence in gene Bank (Benga *et al.*, 2014). Bioinformatics analysis of 16S rRNA sequences was performed with the gene bank by using the BLAST program (Altsschul *et al.*, 1990).

Results and discussion

Patogenic bacterial as well as discovery of specific species can be accomplished by combining the phenotypic characteristics (Table 1) and 16S rRNA gene. Isolate C4 has rod shape and catalase (Table 1)

The high rate of false positive identification of *S.aureus* as an pathogenic bacterium which could lead to the misuse of antibotics (Ayeni *et al.*, 2016). The use accurate methods for identification of pathogenic bacterium at all times is highly recommended. The isolate was not identified as species level after phenotypic characterization, which can be further used for 16S rRNA gene analyses (Tabel 1).

Table 1. Phenotipic characteristics

Parameter	Results
KOH 3 %	+ (positive)
Catalase	+
Oxidase	- (negative)
Triple Sugar Iron Agar	+
Lysine Iron Agar	-
Oxidation Fermentation	-
Indole	-
Ornithin	-
Esculine	-
Citrate	-
Urea	-
Gelatine	-
Methyl Red	+
Voges Preskauer	+
Motility	+
Dextrosa	-
Glucosa	+
Sucrosa	-
Maltosa	-
Inositol	-
Lactose	-
Arabinosa	-
Manitol	-

The 16S rRNA gene analysis

Use of 16S rRNA as a marker molecular for identification of bacteria is possible because the 16S rRNA gene is highly conserved and present in all bacteria. The molecular analysis of 16S rRNA may be used in identification when identification of the bacterial rRNA gene to species level is required (Jenkins *et al.*, 2012).

The phylogenetic studies of *Bacillus* based on the sequence similarity of 16S rRNA gene desiphers the genus with two phylogenetic liniages i.e. first one including *Bacillus* group from NCBI database and the second consisting of only isolate C3 (Fig. 2). The major advantage of this 16S rRNA gene analysis includes accurate identification and represents a unique technology yielding the result in considerably less time (Das *et al.*, 2014).

The phylogeny analysis (Fig. 2) of bacterium as *Bacillus cereus* was confirmed by subjecting its amplification results to 16S rRNA gene analysis and pairwise lignment through Basic Local alignment Statistic Tool (BLAST).

This isolate C4 was used to systematically evaluate the ability of 16S rRNA gene (Fig 2.) for Phylogenetic study and species level identification. Multiple cases of clinical misidentification eith traditional culture based identification across a wide range of Gram-negative rods and Gram-positive cocci as well common Gram-negative cocci (Srinivasan *et al.*, 2015).

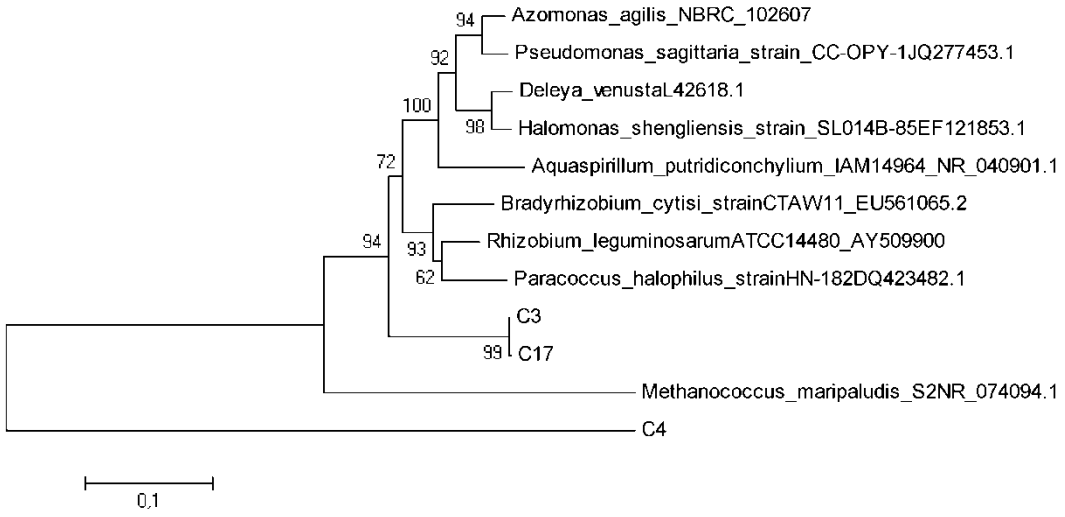

Fig. 2. The phylogenetic study of isolate C4.

Conclution

The 16S rRNA gene and phylogenetic study is standard identification for *Bacillus cereus* strain C4

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