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Prospects and problems

by Sundari Su

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Prospects and problems the amplification of DNA metagenomic from clove plant collected from plantation community in ternate island

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Abstract. DNA content in total microorganisms in plants can utilized for DNA fingerprint studies and a description of the physiological conditions of the plant. The metagenomic technique represents a novel approach in complex genomic analysis of total non-cultured microorganisms. The purpose of this study was to obtain metagenomic DNA isolated from clove plantations of communities in Ternate. The method used in this research is total DNA isolated from leaf and stem samples directly using ZymoBiomic (Zymo Research DNA Extraction) Kit, then amplified DNA based on 16s RNA with forward sequence 27F (5'-AGAGTTTGATCATGGCTCAG-3') sequence and sequence reverse ie 1492R (5'-GGTTACCTTGTTACGACTT-3 '). The results of this study show that of four samples only one sample from fresh leaves have 1400bp. The metagenomic technique of plant tissue is less effective because of many contaminants that are amplify resulting in a double peak amplicon from the first base up to the last base.

1. Introduction

Clove is one of the main spice commodities in North Maluku. Ternate Island is a one centre of clove production. One effort to maintain clove productivity in North Maluku is to control the balance of the ecosystem and reduce the attack of pests and pathogens of clove plants. At present there is a phenomenon of dry clove plant conditions on community plantations in Ternate island. There are no scientific data reveals with this phenomenon. This study aims to explore the first data by obtaining metagenomic DNA isolated directly from samples of fresh clove leaves and dried dead of stems cloves from clove plantations in the Ternate community.

Metagenomics techniques are developing new method for isolation and identification of collective genomes from members of microbial communities that cannot cultured with standard techniques. At this time, it was inform that microorganisms that could not culture with standard techniques were estimate to be around 99%. Metagenomic method begins with the isolation of total DNA from all microorganisms in the sample without being cultured [1]. Metagenomic techniques can use for sequencing processes without going through culture and analysis of DNA obtained from samples of

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known and new microorganisms. The method used in metagenomic DNA isolation is an important step that determines the total DNA quality obtained. The total DNA qualities related with the success of the next process, namely polymerase chain reaction (PCR) to analyse the sequence typical of the test sample. Based on the explanation above, in this study the detection of microorganisms that cause dryness in clove plants on Ternate Island from metagenomics DNA of stem and leaf tissue were carrying out. Metagenomic DNA obtained from leaf and dry stem tissue were amplified with the metagenomic universal primer, 16S RNA with PCR technique, so it is expected that the detection of microorganisms that cause drought in this clove plant can be done fast and simple.

2. Material and Methods

2.1 DNA isolation and Amplification of 16sRNA gene

DNA isolation was carrying out using DNA Presto TM Mini gDNA Bacteria KIT kit (Geneid). The amplification process uses MyTag Red Mix (Bioline). 16SRNA gene amplification with 16s RNA primary sequence sequences with forward 27F sequence sequence (5′-AGAGTTTGATCATGGCTCAG-3 ′) and reverse sequence which is 1492R (5′-GGTTACCTTGTTACGACTT-3 ′). A total of 30 mL PCR reaction mixture (MyTag Red Mix, Primer, ddH2O, and DNA template). The PCR process was carried out under denaturation at 95 0C, annealing at 55 0C, and extension at 720C and post extension at 720C. PCR products were purified using Zimoclean TM gel DNA recovery KIT (Zimo research). Bidirectional sequencing uses 1st Base Malaysia services.

2.2 Phylogenetic Analisys

The data obtained was analysed with the help of computer programs, including: analysis. DNA alligment with MEGA 5 program, BlASTn DNA samples with DNA sequences in GenBank, DNA sequencing from GenBank, and Phylogenetic Neighbor Joint (NJ) tree construction using MEGA 5 program.

3. Result And Discussion

DNA isolation is the initial stage for the PCR process. Whole genome DNA is isolated from the leaves and stems of clove plants. The results of isolation obtained two whole genome DNA. To determine the profile and quality of DNA, agarose electrophoresis and quantitative measurements of DNA were carried out. Results of measurements of concentration and purity of DNA obtained (Table 1).

Table 1. Result of DNA Isolation

No	Sample name	Conc (ng/ml)	A260/280	A260/230	Volume (microlit)
1	MB1	37	1,83	0,17	30
2	SD1	21,7	1,92	0,32	30
3	MB2	77,6	1,83	0,80	30
4	SD2	52,1	1,51	0,72	30

The results of the quantitative analysis revealed that from two samples of clove plants had a good DNA concentration with purity close to 1.8. DNA amplification was then performed using the 16sRNA gene. Amplification of the 16SRNA gene fragments from clove plants The results of 16sRNA gene amplification from clove plant samples were tested by electrophoresis as in Figure 1.

Based on the analysis of the quality of metagenomic DNA amplicons from 4 samples sequenced only 1 sample were amplify well, namely SD1 sample. The sample sequencing results are "MB1_16s", "MB2_16s", "SD2_16s", and there is a double peak from the first base up to the last base. This indicates that there are multiple templates in a PCR reaction, this can occur because the sample is not

pure (single colony) so it cannot proceed to the sequence assembly stage and BLAST analysis. Furthermore, a search analysis of BLASTn (NCBI genebank) was carried out, it was found that SD1 isolate was identical to *Corymbia citriodora* Syn. *Eucalyptus citriodora* Lemon Scented Gum, and synonymous with *Psidium guajava* with 99% identic value. Results of BLAST analysis of 16SRNA sequences from clove leaf tissue samples did not produce similarities to microorganism species in NCBI genebank data. This shows that the primers used as markers are not specific for metagenomic DNA amplification so the results are not effective.

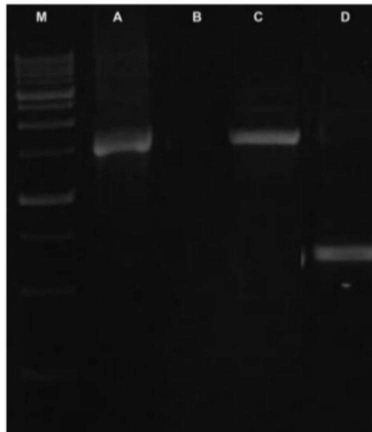

Figure 1. Electropherogram from 16SRNA gene amplification on clove plants from Ternate.

The 16s rRNA amplifications show DNA bands with a size of ± 1400 bp. To find out the name of species from microorganisms in clove plants, phylogenetic analysis of selected samples was compared with data from genebank BLAST search results on NCBI. From this phylogenetic analysis can be known the position of taxon from the identified specimen.

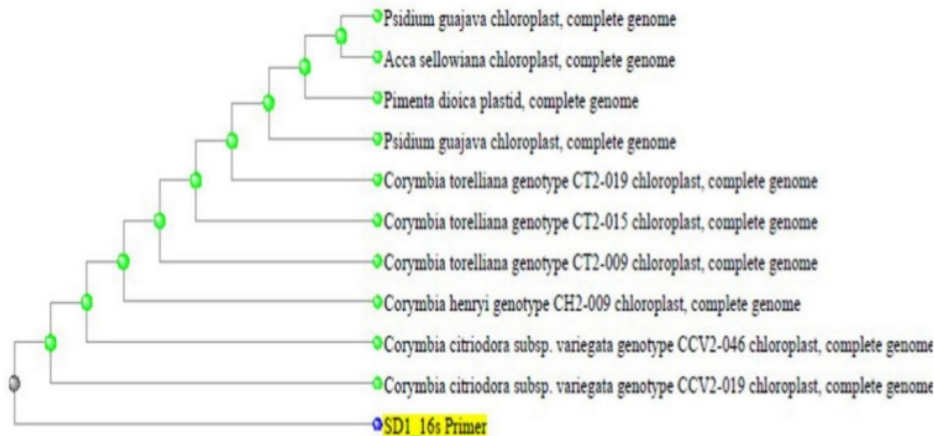

Figure 2. Relationship analysis (phylogenetic) of specimens in cloves

Phylogenetic analysis using neighbor Join (NJ) method the phylogenetic tree shows the relationship of species based on genetic similarity. SD1 samples from clove plants are located at the end of the

cluster and are adjacent to *Corymbia citridora* (Figure 2), it can be seen that the identification of a species, the difference is limited by a node and clusters.

The results of metagenomic DNA amplification with 16 S RNA gene in Ternate clove plant samples showed that only SD1 1 samples were successful. In another sample there is a double seen double peak from the first base to the last base. This indicates that there are multiple templates in one PCR reaction, because the samples are not pure (single colony). Metagenomics are a very appropriate way to know microbial communities that cannot be cultured or unculturable in certain environments. The principle of metagenomic diversity analysis is based on DNA analysis taken directly from a community or ecosystem. DNA from a community can be directly analyzed for diversity and identified using phylogenetic markers such as 16s rRNA (ribosome-RiboNucleic Acid) [2,3].

In this study showed that metagenomic techniques have the prospect of being used as metagenom DNA isolation procedures. The results of this study also show that metagenom techniques have disadvantages of poor amplification products with double peak and multiple templates. In addition, metagenomic techniques have a weakness in sequencing products not on metagenomic DNA from microorganisms but metagenomic DNA from the plant itself. It can see from the results of BLAST that the 16SRNA sequence of metagenomic DNA of clove plants is identical to *Corymbia citridora* a type of eucalyptus plant.

4. Conclusion

The application of metagenomic DNA techniques on four samples of clove plants at the DNA isolation stage can be carried out well, but at the DNA amplification stage of the 4 samples only 1 sample of well-amplified leaves was successful. The size of the amplicon obtained is 1400bp. Metagenomic techniques from plant tissues are less effective because many contaminants are amplified to produce double peak amplicons from the first base to the last base.

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