Optimization of Surface Sterilization Method for Endophytic Bacteria Isolation from Fruit and Leaf Parts of Chilli Plant

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ABSTRACT

Anthracnose caused by the fungus *Collelotrichum spp*. is known to be a major problem in chili cultivation both in the field and post-harvest handling. Anthracnose is a type of disease that is soil borne, air borne, water borne and even seed borne The ability of endophytic bacteria to inhibit fungal growth is from their ability to produce chitinase enzymes that can degrade chitin, the main constituent of fungal cell walls. The isolation of endophytes from the plant require a certain treatment to minimize the contamination from the surface microorganism. The use of endophytes as biocontrol agent will decrease the negative impact of the disease and also to ensure that the chilli is save to consumed. The aim of this research was to determine the suitable surface sterilization method for leaf and fruit part of chilli plant to obtain useful endophytes for controlling *Collelotrichum spp*.
The chilli plants used in this research were collected from a farmers' field in Turi, Sleman regency, Yogyakarta. The plants were collected directly from the field in the morning and transported to Microbiology Laboratory of Faculty of Mathematics and Natural Sciences, Universitas Negeri Yogyakarta. Each part was clean as necessary before treated with several surface sterilization methods. The leaf and fruit from the chilli plant were washed thoroughly under tap water before excised into parts of approximately 2 cm in length. The four methods of sterilization methods used were all washed with sterile distilled water 5 times in the first step. The differences were in steps 2-4 of sterilization: Method A used 20% NaOCL for 20 minutes, then washed with sterile distilled water 3 times. Method B used 95% ethanol for 5 minutes, then immersion in 0,1% $HgCL_2$ for 20 minutes then washed with sterile distilled water 5 times. Method C used 70 % ethanol for 5 minutes, then immersion in 0,1% HgCL for 10 minutes then washed with sterile distilled water 5 times and method D used immersion in 0,1% $HgCL_2$ for 20 minutes then washed with sterile distilled water 3 times. The samples were then grinded and the isolation done in nutrient agar (NA) media by using quadrant streak plate. The distilled water from the last rinse was cultured to know the possibility of the microorganism contamination.
The results showed that the efficiency of methods A and B were low because there were still many colonies found in the last rinse sterile distilled water cultured in NA media. While method C was completely inefficient as was nothing grew on the NA plate. Method D was found more efficient to clean the surface microbes and many endophytes also found especially from the leaf cells. The NaOCl used in method A does not sporadically eliminate the unwanted microbes from the surface of the chilli leaf and fruit. Similar result was also found using method B using ethanol and mercury chloride. The ethanol alone was not effective in sterilizing the surface of many plant samples. We found that the $HgCl_2$ when combined with ethanol in method C, could damage the cells and kill the endophytes so there were no colonies found in the NA plate. However, the single exposure of $HgCl_2$ 0.1% for 20 minutes in method D was found to reduce the amount of bacterial colonies on the plant surface and at the same time efficient to increase the number of endophytic bacteria isolated from chilli leaf and fruit. In conclusion, our findings showed that method D was the most efficient and effective method of surface sterilization method on chilli leaf and fruit for the isolation of endophytic bacteria.

Kata Kunci: *chilli, antrachnose, endophytic bacteria, sterilization method*