

論文 / 著書情報
Article / Book Information

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Title(English)	Detection of Escherichia coli O157:H7 through development of a bacteriophage-based colorimetric method for the prevalence of organic farming
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種別(和文)	論文要旨
Type(English)	Summary

論文要旨

THESIS SUMMARY

専攻 : Department of	International Development Engineering	専攻	申請学位 (専攻分野) : Academic Degree Requested	博士 Doctor of	(Philosophy)
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要旨 (英文 800 語程度)

Thesis Summary (approx.800 English Words)

Organic farming is a form of agriculture that relies on techniques such as crop rotation, green manure, compost and biological pest control. The organic farming produce high quality products and it is environmentally friendly. The market for the organic foods and drink had grown rapidly and reached almost 63 billion US dollars in 2012. Among the factors involving in the organic farming, compost is considered as one of the main factors that has been used on farming for thousands of years ago. The compost is product of composting process in which organic matters were degraded and pathogens possibly containing in the organic matters were eliminated to ensure the safety of compost product that is directly used in agriculture to produce agricultural products. If the composting operation is not adequate, compost can be a source of pathogen contamination especially *E. coli* O157: H7 since animal manures that are usually used as raw materials in composting are the original source of *E. coli* O157: H7. As a result, human is easily infected by the *E. coli* O157: H7 through using agricultural products. Therefore, detection of *E. coli* O157: H7 in compost samples as well as in agricultural and food products are very important to prevent the infection of *E. coli* O157: H7. Although some methods were developed to detect *E. coli* O157: H7, those methods are expensive and/or unquantifiable. Therefore, the goal of this study is to quantitatively evaluate the concentration of *E. coli* O157: H7 in various materials by developing a colorimetric bacteriophage-based method.

Firstly, a new rapid and simple method was developed for the detection of *Escherichia coli* K12 by constructing a recombinant T4 bacteriophage (phage) carrying the *cytochrome c peroxidase* gene derived from *Saccharomyces cerevisiae* (T4ccp) using which, the colorimetric detection of *E. coli* K12 was examined. The oxidation activity towards the chromogenic substrate cytochrome c was demonstrated by the cytochrome c peroxidase (CCP) produced from the T4ccp genome. The color change caused by the oxidation of the substrate could be visually perceived. The possibility of interference in the detection by the coexistence of other bacteria was assessed and it was indicated that the coexistence of other bacteria caused no interference in the detection of *E. coli* K12. Therefore, development of a new colorimetric phage-based for detection of *E. coli* K12

was successful.

Secondly, quantitative detection of *E. coli* O157: H7 using a combination of the colorimetric phage-based assay and most probable number technique was developed. A recombinant phage PP01ccp carrying the *ccp* gene was constructed to detect the pathogenic *E. coli* O157: H7. The PP01ccp was used to detect *E. coli* O157: H7 in broth and color change derived from the experiment could be easily recognized by eyes. Next, a combination of the detection method based on PP01ccp and the MPN technique (abbreviated as MPN-phage assay) was developed to quantify *E. coli* O157: H7 concentration in various samples. The MPN-phage assay was successful in quantitative detection of *E. coli* O157: H7 in apple juice, milk that were considered to contain low background bacteria concentration. Next, the MPN-phage assay was tested in quantitative detection of *E. coli* O157: H7 in fecal sample. Fecal sample was considered to contain high background bacteria concentration that may suppress growth of *E. coli* O157: H7 during pre-cultivation for the detection. To partly eliminate the background bacteria, antibiotics of cefixime and vancomycin were finally selected to use in the MPN-phage assay and the MPN-phage assay enabled quantitative detection of *E. coli* O157: H7 in fecal sample.

Lastly, the fate of *E. coli* O157: H7 during a cattle manure composting was investigated using the MPN-phage assay. Two types of composting runs, with and without turning, were conducted with the temperature of composting was set at 30, 50, and 70 °C to simulate the temperature condition in the large composting pile. For the runs with turning, the material from the reactors was removed, mixed each other and redistributed daily, whereas for those without turning, the compost was agitated inside the reactors. The MPN-phage assay revealed that the *E. coli* O157: H7 was rapidly eliminated at composting at 70 °C and 50 °C for both with and without turning. For the composting at 30 °C, the concentration of *E. coli* O157: H7 in the composting with turning was much lower than that in the composting without turning. It indicated that the fate of *E. coli* O157: H7 was accelerated by effect of turning. And it would be expected that *E. coli* O157: H7 in composting with turning would be completely eliminated if the composting was conducted for more few days.

備考：論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。

Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1 copy of 800 Words (English).