CHAPTER I
INTRODUCTION

Thailand exports fresh vegetables to Japan, USA, United Kingdom, Germany, Australia and other countries (Katenil, 2000), with the value of approximately 3,300 million Baht/year and it tends to increase in the future (Department of Foreign Trade, 2007). In January 2010, export of fresh, chilled, frozen, and dried vegetables amounted to 537.08 million baths. The obstruction of fresh vegetable exporting was Nontariff Barrier (NTB), and Sanitary and Phytosanitary Measures (SPM), especially contaminated *E. coli* and *Salmonella* spp. which are the major accusation in obstruction. Both species of pathogens are one of the most important foodborne human pathogens (Altekuse *et al.*, 1997). The pathogens can be transported from field plots where vegetables are grown or through storm water after a heavy rainfall washing infected manure into the wells of the farming community and contaminating ground water and soil. The application of animal waste to agricultural land, may result in large numbers of pathogenic bacteria being released into the environment. In Thailand, 118,292 people fell ill with diarrhea and 96,383 people with food poisoning, with the 1,794.8 and 152.1 per hundred thousand populations, respectively. Two more died in the four provinces in Northeast Lower (in 2009). In 2010, it expected to be more patient than a year ago because the weather is very hot (Department of Mental Health, 2010).

Detection of these two pathogenic bacteria by conventional method, rapid method for *E. coli* and ELISA assay for *Salmonella* is the responsibility of Department of Agriculture who has authority to certify acetic products for export but these detection methods are time-consuming and take several days. Nowadays, the advanced molecular techniques especially real-time PCR (RT-PCR) is popular for estimating the quality and quantity of bacterial population. It does not require the post PCR analysis such as gel-electrophoresis (An *et al.*, 2006), eliminates the risk of cross-contamination, reducing both the amount of work and analysis time (Omiccioli

et al., 2009). The advantage of such technique is higher precision and quicker and it is also decrease laboratory work. It consumes not more than 24 hours in a detection time so that this method tends to be an appropriate for alternative one (Hein *et al.*, 2001; Stubner 2002; Newby *et al.*, 2003; Morillo *et al.*, 2003;De Medici *et al.*, 2003 refer to Grattepanche *et al.*, 2005). Klerks *et al.* (2004) studies were to compare different the RT-PCR based methods detected either *Salmonella* spp. or *E. coli* O157:H7 with respect to sensitivity, precision and accuracy. In addition, a general internal amplification control (IAC) is presented, allowing prevention of false negative results. The IAC allows insight in amplification efficiency and enables a more accurate quantification with the evaluated RT-PCR methods. The quantification threshold of the methods in which the IAC was determined at 1 pg of target DNA (equal to 200 CFU) per reaction. Qualitative detection was feasible down to 10 fg of target DNA per reaction using both methods in which the IAC was incorporated. The adjusted methods have the potential to provide fast and sensitive detection of *Salmonella* spp. or *E. coli* O157:H7, enabling accurate quantification and preventing false negative results by using the general IAC. The real-time PCR-based assay enabled sensitive and rapid quantification of *E. coli* O157:H7, *Salmonella* spp.and *Listeria monocytogenes* in environmental, soil and milk (Ibekwe and Grieve, 2003; Omiccioli *et al.* 2009). Thus, this method could detect of several pathogens and products.

Many potential pathogens for livestock as well as humans actually contaminate within their manures. Commonly of which, are *E. coli* and *Salmonella* spp. When these potential pathogens move through the slaughterhouse on livestock of poultry, they may carriage significant risks to consumers. Prevalence of *E. coli* O157:H7 in humans, cattle, pigs and poultry were 1%, 16%, 0.4% and 1.3% respectively and *Salmonella* spp. were 1%, 0-13%, 0.38% and 10-100%, respectively (Kirk, 2011). Contamination of *E. coli* O157:H7 in beef feces was high levels ($>10^4$ CFU/g) (Arthur *et al.*, 2010). Islam *et al.* (2004a) reported that the persistence of *S. enteric* serovar Thyphimurium on lettuce and parsley and soils on which they were grown in fields treated with contaminated manure composts. It persisted for 161 and up to 231 days in amended soil with contaminated composts on which lettuce and parsley, respectively. It was detected for up to 63 days and 231 days on lettuce and parsley, respectively.

Therefore, in order to reduce trade barriers and increase product safety for consumers, reduction of contamination for both pathogens bacteria should be previously treat at the site of vegetable growing farms with the correct and the proper management. One of that, the modified molecular biological technique for detection the pathogenic bacteria is an advantage method for save time and labor.

Research Objectives

The objectives of this study were to evaluate the appropriate detection technique for *E. coli* and *Salmonella* spp. and to decrease contamination of both bacteria in vegetable cultivation system. The specific objectives consist of:

1.1 To evaluate the appropriate detection techniques for *E. coli* and *Salmonella* spp. by using conventional methods (FDA/BAM), 3M Petrifilm E.coli/Coliform Count plate (AOAC 2000: 991.14) and real time PCR (Modified from O'Hanlon *et al.*(2004) and Malorny *et al.* (2004)).

1.2 To quantify the contamination of *E. coli* and *Salmonella* spp. in animal manure during compost process.

1.3 To quantify *E. coli* and *Salmonella* spp. in vegetable cultivation when growing soil amended with completely composted animal manure.