ศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคเหนือตอนล่าง
กรมปศุสัตว์ กระทรวงเกษตรและสหกรณ์ ปีที่ 14 ฉบับที่ 54 ต.ค.-ธ.ค. 60 ISSN 1685-9952

Rapid detection of Lactic Acid Bacteria using the Soleris detection system

Daranee Nak-opat
MSc food Science, Safety and Health

Abstract

Lactic acid bacteria (LABs); a large group bacteria in which responsible for production of lactic acids as well as major spoilage organism in various food industries. The Soleris microbial detection system, an optical sensor combined with dye technology based upon the detection of metabolic changes due to microbial activities was evaluated the capacity to detect the presence of LABs contamination. The purpose of this study was to determine the Soleris sytem for its ability to identify LABs in food samples *i.e.* raw chickens and mixed fruit juices. Nine species of LABs and nine species of non-LABs including bacterial and fungal strains, were examined by inclusivity and exclusivity testing. The results were compared with standard plating method. The Soleris system specifically detected four species of LABs and almost of non-LABs species except S. *typhimurium* at inoculation levels of 10^2-10^3 cfu/ml. In addition, the detection time of the Soleris system was faster than culture method. For the alternative validation testing, the sensitivity values of the Soleris and standard plate count method were evaluated; (100%-Soleris system, 94%-plating method; raw chickens inoculated with *L. salivarius*). This system

วัตถุประสงค์

1. **เพื่อเผยแพร่ข้อมูลวิชาการด้านสุขภาพสัตว์**
2. **เพื่อเป็นแหล่งข้อมูลด้านการปศุสัตว์**
3. **เพื่อเป็นสื่อกลางในการแลกเปลี่ยนความคิดเห็นระหว่างชาวปศุสัตว์**

provided accurate and rapid results which is useful for an early detection and high through put examination.

1. Introduction

Lactic acid bacteria (LABs) have been defined as a group of bacteria that responsible for production of lactic acids as the final product via fermentation metabolism (Munoz, Moreno-Arribas and Rivas, 2011) due to their inability to synthesize the energy from respiration. Thus, LABs produce ATP by using sugar fermentation, decarboxylation and deamination pathways (Pessione, 2012). LABs are generally found in two main phyla *i.e. Actinobacteria* and *Firmicutes* that are differentiated by GC content in the genome (*Firmicutes* phylum has low GC content (31-49%) while *Actinobacteria* phylum is high GC content organism (58-61%). The most important LAB genera that can be generally found are *Bifidobacterium*,*Enterococcus, Lactobacillus, Lactococus, Leuconostoc, Pediococcus, Streptococcus* and *Weissella*. These genera vary in some characteristics such as morphology, habitats,pathogenic capability. Generally, LABs have been described as Gram-positive rods (bacilli)or cocci shaped, typically catalase and oxidase negative, nonmotile, nonsporulation, microaerophilic organism that can tolerate oxygen and acid (Sun et al, 2014). LABs are ubiquitous organisms and play an essential role in several applications. They are widely used in a variety of human endeavours, ranging from industrial fermentation processes to human health. With regards to the latter, the genera *Lactobacillus* and *Bifidobacterium*, have been designated “generally regarded as safe”(GRAS) organisms and are mainly used as probiotics (Galat et al, 2016; Pessione, 2012).Some LABs are well known as probiotic strains which are stated by FAO/WHO, 2002 as “live microorganism which, when administered in adequate amount, confer a health benefit on the host”. Probiotic strains have the capacity to regulate gut microflora and therefore maintain the equilibrium between useful bacteria and harmful bacteria hence, enhance the protective immune responses (Pringsulaka et al, 2015). Some strains of LABs, such as *L. acidophilus*,*L. rhamnosus* (Kechagia et al, 2017) are frequently used as probiotics in the form of dietary supplements and, or functional foods to increase or retain their presence in the human gastrointestinal tract and consequently present benefits to human health (Messaoudi et al,2013). In addition, LABs are also important in several industrial fermentation processes as starter cultures. Some groups of LABs such as *Pediococcus* spp. and *Lactobacillus* spp. Are usually responsible for the production of cultured dairy and fermented meat products.They can provide some particular characteristics, not only in their primarily function as producers of lactic acid but also promote changes in sensory properties such as aroma, flavour, colour, texture, proteolytic and lipolytic activities (Castilho et al, 2015; Mullan, 2014). Furthermore, some LABs can generate antibacterial substances called bacteriocins which inhibit the growth of a broad spectrum of both undesired Gram-positive and Gramnegative pathogenic strains, and in some cases of yeast and fungi. For example, *Lactobacillus salivarius* is a well-known bacteriocins producer (Dobson et al., 2011). Bacteriocins produced by LABs can be applied in food as a natural preservative to reduce the contamination of food borne pathogens. A study by Sakaridis (2012) showed that ninety-two LAB isolates exhibited antimicrobial activity against pathogenic strains such as *Salmonella* spp. and *Listeria monocytogenes*. Moreover,

bacteriocins from LABs have the appropriate characteristics to be used as food biopreservatives. For instance, they exhibit an ability to be active agents against other bacterial strains at concentrations as low as nanomolar, they are non-toxic to eukaryotic cells and have no, or only slight effects, on the microbiota in the gastrointestinal tract because they can be digested by proteolytic enzymes (Woraprayote et al, 2016). Nevertheless, LABs have been reported as the spoilage microorganism in food products. LABs are the main group of bacteria that responsible for spoilage of meat products, such as cooked ham, due to their ability to grow in the conditions that usually limit bacterial growth; *i.e.*in the presence of limited oxygen, the presence of salt and low water activity values. For example, the population of the LAB genera *Leuconostoc* was shown to expand during storage process of meat products (Duskova et al, 2016). In the case of wineries, LABs can cause negative effects during malolactic fermentation, resulting in changes of the organoleptic properties of the final products, thus, reducing the quality of wine (Munoz, Moreno-Arribas and Rivas, 2011). In breweries, *Lactobacillus brevis* is well known as the most harmful beer spoiler, often penetrating through the filter membrane, resulting in contamination in beer products (Asano et al, 2009). In addition, some strains of LABs can survive in all processes of beer production due to their ability to grow in restricted conditions, such as the absence of air and the presence of ethanol and acid (Huhtamella et al, 2007). Although the most important organisms that are associated with spoilage in soft drinks and fruit juices are yeasts, some LABs also have been reported as responsible for spoilage in the beverage production include *Lactobacillus, Leuconostoc* spp. (Wareing and Davenport, 2007). Currently, microbial contamination causes concern relating to compromising the safety and quality of food. Detection of LABs is important in many industries to ensure the quality of the products. To overcome these problems, several methods have been used to detect the contaminants in food. Nowadays, the methods used to analyse the contamination of LABs are commonly based on cultivation techniques and followed by some fundamental phenotypical and physiological tests. Although the traditional cultivation methods are mainly used, and are the routine methods in many analytical laboratories, they have some limitations in that nonculturable organisms are not detected and they are sometimes insufficiently accurate to identify to species level (Przybyt et al, 2010). They also take a long period of time and require labour (Huhtamella et al, 2007). Molecular biological techniques (mostly PCR based) have been developed such as DNA-fingerprint and 16S-rRNA to confirm the identification of strains. However, these methods require high standardization, well trained staffs, and are relatively expensive (Galat et al, 2016). For reduction of the time requirement, miniature kits and novel rapid detection assays have been employed in several industries. For instance, chromogenic media (PetrifilmTM) that used specific selective substances to facilitate identification and enumeration of LABs (Castilho et al, 2015). Optical detection instruments such as VITEK (bioMerieux) and Microfoss (Biosys) which can detect the metabolic changes resulting from microbial metabolism can also be used to monitor the presence of microbial contamination in food (Firstenberg-Eden et al, 2002). The Soleris automated system (Neogen) combines chromogenic and optical sensors technologies together to detect LABs in a variety of sample types (including food, dairy, beverage, nutraceutical and environmental) by their metabolic activities based on monitoring changes in the chemical

characteristics of a microbial growth medium in which the target microorganisms grow. The growth of LABs produce CO2 causing a pH/ colour change in the indicator dye which was, then monitored by optical sensors system. The Soleris detection system can provide the semi-quantitation results that are negative and positive above the background level. Recently, this technology has been widely employed (Pereault et al, 2014) in order to detect the early contamination of LABs in the production processes, and to ensure the quality of final food products.

This study aimed to determine the rapid detection method based upon an optical technology to identify LABs in food samples; raw chickens and mixed fruit juices compare with the conventional standard method based on cultivation. Additionally, the potential of this detection system to specifically detect LABs species also were examined by inclusivity as well as exclusivity testing.

2. Materials and Methods

2.1. Materials

2.1.1. Microorganism species and culture conditions

Several species of either bacteria or fungi were selected (shown in table1). In order to culture Lactic acid bacteria (LABs), it is necessary to use suitable growth media such as de Man Rugose Sharpe (MRS) medium (Munoz, Moreno-Arribas and Rivas, 2011) either as broth cultures or on agar plates. (LAB 094-A and LAB 223-A respectively, Lab M Neogen Ltd.) MRS was also used for the pour plating technique. LAB cultures were incubated at 30°C. For the other (non-LABs) species such as *Salmonella* spp. and *Bacillus* spp. were cultured in buffer peptone water (BPW) (LAB 204-A, Lab M Neogen Ltd.) and plated on Nutrient agar (CM0001, Oxoid Ltd.), and Standard Plate Count Agar (APAH) (CM04630, Oxoid Ltd.) for enumeration. In case of yeasts and moulds, they were cultured in Potato dextrose broth (7585A, Neogen Ltd.) plated on Dichloran Rose Bengal Chlortetracycline (DRBC) Agar (7591A, Neogen Ltd.) for isolation and enumeration. The optimum temperature for incubation of all yeasts and moulds is 25°C.

2.1.2. Food samples

Two samples, one liquid and one solid, were selected to test the Soleris detection in this study. Mixed fruit juice (Tesco 100% pure pressed apple 73%, peach 14%, mango 8%, and passion fruit 4%) was adjusted from initial pH 3.48 to final pH 5.33 by adding 10M NaOH. The solid sample selected was chicken (Sainbury's British chicken diced breast and Asda British breast fillets). It was tested by adding buffer peptone water (BPW) (Lab M Neogen Ltd., LAB 204-A) and homogenised using a stomacher.

2.2. Methods

2.2.1 Soleris detection system

Soleris microbial detection system (Neogen) was used to detect the contamination of LABs in the samples. The Soleris vials (DLA-109) contained a broth growth media, separated by a gas-permeable membrane from an agar plug with pH dye indicator. The growth of LABs produced CO_2 causing a pH/ colour change in the indicator dye which was, then detected by optical sensors in the Soleris instrument. This system therefore measured the growth of LABs in real time when the

change of colour is higher than the background for three consecutive readings. The Soleris vials were prepared by firstly, removing the vials from the refrigerator and allowing them to equilibrate at room temperature. In order to improve the selectivity of the assay and prevent the growth of background contamination such as yeasts, moulds and bacteria such as *Bacillus* spp, antifungal and antibacterial supplements were added to the vials;

• Antifungal supplement preparation Weigh 0.01g of AmphoteracinB (A9528-50mg, Sigma-Aldrich Ltd.) into steriled test tube containing 10 ml of steriled water and mix until well dissolved. The final concentration was 0.0001 g/ml. 120 µl of 0.0001 g/ml AmphotericinB was added to the vials.

• Antibacterial supplement preparation Weigh 0.1g of Vancomycin. (V2002-1g, vancomycin hydrochloride from *Streptomyces orientalis*, Sigma-Aldrich Ltd.) into 10 ml of steriled water. 0.45 µm filter was used to sterilise the solution (stock solution 0.01 g/ml). To prepare the working solution of Vancomycin, 100 µl of 0.01g/ml stock solution was aceptically added to 9.9 ml of steriled water. 100 µl of 0.0001 g/ml working solution Vancomycin was added to the vials.

1 ml of each sample was transferred to a vial, then cap the vial and gently invert 3 times to mix the sample, and slightly loosen the cap to allow air and gas exchange. The vials were introduced into the Soleris instrument with the temperature in the chamber set at 30°C and the assays was run for 72 hours. In case of positive result, Soleris vials change from blue to yellow colour result from CO_2 production by LABs.

2.2.2. Inclusivity

For testing the inclusivity of the Soleris DLA-109 vials, nine species of LABs; *L. lactis* spp. Lactis NU-136, *L. paracasei* ATCC BAA-52, *L.casei* ATCC 334, *L. salivarius* NCIMB 11975, *L. leichmannii* ATCC 4797, *L. acidophilus* ATCC 4356, *L. delbrueckii* 11778, *L. fermentum* ATCC 11739, *Leuconostoc mesenteroides* subsp. Mesenteroides ATCC 8293 were selected for analysis. The vials were tested by directly inoculating 1ml of a 10^2-10^3 cfu/ml cultures into the vial. The vials were introduced into the Soleris instrument with the test parameters set up as follows; Threshold 8, Skip 2, Shuteye 50, temperature 30°C and the assays was run for 72 hours. The pour plating technique also was tested in parallel to compare the results by adding 1 ml of a 10^2-10^3 cfu/ml cultures into the plates and mixing with approximately 15 ml of MRS agar. Allow the plates were solidified and incubated at 30°C for 72 hours. Both the Soleris detection method and pour plating method were tested in duplicate.

2.2.3. Exclusivity

To determine the exclusivity of the Soleris system, nine species of bacteria and fungi were used to test the Soleris DLA-109 vials *i.e. B.cereus, B. alvei, B. macerans, B. pumilus, B.thuringiensis, S. enterica* serovar. Typhimurium ATCC 14028, *C. krusei, C. tropicalis* and *S.cerevisae*. The vials were tested by directly inoculating 1ml of a 10^2-10^3 cfu/ml cultures into the vial. The vials were introduced into the Soleris instrument with the test parameters set up as follows; Threshold 8, Skip 2, Shuteye 50, temperature 30 °C and the assays was run for 72 hours. The pour plating technique also was tested in parallel to compare the results by adding 1 ml of a 10^2-10^3 cfu/ml *B.*

cereus and *S. enterica* serovar Typhimurium ATCC 14028 cultures into the plates and mixing with approximately 15 ml of Standard Plate Count Agar (APAH). Allow the plates were solidified and incubated at 30°C for 72 hours. For all of yeasts and moulds, plated on Dichloran Rose Bengal Chlortetracycline (DRBC) Agar and incubated at 25°C. Both the Soleris detection method and pour plating method were tested in duplicate.

Table 1. List of microorganisms used to test the Soleris detection system.

Bacterial species	**Yeasts and moulds**
Bacillus cereus ATCC 11778	*Candida krusei*
B. alvei	*C. tropicalis*
B. macerans	*Saccharomyces cerevisae*
B. pumilus	
B. thuringiensis	
Lactobacillus acidophilus ATCC 4356	
L. casei ATCC 334	
L. delbrueckii 11778	
L. fermentum ATCC 11739	
L. lactis spp. Lactis NU-136	
L. leichmannii ATCC 4797	
L. paracasei ATCC BAA-52	
L. salivarius NCIMB 11975	
Leuconostoc mesenteroides subsp. Mesenteroides ATCC 8293	
Salmonella enterica serovar Typhimurium ATCC 14028	

2.2.4. Alternative validation protocol

To indicate an ability of the Soleris detection method to detect the presence of LABs species in samples *i.e.* mixed fruit juice and raw chicken were analysed at eight different levels of contamination using a serial dilution technique. The fruit juice itself was used as the diluent. Eight sterile universal tubes were filled with 9ml of fruit juice with pH that had been adjusted to a range of 5-5.5. Then 100µl of an overnight culture of *Leuconostoc mesenteroides* in MRS broth was added into the first tube (containing 10ml of fruit juice) and missed thoroughly. Subsequently 1ml was removed and innoculated into the next tube and a 1 in 10 serial dilution performed in the eight tubes. In the case of the solid chicken sample, eight sterile stomacher bags were filled will 5g of chicken (cut by using sterile scissors and forceps) and 40ml of BPW (except the first bag which was filled with 45ml of BPW). An inoculum of 500µl from an overnight culture of *L. salivarius* was added to the first bag. A 1 in 10 serial dilution was performed in much the same way as for the fruit juice, except that 5ml was removed from the first bag, and the mixing was performed using a stomacher, and 5 ml transferred to the next bag etc. From each level of dilution, 1ml was transfered into a Soleris vial (DLA-109 vial) and a further 1 ml added to a plate for pour plating with approximately 15ml of molten (50°C) MRS agar. All dilutions were assayed in duplicate.

2.2.5. Relative Limit of Detection (RLOD) study

Two lactic acid bacteria were selected to spike into the samples; *Leuconostoc mesenteroides* was spiked into the mixed fruit juice sample and *L. salivarius* was inoculated into raw chicken sample. Samples were to be tested in parallel assays using Soleris detection system and ISO standard or an alternative validation protocol. The protocol is based on ISO 16140 *(ISO16140-2:2016,* Microbiology of the food chain-Method Validation-Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method*)*

3. Results

3.1. Inclusivity testing

The purpose of this study was to test the potential of the DLA-109 vials of the Soleris microbial detection system (Neogen) to detect LAB species. Nine species of LABs were selected to test in parallel with the Soleris detection method and pour plating techniques (Table 2).

Table 2. The result of inclusivity testing to detect LAB species by the Soleris detection system and the pour plating method at inoculation levels of 10^2-10^3 cfu/ml.

LABs species	Soleris system		Pour plating
	DT*	results	
L. acidophilus ATCC 4356	ND	-	+
L. casei ATCC 334	14.4	+	+
L. delbrueckii 11778	ND	-	+
L. fermentum ATCC 11739	16.6	+	+
L. lactis spp. Lactis NU-136	ND	-	+
L. leichmannii ATCC 4797	ND	-	+
L. paracasei ATCC BAA-52	12.9	+	+
L. salivarius NCIMB 11975	10.9	+	+
Leuconostoc mesenteroides ATCC 8293	55.7	+	-

Four species; *L. casei*, *L. fermentum*, *L.paracasei* and *L. salivarius* were positive in either pour plating or the Soleris system with detection times of 14.4h, 16.6h, 12.9h and 10.9h (table 2). Four LABs; *L. acidophilus, L.delbrueckii, L. lactis* and *L. leichmannii* were not detected by the Soleris but were positive by cultivation, while *Leuconostoc mesenteroides* was detected by the Soleris with detection time of 55.7h but did not grow on plates.

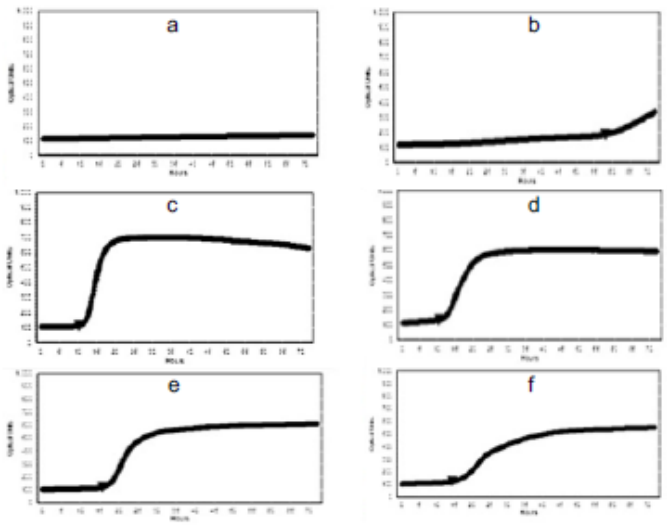

figure 1. The growth curve of LABs species by the Soleris detection system. (a) not detected, (b) *Leuconostoc mesenteroides*, (c) *L. salivarius*, (d) *L. paracasei*, (e) *L. casei* and (f) *L. fermentum*

3.2. Exclusivity testing

To examine the selectivity of the Soleris detection system, nine species of non-LABs including bacterial, yeasts and moulds species were tested in the same way as the inclusivity testing. The results of both the Soleris system and the plating method are shown in table 3. All non-LABs species, except *S. typhimurium*, which was detected in 4.6h, were not detected by the Soleris system but positive with culture method while our sample of *C. tropicalis* was negative in both detection methods.

Table 3. The result of exclusivity testing to detect non-LAB species by the Soleris detection system and pour plating methods at inoculation levels of 10^2-10^3 cfu/ml.

species	Soleris system		Pour plating
	DT*	results	
Bacillus alvei	ND	-	+
B. cereus	ND	-	+
B. macerans	ND	-	+
B. pumilus	ND	-	+
B. thuringiensis	ND	-	+
Salmonella typhimurium	4.6	+	+
Candida krusei	ND	-	+
C. tropicalis	ND	-	-
Saccharomyces serevisae	ND	-	+

+ = positive, - = negative, ND = Not detected, DT = Detection time (h)

3.3. Alternative validation method

To indicate an ability of the Soleris detection method to detect the presence of LABs species in samples, sensitivity value is used to determine the assay by following formulas;

$$SE_{alt} = \frac{(PA + PD)}{(PA + ND + PD)} \times 100\%$$

$$SE_{ref} = \frac{(PA + ND)}{(PA + ND + PD)} \times 100\%$$

When, SEalt = sensitivity of alternative method, SEref = sensitivity of reference method, PA = Positive Agreement; number of dilutions giving positive results for the plating method, that are also positive for the Soleris, PD = Positive Deviation; number of dilutions giving negative results for the plating method, that were positive for the Soleris, NA = Negative Agreement; number of dilutions giving negative results for the plating method, that are also negative for the Soleris, ND = Negative Deviation; number of dilutions giving positive results for the plating method that were negative for the Soleris.

A liquid sample, mixed fruit juice inoculated with *Leuconostoc mesenteroides,* and a solid sample, raw chicken inoculated with *L.salivarius* were selected to examine the sensitivity of the given detection method, the Soleris detection system compared to the reference method, which was the plating method. The results of mixed fruit juice samples inoculated with *Leuconostoc mesenteroides* in which were tested in duplicate are shown in table 4. All of dilution levels were detected by the Soleris system but did not grow on plates hence, the sensitivity values were 100% (SEalt) and 0% (SEref). For chicken samples, they were repetitively tested by two different manners; as received samples and naturally contaminated samples. The Soleris system detected

all of dilution levels while plating method could not detect at dilution level at 10-8 cfu/g (Table 5.) In addition, all dilution levels of an as received raw chicken inoculated with *L. salivarius* were detected by both the Soleris system and plating method (the results are not shown). Thus, the sensitivity value of the alternative method, Soleris system was 100% while the reference plate count method was 94%

Table 4. The result of alternative validation testing; mixed fruit juice inoculated with *Leuconostoc mesenteroides.*

Dilutions	DT (h)	Results	Plate counts (cfu/g)
10^{-1}	32.6	+	-
10^{-2}	50.1	+	-
10^{-3}	55.7	+	-
10^{-4}	64.8	+	-
10^{-5}	49.8	+	-
10^{-6}	53.4	+	-
10^{-7}	51.4	+	-
10^{-8}	53.8	+	-

Table 5. The result of alternative validation testing; naturally contaminated raw chicken inoculated with *L. salivarius.*

Dilutions	DT (h)	Results	Plate counts (cfu/g)
10^{-1}	5.2	+	TNTC
10^{-2}	7.9	+	TNTC
10^{-3}	10.2	+	TNTC
10^{-4}	12.5	+	TNTC
10^{-5}	14.4	+	233
10^{-6}	17.1	+	17
10^{-7}	22.0	+	1
10^{-8}	22.7	+	-

+ = positive, - = negative, DT = Detection time (h), TNTC = Too numerous to count

4. Discussion

LABs play an important role in various food productions ranging from industrial fermentation processes; dairy and beverages, to human health as probiotic microorganism. However, LABs can cause the negative effects. Some strains of LABs have been defined as the major spoilage organism in food processes. Consequently, the detection of LABs during the processes of the production and as well as finished products is a matter of concern. Recently, several detection methods have been introduced to identify the presence of LABs contamination such as standard cultivation method or molecular methods based upon genotypical identification. These methods are relatively time consuming and provide ambiguous results (Ivnitski et al, 1999). Rapid alternative methods for detection of LABs species are deliberately used to replace the traditional method based on culture technique in many food manufactures. In this study aimed to determine the potential of rapid microbial detection method, the Soleris microbial detection system to detect LABs in food samples. As exhibited in the results part; inclusivity testing, the Soleris system showed an ability to detect various strains of LABs compared to plating technique except the following LABs species; *L. acidophilus*, *L. delbrueckii, L. lactis* and *L. leichmanii* were

not detected by the Soleris system but were positive by culture method. According to Harutoshi, 2013 indicated that some strains of LABs such as *L. lactis, L. delbrueckii* and *Leuconostos* spp. can produce exopolysaccharides (EPS) that normally used as thickener or stabilizer (Welman and Maddox, 2003) can cause slime and disrupt an agar plug in the vials resulting in misreading the signals. Moreover, in the case of *Leuconostoc mesenteriodes* which is nutritionally fastidious organism (Holland and Liu, 2011) and weakened capacity to downgrade the 2,5,5-triphenyl tetrazolium chloride (TTC) dye (Castilho *et al*, 2015) thus cannot properly grow on plates. The Soleris detection system presented an ability to early detect the presence of LABs over the plating method showed as detect time in table 2. The Soleris system required less analytical time to detect the LABs except *Leuconostoc mesenteroides* while plating technique required up to 72 hours to develop the colonies (Ivnitski et al, 1999). For exclusivity testing in which was used to test the specificity of the given alternative method, the Soleris detection system expressed the results as demonstrated in table 3. that could not detect other (non-LABs) species such as *Bacillus* spp. as well as yeasts and moulds due to the consequence of supplements were added into the vial to suppress the growth of other organisms. Excluding, *S. typhimurium* could be detected by both plating method and the Soleris system with the detection time of 4.6 hours. Due to the effect of antimicrobial supplement, Vancomycin which widely used against the growth of Gram-positive (Allen & Nicas, 2003) was added to inhibit the growth of particularly *Bacillus* spp. hence *S. typhimurium* could survive. Alternative validation analysis was used to indicate an ability of the given method to detect the presence of LABs species in samples. The sensitivity values were used to determine the alternative method, the Soleris detection system compared to the reference method, standard plate count method. In the case of the inadequate growth of *Leuconostoc mesenteroides*, to analyse the data of the alternative validation method by using mixed fruit juice inoculated with *Leuconostoc mesenteriodes* was not straightforward. A study by Kaklamanou (2017) determined the sensitivity of the Soleris detection system by using mixed fruit juice inoculated with *L. brevis,* and outlined the result as 77% of SEalt. The study of relative limit of detection was not examined due to the problem with the inappropriate growth of *Leuconostoc mesenteroides* as mentioned above, as well as the RLOD study in *L. salivarius* was not tested because raw chicken samples were naturally contaminated in high level. In conclusion, this study has shown that the Soleris detection system is comparable to standard base on culture method. In addition, this rapid alternative detection method provides fast results, simple and high throughput assay.

5. Acknowledgments

I would like to thank Assistant Prof. Ruth Fowler for all supports and advices in this study project. I am also grateful to Antonio Deban Valles, microbiology application specialist from Neogen Ltd. and Ilincheta Saioa for providing technical laboratory guidances. In addition, staffs of school of Institute of Earth & Life Sciences, Heriot-Watt university for all helping and lastly, the Royal Thai Government for financial supports.

6. References

Allen N, Nicas T (2003) Mechanism of action of oritavancin and related glycopeptide antibiotics. *FEMS Microbiology Reviews* **26:** 511-532

Asano S, Iijima K, Suzuki K, Motoyama Y, Ogata T, Kitagawa Y (2009) Rapid detection and identification of beer-spoilage lactic acid bacteria by microcolony method. *Journal of Bioscience and Bioengineering* **108:** 124-129

Castilho N, Okamura V, Camargo A, Pieri F, Nero L (2015) Adequacy of Petrifilm™ Aerobic Count plates supplemented with de Man, Rogosa & Sharpe broth and chlorophenol red for enumeration of lactic acid bacteria in salami. *Meat Science* **110:**253-261

Dobson A, Cotter P, Ross R, Hill C (2011) Bacteriocin Production: A Probiotic Trait? *Applied and Environmental Microbiology* **78:** 1-6

Duskova M, Kamenik J, Lacanin I, Sedo O, Zdrahal Z (2016) Lactic acid bacteria in cooked hams - Sources of contamination and chances of survival in the product. *Food Control* **61:** 1-5

FAO/WHO, 2002. Guidelines for the evaluation of probiotics in food. Report of a Joint FAO/WHO Working Group on Drafting Guidelines for the Evaluation of Probiotics in Food: 1–11

Firstenberg-Eden R, Foti D, McDougal S, Baker J (2002) Optical instrument for the rapid detection of microorganisms in dairy products. *International Dairy Journal* **12:**225-232

Galat A, Dufresne J, Combrisson J, Thepaut J, Boumghar-Bourtchai L, Boyer M, Fourmestraux C (2016) Novel method based on chromogenic media for discrimination and selective enumeration of lactic acid bacteria in fermented milk products. *Food Microbiology* **55:** 86-94

Harutoshi T (2013) Exopolysaccharides of Lactic Acid Bacteria for Food and Colon Health Applications *Lactic Acid Bacteria R&D for Food, Health and Livestock Purposes*

Holland R, Liu S (2011) Lactic Acid Bacteria:Leuconostoc spp. *Encyclopedia of Dairy Sciences*: 138-142

Huhtamella S, Leinonen M, Nieminen T, Fahnert B, Myllykoski L, Breitenstein A, Neubauer P (2007) RNA-based sandwich hybridisation method for detection of lactic acid bacteria in brewery samples. *Journal of Microbiological Methods* **68:** 543-553

Ivnitski D, Abdel I, Atanasov P, Wilkins E (1999) Biosensors for detection of pathogenic bacteria. *Biosensors&Bioelectronics* **14:** 599-624

Kaklamanou P (2017) Evaluation of the Soleris lactis acid bacteria detection system compared with ISO 15214: *MSc research project*

Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, Fakiri E (2013) Health Benefits of Probiotics: A Review. *ISRN Nutrition* **2013:** 1-7

Messaoudi S, Manai M, Kergourlay G, Prevost H, Connil N, Chobert J, Dousset X (2013) Lactobacillus salivarius: Bacteriocin and probiotic activity. *Food Microbiology* **36:** 296-304

Mullan, W (2014) Starter culture: Importance of Selected Genera. *Encyclopedia of Food Microbiology*: 515-521

Munoz R, Moreno-Arribas M, Rivas B (2011) Lactic Acid Bacteria. *Molecular Wine Microbiology*: 191-226

Pereault M, Alles S, Caballero O, Sarver R, McDougal S, Mozola M, Rice J (2014) Validation of the Soleris Direct Yeast and Mold Method for Semi-quantitative Determination of Yeast and Mold in a Variety of Foods. *Journal of AOAC International* **97:** 1084-1091

Pessione E (2012) Lactic acid bacteria contribution to gut microbiota complexity: lights and shadows. *Frontiers in Cellular and Infection Microbiology* **2**:

Pringsulaka O, Rueangyotchanthana K, Suwannasai N, Watanapokasin R, Amnueysit P, Sunthornthummas S, Sukkhum S, Sarawaneeyaruk S, Rangsiruji A (2015) In vitro screening of lactic acid bacteria for multi-strain probiotics. *Livestock Science* **174:** 66-73

Przybyt M, Iciek J, Papiewska A, Biernasiak J (2010) Application of biosensors in early detection of contamination with lactic acid bacteria during apple juice and concentrate production. *Journal of Food Engineering* **99:** 485-490

Sakaridis I, Soultos N, Dovas C, Papavergou E, Ambrosiadis I, Koidis P (2012) Lactic acid bacteria from chicken carcasses with inhibitory activity against Salmonella spp. and Listeria monocytogenes. *Anaerobe* **18:** 62-66

Sun Z, Yu J, Dan T, Zhang W, Zhang H (2014) Phylogenesis and Evolution of Lactic AcidBacteria. *Lactic Acid Bacteria*: 1-101

Wareing P, Davenport R (2007) Microbiology of Soft Drinks and Fruit Juices. *Chemistry and Technology of Soft Drinks and Fruit Juices*: 279-299

Welman A, Maddox I (2003) Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology* **21:** 269-274

Woraprayote W, Malila Y, Sorapukdee S, Swetwiwathana A, Benjakul S, Visessanguan W (2016) Bacteriocins from lactic acid bacteria and their applications in meat and meat products. *Meat Science*, **120:** 118-132

การจัดการขยะ : การจัดการขยะมูลฝอยชุมชนอย่างครบวงจร ตอน 1

การดำเนินงานเพื่อจัดการขยะมูลฝอยที่ผ่านมา ท้องถิ่นส่วนใหญ่สามารถให้บริการเก็บรวบรวมขยะมูลฝอยได้มากขึ้น ทำให้ปัญหาขยะตกค้างน้อยลง แต่ยังมีปัญหากำจัดขยะมูลฝอยไม่ถูกสุขลักษณะอยู่มากตกค้างน้อยลง แต่ยังมีปัญหากำจัดขยะมูลฝอยไม่ถูกสุขลักษณะอยู่มาก แม้จะมีการจัดสรรงบประมาณ เพื่อก่อสร้างระบบกำจัดที่ถูกสุขลักษณะมากขึ้น แต่ยังมีหลายพื้นที่ที่ยังไม่ได้รับการสนับสนุนงบประมาณ และยังมีท้องถิ่นหลายแห่งที่มีระบบแล้วก็ไม่สามารถกำจัดขยะมูลฝอยได้ถูกสุขลักษณะตามที่ออกแบบไว้ได้ และบางแห่งได้รับการต่อต้านคัดค้านจากประชาชนจนไม่สามารถเข้าใช้พื้นที่ได้

ทั้งนี้เนื่องจากการทำโครงการที่ผ่านมามักไม่ได้คำนึงถึงการเตรียมพร้อมที่จะดำเนินงานดูแลรักษาระบบอย่างต่อเนื่องทำให้มีข้อจำกัดทางด้านบุคลากรและองค์กรบริหารจัดการที่ชัดเจน นอกจากนี้การดำเนินงานที่ผ่านมา ส่วนใหญ่มีลักษณะต่างคนต่างทำ ทำให้มีสถานที่กำจัดมูลฝอยที่ไม่ถูกสุขลักษณะขนาดต่าง ๆ กันกระจายทั่วไป ทำให้สิ้นเปลืองงบประมาณโดยเฉพาะอย่างยิ่งในช่วงวิกฤติเศรษฐกิจของประเทศ มีผลทำให้การจัดสรรงบประมาณจากส่วนกลางมีจำกัดและไม่ต่อเนื่องซึ่งเป็นอุปสรรคต่อการจัดการขยะมูลฝอย

จากการศึกษาความเป็นไปได้ของการจัดตั้งศูนย์กำจัดขยะมูลฝอยในหลาย ๆ พื้นที่ของประเทศได้ข้อสรุปว่า รูปแบบศูนย์การจัดการขยะมูลฝอยชุมชน เป็นแนวทางหนึ่งในการจัดการขยะมูลฝอยที่จะแก้ไขสภาพปัญหาที่เกิดขึ้น โดยเป็นการมุ่งเน้นให้ชุมชนที่อยู่ใกล้เคียงกันนำขยะมูลฝอยมากำจัดร่วมกัน ซึ่งแนวทางนี้จะช่วยแก้ไขปัญหาได้ในระยะยาว และเพื่อเป็นการลดภาระของรัฐบาลด้านการลงทุนและการบริหารจัดการ อีกทั้งสนับสนุนให้ภาคเอกชนเข้ามามีบทบาทร่วมลงทุนและดำเนินการ โดยรูปแบบการลงทุนและดำเนินการศูนย์กำจัดขยะมูลฝอยอาจทำได้หลายวิธี อาทิ เอกชนเป็นผู้ลงทุนและดำเนินการเองทั้งหมด รัฐร่วมลงทุนกับภาคเอกชน รัฐลงทุนการก่อสร้างระบบและให้เอกชนดำเนินการ เป็นต้น

อย่างไรก็ตามการดำเนินการจัดการขยะมูลฝอยร่วมกันต้องอาศัยความร่วมมือจากหน่วยงานต่าง ๆ ดังนั้นจำเป็นต้องกำหนดแนวทางและทิศทางในการดำเนินงานและการจัดสรรงบประมาณโดยใช้รูปแบบศูนย์กำจัดขยะมูลฝอย ร่วมกันเพื่อนำไปสู่การปฏิบัติอย่างเป็นรูปธรรมในโอกาสต่อไป และเป็นการแก้ไขปัญหาผลกระทบอันเนื่องจากการจัดการขยะในระยะยาวอย่างมีประสิทธิภาพ และถูกหลักสุขาภิบาล สามารถช่วยแก้ไขปัญหาการขาดแคลนสถานที่กำจัดขยะมูลฝอยของชุมชนต่าง ๆ โดยเฉพาะชุมชนขนาดเล็ก และยังประหยัดงบประมาณ บุคลากร พื้นที่ในการทำจัดขยะมูลฝอย ทั้งนี้เพื่อคุณภาพชีวิตและสุขนามัยของประชาชน

การจัดการขยะมูลฝอยชุมชนอย่างครบวงจรคู่มือสำหรับผู้บริหารองค์กรปกครองส่วนท้องถิ่นนี้จะประกอบด้วยหลักเกณฑ์การพิจารณาระบบการจัดการขยะมูลฝอยครบวงจรตั้งแต่ระบบการเก็บรวบรวม การคัดแยก การนำกลับไปใช้ประโยชน์ และรูปแบบศูนย์กำจัดขยะมูลฝอย แนวทางของการจัดการขยะมูลฝอยโดยรูปแบบศูนย์กำจัดรวมนั้น ผู้บริหารขององค์กรปกครองส่วนท้องถิ่น ควร

พิจารณารูปแบบของการจัดการขยะมูลฝอยอย่างครบวงจร เพื่อดำเนินการจัดการในเขตพื้นที่ความรับผิดชอบโดยเริ่มจากการสำรวจข้อมูลปริมาณขยะมูลฝอยที่เกิดขึ้นจากแหล่งกำเนิดต่าง ๆ และการคาดการณ์ในอนาคต ตลอดจนสัดส่วนหรือลักษณะองค์ประกอบของขยะมูลฝอยทางด้านกายภาพ เคมี และอื่น ๆ โดยข้อมูลเหล่านี้ นำไปใช้ในการวางแผนและออกแบบระบบการจัดการขยะมูลฝอยที่สามารถรับได้ในระยะเวลา 20 ปี ตั้งแต่ขั้นตอนการคัดแยกการเก็บรวบรวม การขนส่ง การนำไปใช้ประโยชน์และการกำจัด โดยคำนึงถึงเทคโนโลยีที่มีความเหมาะสม รวมทั้งการคัดเลือกพื้นที่ รูปแบบของการบริหารจัดการ และการมาตรการลดปริมาณขยะมูลฝอย โดยจัดให้มีการรณรงค์และนำระบบการนำวัสดุกลับคืนมาใช้ให้มากขึ้น พร้อมทั้งพิจารณาค่าลงทุนและค่าใช้จ่ายในการดำเนินงานด้านต่าง ๆ อย่างครบถ้วนตลอดอายุโครงการ

ที่มา : http://www.pcd.go.th/info_serv/waste_garbage.html

โปรดติดตามตอนต่อไปของ "การจัดการ" นะค่ะ ^^_^^

กองบรรณาธิการ

รายงานการชันสูตรโรคสัตว์

ตุลาคม – ธันวาคม 2560

ชนิดสัตว์	จำนวนตัวอย่างที่ส่งตรวจ				โรคที่ตรวจพบ	จำนวนตัวอย่างที่พบ
	ซาก, มีชีวิต	อุจจาระ	เลือด, ซีรั่ม	เชื้อป้ายสำลี		
โค	1	45	1,691	13	- Anaplasmosis - Foot and mouth disease type O - Theileriosis	19 7 10
กระบือ	-	-	90	-	- Foot and mouth disease type O - Theileriosis	2 1
สุกร	2	-	1,195	-	Classical swine fever	1
แกะ	-	-	264	-	-	-
แพะ	22	-	5,514	-	Melioidosis	6
กวาง	-	-	2	-	Theileriosis	9
ไก่	1,724	-	1,160	3,506	- Salmonellosis - Infectious bronchitis	4 2
เป็ด	33	-	-	567	New duck syndrome	4
นกธรรมชาติ	-	-	-	6	-	-
สัตว์ปีกสวยงาม	-	-	-	2	-	-
นกกระทา	-	-	-	12	-	-
นกกระจอกเทศ	-	-	-	-	-	-
ห่าน	-	-	-	-	-	-
ม้า	-	-	31	-	-	-
สัตว์ป่า	2	-	41	20	Theileriosis (ลิง)	1
สัตว์น้ำ	-	-	-	-	-	-
สัตว์เลี้ยง	54	-	-	-	Rabies (สุนัข)	1
สัตว์ครึ่งบกครึ่งน้ำ	-	-	-	-	-	-
สัตว์ทดลอง	7	-	26	-	-	-

ศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคเหนือตอนล่าง

อ.วังทอง จ.พิษณุโลก 65130 โทร 0-5531-3137

E-mail : vrd_sn@dld.go.th

ชำระค่าฝากส่งเป็นรายเดือน
ใบอนุญาตเลขที่ 60/2542
ไปรษณีย์วังทอง

เหตุขัดข้องที่นำจ่ายผู้รับไม่ได้
0จ่าหน้าไม่ชัดเจน
0ไม่มีเลขที่บ้านตามจ่าหน้า
0ไม่ยอมรับ
0ไม่มีผู้รับตามจ่าหน้า
0ไม่มารับภายในกำหนด
0ตาย
0เลิกกิจการ
0ลาออก
0ย้าย ไม่ทราบที่อยู่ใหม่
0เลขทับมีไม่ถึง
0บ้านรื้อถอน
0เลขขาดหายไป
0อื่นๆ .....
ลงชื่อ.....

ที่ปรึกษา: ผู้อำนวยการศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคเหนือตอนล่าง
เจ้าของ: ศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคเหนือตอนล่าง
บรรณาธิการ: นางสาววิลาวรรณ บุตรกูล

กองบรรณาธิการ: น.สพ.เสกสิทธิ์ สิงห์แจ่ม
สพ.ญ.ปาริชาติ มัธยม
นางสาวสุวรรณี ตันรัตนวงศ์
นายชัยณรงค์ กุลฉิม
น.สพ.สืบชาติ สัจจวาทิต
น.สพ.ธีรพงษ์ พรมปัญญา
นางสาวโยธกานต์ สิงห์วงศ์
นางสาวดารณี นาคโอภาส
สพ.ญ.อังคณา ขันทะบุตร
นายประสิทธิ์ วานิชสวัสดิ์วิชัย
นางนงลักษณ์ แสงแก้ว

กำหนดออก : ทุก 3 เดือน

ศูนย์วิจัยและพัฒนาการสัตวแพทย์ภาคเหนือตอนล่าง
9 ม.15 ต.วังทอง อ.วังทอง จ.พิษณุโลก 65130
โทรศัพท์ 055-313137, 39 e-mail : vrd_sn@dld.go.th website: vrd-sn.dld.go.th