MICROBIOLOGICAL PROFILING OF FOOD WASTE COMPOST

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FACULTY OF SCIENCES
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MICROBIOLOGICAL PROFILING OF FOOD WASTE COMPOST

ABSTRACT

Increase in food waste is a growing problem in most developing countries including Malaysia. The improper and inadequate use of landfill treatment systems in the country elucidate high release of various environmental contaminants such as leachate which contaminate groundwater, potential release of toxic gases and other odour producing organic pollutants. Therefore, employing composting as a potentially viable means to reduce waste disposed through landfilling may yield fruitful results. However, this composting has its limitations because the process may fail to inactivate all of the pathogenic microorganisms that may be present in the food waste. The resulting food waste compost may then pose a public health risk by cross contaminating the food crops. Thus, this study aims to assess the microbial risk by identifying and characterising the potential microbial hazards and risk associated with food waste compost, as well as to isolate beneficial bacteria with antimicrobial activity against the major foodborne pathogens associated with the food waste compost. The microbial profiling identified *Clostridium* sp. as a potential high risk microbial hazard associated with food waste compost, followed by *Listeria monocytogenous*, *Escherichia coli*, *Salmonella*, *Bacillus cereus* and *Vibrio* sp. to be categorised as the microbial hazards with medium risk and *Staphylococcus aereus* as low risk. The matured food waste composts tested in this study demonstrated high count of heterophilic bacteria (Mean = 2.5×10^4 CFU/g) and fungi (Mean = 3.1×10^4 CFU/g). Although Enterobacteriaceae (Mean = 1.6×10^4 CFU/g) and total coliforms (Mean = 1.1×10^4 CFU/g) were detected in the samples, *E. coli*, *Salmonella,* and *Vibrio* sp., were not detected in the matured food waste compost. Of the 91 isolates collected from the composts, results showed that about two-third of the isolates

(n=56; 62%) were Gram positive bacteria. Maldi-ToF was only able to identified 21 isolates (23%) of the compost-borne bacteria. *Bacillus* sp. is the most commonly identified bacterial genera of these identified bacteria. From the 91 compost-borne isolates that were tested to determine the antimicrobial activity against the nine selected foodborne pathogens (various type of pathogenic *E. coli*, *B. cereus*, *P. mirabilis and Salmonella*), nine isolates (10%) showed antimicrobial activity against the foodborne pathogens tested. These nine isolates were identified to be *Klebsiella aerogenes*, *Lysinibacillus fusiformis*, *Shigella flexneri, Klebsiella pneumoniae, E. coli, Enterobacter aerogenes, Shigella dysenteriae, Enterobacter cloacae* and *Pantoea agglomerans*. In conclusion, this study showed that improperly composted food waste could pose a significant public health risk if the compost is used for food crops farming. Although the thermophilic stage during the composting has been thought to be the major inactivation mechanisms of pathogenic microorganisms, the survival of pathogens during the composting process could be very complex and involves numerous physicochemical factors as well as the antagonistic effect of other microorganisms present in the compost. Thus, this study clearly showed that the anti-pathogenic bacteria are present in the food waste compost. These beneficial bacteria could be further explored for application to enhance the inactivation rate of pathogenic bacteria during food waste composting.

Keywords: food waste compost, microbial risk assessment, beneficial bacteria, antimicrobial.

PROFIL MIKROBIOLOGI KOMPOS SISA MAKANAN

ABSTRAK

Peningkatan sisa makanan telah menjadi salah satu masalah yang semakin meningkat di kebanyakan negara membangun termasuk Malaysia. Sistem rawatan tapak pelupusan sampah yang tidak teratur dan tidak mencukupi di negara ini menyebabkan pelepasan tinggi pelbagai pencemaran alam sekitar seperti lindi yang mencemari air bawah tanah, potensi pelepasan gas toksik dan bahan pencemar organik bau yang lain. Oleh itu, penggunaan cara pengkomposan sebagai cara yang berpotensi untuk mengurangkan sisa yang dilupuskan melalui sistem tapak pelupusan sampah boleh memberi hasil yang memuaskan. Walaubagaimanapun, pengkomposan ini mempunyai batasannya kerana prosesnya mungkin gagal untuk menyahaktifkan semua mikroorganisma patogen yang terdapat dalam sisa makanan. Kompos makanan yang dihasilkan kemudiannya akan menimbulkan risiko kesihatan awam dengan pencemaran silang terhadap tanaman makanan. Oleh itu, kajian ini bertujuan untuk menilai risiko mikrobial dengan mengenalpasti dan mencirikan potensi bahaya mikroba dan risiko yang berkaitan dengan kompos sisa makanan, serta mengasingkan bakteria berfaedah dengan aktiviti antimikrobial terhadap patogen makanan yang utama yang berkaitan dengan kompos sisa makanan. Profil mikroba mengenalpasti *Clostridium* sp. berpotensi sebagai mikrob risiko tinggi yang berkaitan dengan kompos sisa makanan, diikuti oleh *Listeria monocytogenous, Escherichia coli, Salmonella, Bacillus cereus* dan *Vibrio* sp. yang dikategorikan sebagai mikrob berrisiko sederhana dan *Staphyloccus aereus* sebagai risiko yang rendah. Kompos sisa makanan matang yang diuji dalam kajian ini menunjukkan kiraan heterophilic yang tinggi (Min = 2.5×10^4 CFU/g) dan kulat (Min = 3.1×10^4 CFU/g). Walaupun Enterobacteriaceae (Min = 1.6×10^4 CFU/g) dan jumlah koliform (Min = 1.1×10^4 CFU/g) dikesan dalam sampel, *E. coli*, *Salmonella* dan *Vibrio* sp. tidak dikesan

dalam kompos sisa makanan yang matang. Dari 91 isolat yang dikumpul dari kompos, hasil identifikasi menunjukkan bahawa kira-kira dua pertiga daripada isolat (n=56; 62%) adalah bakteria Gram positif. Maldi-ToF hanya dapat mengenalpasti 21 isolat (23%) daripada bakteria bawaan kompos. Daripada bakteria yang dikenal pasti ini, *Bacillus* sp. adalah genera bakteria yang biasa dikenalpasti. Daripada 91 isolat bawaan kompos yang telah diuji untuk menentukan aktiviti antimikrobial terhadap sembilan patogen bawaan makanan yang tertentu (pelbagai jenis patogen *E. coli, B. cereus, P. mirabilis dan Salmonella*), sembilan isolat (10%) menunjukkan aktiviti antimikrobial terhadap patogen bawaan makanan yang diuji. Sembilan jenis isolat yang dikenal pasti adalah (*Klebsiella aerogenes, Lysinibacillus fusiformis, Shigella flexneri, Klebsiella pneumoniae, E. coli, Enterobacter aerogenes, Shigella dysenteriae, Enterobacter cloacae dan Pantoea agglomerans*). Kesimpulannya, kajian ini memperlihatkan bahawa sisa makanan yang tidak dikompos dengan elok boleh menyebabkan risiko kesihatan awam yang ketara jika kompos digunakan untuk pertanian tanaman makanan. Walaupun tahap thermophilic semasa proses pengkomposan dianggap sebagai mekanisma nyahaktif utama mikroorganisma patogenik, kewujudan patogen semasa proses pengkomposan boleh menjadi sangat rumit dan melibatkan banyak faktor fizikokimia serta pengaruh antagonistik mikroorganisma lain yang terdapat dalam kompos. Oleh itu, kajian ini dengan jelas menunjukkan bahawa terdapat bakteria antipatogen dalam kompos sisa makanan. Bakteria yang bermanfaat ini boleh diterokai dengan lebih lanjut untuk aplikasi untuk meningkatkan kadar nyahaktif bakteria patogen semasa pengkomposan sisa makanan.

Kata Kunci: kompos sisa makanan, penilaian risiko mickrob, bakteria berfaedah, antimikrobial

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LIST OF SYMBOLS AND ABBREVIATIONS

µl	:	Microliter
µm	:	Micrometer
°C	:	Temperature
%	:	Percentage
a_w	:	Water activity
n	:	Number of samples
C/N	:	Carbon / Nitrogen
CFU/g	:	Colony forming unit/gram
CFU/mL	:	Colony forming unit/mililiter
DNA	:	Dinucleotide
dNTP	:	Deoxyribonucleotide triphosphate
DAEC	:	Diffusely adherent *E. coli*
ddH_2O	:	Doubled distilled water
EC	:	Electrical Conductivity
EAEC	:	Enteroaggregative *E. coli*
EHEC	:	Enterohaemorrhagic *E. coli*
EIEC	:	Enteroinvasive *E. coli*
EPEC	:	Enteropathogenic *E. coli*
ETEC	:	Enterotoxigenic *E. coli*
ECC	:	*E. coli* and Coliform agar
GHG	:	Greenhouse gases
G + C	:	Guanine-Cytosine
H_2O_2	:	Hydrogen peroxide

MALDI-ToF	:	Matrix Assisted Laser Desorption ionization - Time of Flight
$MgCl_2$	:	Magnesium chloride
MSW	:	Municipal Solid Waste
MC	:	Moisture content
MAC	:	MacConkey Agar
NaCl	:	Sodium chloride
NH_3	:	Ammonia
N_2O	:	Dinitrogen monoxide
NA	:	Nutrient Agar
NA	:	Not available
NPF	:	No peak found
ORI	:	Origin
PCR	:	Polymerase Chain reaction
pH	:	Potential of Hydrogen
PDA	:	Potato Dextrose Agar
PALCAM	:	Listeria Identification Agar Base
PCA	:	Plate Count Agar
rRNA	:	ribonucleic acid
RTE	:	Ready-to-eat
TN	:	Total nitrogen
UV	:	Ultraviolet
VOCs	:	Volatile organic compounds
VRBG	:	Violet red bile glucose agar
WHO	:	World Health Organization
XLD	:	Xylose Lysine Deoxycholate agar

LIST OF APPENDICES

CHAPTER 1: INTRODUCTION

1.1 Food Waste

Food waste is defined as leftover organic matter including food thrown unfinished, food wasted during preparation and unconsumed food. One third of the worldwide food production is lost or wasted everywhere from crop to food (Stenmarck et al., 2016). More than one billion tonnes of food waste are generated annually which needs a proper disposal management. Tonnes of food are wasted daily from vastly populated areas like housing residences, restaurants, food courts and cafes, schools and universities canteens, industrial cafeterias, supermarkets and shopping malls, and hospitals (Jereme et al., 2016). Food waste is characterized as major growing concern due to problem in handling and storing. By character, they produce offensive odour, decay, high moisture content, solid content and volatile solid content during collection and transportation, (Zhang et al., 2017). Food waste is commonly disposed through dumping landfill, combustion, gasification as well as, composting which is the most recommend method used lately.

Composting is an aerobic microbiological process that is facilitated by bacteria and fungi to produce fertilizer and act as soil conditioner (Partanen et al., 2010). Typical compost is rich with bacteria community, which are good for soil conditioning, however, some bacteria that are detrimental to health can tag along if the composting process is not done properly. Greater temperature is used to generate mature compost because natural decay does not abolish pathogens and weed seeds present in the food waste (US EPA, 2016). Composting process is carried out under aerobic setting and little moisture for microorganisms to decay organic matter producing an end product rich in nutrients and soil supplement that can be utilized as fertilizer, peat and compost in agricultural activities (Saer et al., 2013).

1.2 Foodborne bacteria associated with incomplete composting

Pathogenic microorganisms are microorganisms that are bad and can be hazardous to human health. Foodborne pathogens are a main threat to food safety lately, especially in developing nations where there is public concern to the danger of pathogens associated with food waste compost (Larney et al., 2003; Pandey et al., 2016). The alternate use of food waste compost as fertilizer for agricultural use is still questionable due to the vulnerability in pathogen inactivation during composting processes because pathogenic microbes can be actuated if the composting process are improperly done (Millner et al., 1994; Beffa et al., 1996; Beuchat, 2002; Jiang et al., 2002; Islam et al., 2005). Food waste compost is known to contain pathogenic microorganisms like *Salmonella, Listeria, E. coli, Campylobacter, Mycobacteria, Clostridia* and other pathogens that pose significant risk to human health (Sahlström, 2003). For example, *L. monocytogenes* can continue to live for up to half a year in clay like compost and for several weeks in compost (McCrackin et al., 2016). It causes infection in pregnant women and also affect immuno-compromised of people (Gaul et al., 2012).

1.3 Problem Statement

Food waste composting is an emerging green approach to recycle food waste to produce more foods, i.e. using as organic fertilizer to fertilize the soil for food crops farming. While there are lots of benefit to use food waste compost, food waste compost might carry potential harmful foodborne pathogens originated from the food waste. Food waste is known to contain pathogenic microorganisms like *Salmonella, Listeria, E. coli, Campylobacter, Mycobacteria, Clostridia* and other pathogens that can pose risk to human health (Pandey et al., 2016). These microbial hazards could then cross-contaminate the food crops, such as vegetables and fruits when being used as fertilizer in the farms. Pathogen contaminated foods from farms could then pose a public health risk

to the consumers. In fact, there have been many studies and outbreak cases that have clearly identified organic compost as one of the highly potential source of cross-contamination at the farm level (Aureli et al., 2000; Gaul et al., 2012; Huang et al., 2017). The occurrence of foodborne pathogens in the final matured food waste and organic compost could be due to poorly conducted composting process, as well as other factors such as the presence pathogens that resist the composting process. Therefore, this study aims to (i) assess the overall microbial risk associated with food waste compost by using the microbial risk assessment approach, including (ii) detection of foodborne pathogens in the matured food waste compost collected from a food waste composting site. Also, this study aims to (iii) determine the presence of anti-pathogens bacteria in the food waste compost that contribute to the inactivation of foodborne pathogens during the composting process.

1.4 Objectives of Study

The objectives of this study are:

1. to conduct microbial risk profiling of food waste compost.
2. to enumerate and determine total mesophilic bacteria, yeast and mold, Enterobacteriaceae, coliforms, *E. coli*, *Salmonella* and *Vibro* in the food compost.
3. to determine the antimicrobial activity of isolated bacteria against a set of selected foodborne pathogens.

CHAPTER 2: LITERATURE REVIEW

2.1 Municipal Solid Waste Generation in Malaysia

The increase in MSW is as a result of rapid economic growth that increases standard of living standard, consumption patterns and urban population. Globally, MSW in developed nations and developing nations, was estimated to annual increase of 3.2% to 4.5% and 2% to 3% respectively (Agamuthu, 2001; Suocheng et al., 2001).

Statistics on demographics showed that within 15 years, Malaysian inhabitants has increased from 21 million people in 1997 to approximately 29.5 million (40.5) in 2012. Agamuthu et al. (2009) stated that, Peninsular Malaysia amplified consistent waste production from 13,000 tonnes in 1996 to 19,000 in 2006. In 2005, Malaysia generated approximately 7.34 million tonnes of solid waste and it is assumed that 31,000 tonnes of MSW will be generated every day by 2020 (Chandravathani, 2006; Latifah et al., 2009). Kuala Lumpur generates 3,500 tonnes of household and industrial waste daily where 50% of it is organic waste (Bavani, 2009; Jalil, 2010).

Table 2.1 demonstrates the making of MSW in main town areas in Peninsular Malaysia. The MSW generation rate in all the major urban areas increased approximately 13% from 2006 to 2012. Among all the major urban area in Malaysia, Kuala Lumpur recorded the highest MSW generation at 37,000 tonnes /day, far leading other urban areas. Melaka ranked the second; only 755 tonnes /day followed by Klang, the third, 643 tonnes/day (Fauziah et al., 2013).

Table 2.1: Generation of MSW in major urban areas in Peninsular Malaysia from1970 to 2012 Adapted from: Fauziah et al. (2013)

Urban centre	Solid waste generated (tonnes/day)							
	1970	**1980**	**1990**	**2002**	**2006**	**2009**	**2010**	**2012**
Kuala Lumpur	98.9	310.5	586.8	2754.0	3100.0	3387.0	3489.0	3701.0
Johor Bahru (Johor)	41.1	99.6	174.8	215.0	242.0	264.0	272.0	289.0
Ipoh (Perak)	22.5	82.7	162.2	208.0	234.0	256.0	264.0	280.0
Georgetown (Pulau Pinang)	53.4	83.0	137.2	221.0	249.0	272.0	280.0	297.0
Klang (Selangor)	18.0	65.7	122.8	478.0	538.0	588.0	606.0	643.0
Kuala Terengganu (Terengganu)	8.7	61.8	121.0	137.0	154.0	168.0	173.0	184.0
Kota Bahru (Kelantan)	9.1	56.5	102.9	129.5	146.0	160.0	165.0	175.0
Kuantan (Pahang)	7.1	45.2	85.3	174.0	196.0	214.0	220.0	233.0
Seremban (Negeri Sembilan)	13.4	45.1	85.2	165.0	186.0	203.0	209.0	222.0
Melaka	14.4	29.1	46.8	562.0	632.0	691.0	712.0	755.0

Due to economic growth, rising of living standards and change of composition pattern, the solid waste generate per capital has been increasing steadily, from 0.5 kg per day in 1980s to about 1.5 kg per day in 2007 (Agamuthu et al., 2009). This increasing trend indicates that the 3R strategy (reduce, reuse and recycle) is unsuccessful. Recycling campaigns have been conducted for few years but end up with failure due to insufficient public response. However, public knowledge on related issues increased slightly (Agamuthu et al., 2013).

2.2 Municipal Solid Waste Composition in Malaysia

Composition of waste directly influenced the density of the waste and the most efficient waste disposal methods to be applied, for instance high recyclable and compostable content make waste recovery feasible (Al-Khatib et al., 2010). The common

characteristics of solid waste in Asian countries is high organic and moisture content (Visvanathan et al., 2004). The common MSW compositions are food waste, plastic, paper, rubber/ leather, wood, metal, glass and textiles (Chiemchaisri et al., 2007). MSW in Malaysia is very assorted and hold high fraction of organic matter (Agamuthu & Nasser, 2009). Statistics from 9th Malaysia plan, prepared by National Solid Waste Management Department (2005) is in parallel with this statement. Food waste took up almost half of the total waste amount generated in 2005 (45%) followed by plastic (24%) and paper (7%) (Figure 2.2). Organic content in MSW has a direct impact on the moisture content and bulk density.

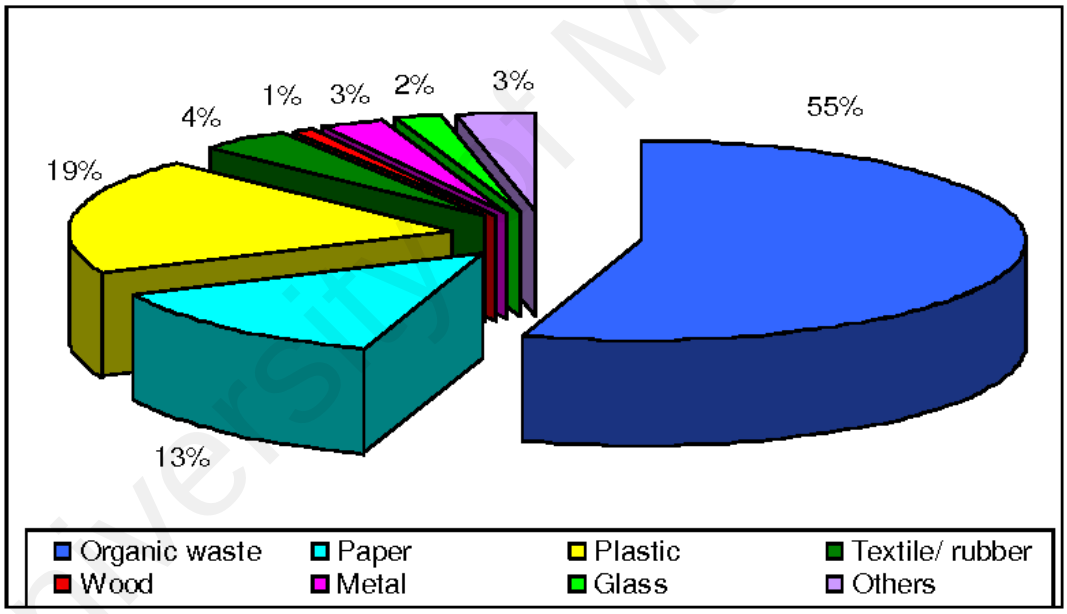

Figure 2.1: Typical municipal solid waste composition in Malaysia from 1975 to 2009 Adapted from: Periathamby & Hamid (2009)

Figure 2.1 depicts the compositions of MSW from 1975 to 2005. Organic waste is always the main composition, followed by paper (Agamuthu et al., 2009). The paper and plastic composition recorded high in 1985 compared to other years resulted from recognition of the materials as safe packaging materials in Malaysia Food Regulations 1985 (Neilsen & Ng, 2004). These compositions decreased due to economic turmoil from

1990 to 1999 but increased again in 2000 resulting from the materials (Agamuthu et al., 2009).

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Table 2.2: Waste composition (percentage of wet weight) in Malaysia from 1975 to 2005 Adapted from: Agamuthu et al. (2009)

Waste composition	1975	1980	1985	1990	1995	2000	2005
Organic	63.7	54.4	48.3	48.4	45.7	43.2	44.8
Paper	7.0	8.0	23.6	8.9	9.0	23.7	16.0
Plastic	2.5	0.4	9.4	3.0	3.9	11.2	15.0
Glass	2.5	0.4	4.0	3.0	3.9	3.2	3.0
Metal	6.4	2.2	5.9	4.6	5.1	4.2	3.3
Textiles	1.3	2.2	N/A	N/A	2.1	1.5	2.8
Wood	6.5	1.8	N/A	N/A	N/A	0.7	6.7
Others	0.9	0.3	8.8	32.1	4.3	12.3	8.4

*N/A, not available

2.3 Municipal Solid Waste Management in Malaysia

Ascribed to its heterogenous characteristics, MSW ought to be overseen utilizing a combined solid waste management as different types of waste could not be handled by single treatment. Many countries have started using recovery-based solution from landfill-based solution to endorse sustainable development (Thanh et al., 2010). In Malaysia, since 95% of the collected solid waste disposed of in landfill, it is estimated to

have 310, 220 t of CH_4 as at 2010, which is estimated to increase to 350,000 tonnes in 2015 and 370,000 tonnes in 2020 (Johari et al., 2012). Various advancements, which can lessen emission of GHG from MSW, are being engaged as vital advancement contrary to environmental alteration. For instance, incineration and landfill gas catch enables energy recuperation to produce power, whereas, organic waste is transformed into compost as soil fertilizer. These new technologies decrease the interest for non-renewable energy source, protect natural resources and lessen the emissions of GHG (Chalita & Shabbir, 2008).

National Solid waste management stated that, till September 2009, there were 176 landfills and disposal sites in Malaysia where only eight are sanitary landfills, for example, Pahang (one), Selangor (three include Bukit Tagar Sanitary Landfill), Johor (one) and Sarawak (three) while 114 landfills and disposal sites had been shut down (Li, 2010). Harmful environment and health effects are of concern when landfills are practiced particularly when it is located near residential region where gas emission and leachate drainage is of concern (Hassan, 2000; Fauziah, 2010).

Advanced countries such as Japan and the United States are strongly promoting zero waste. The concept of zero waste gains more popularity to overcome problems of landfill scarcity, global climate change and resource depletion. Hence, this concept is being promoted as the next greatest innovation in waste management (Russell, 2009). Nevertheless, Malaysia is still lagging behind, as landfilling is the main waste disposal method. MSW disposed of at the landfills is 95% out of total MSW, generating approximately 3 million liters of leachate per day (Periathamby & Hamid, 2009; Frank & Agamuthu, 2010).

Table 2.3 depicts the methods of waste disposal since 2002 and proposed methods in 2020. Introduction of waste disposal through recycling had only a slight increase from 2002 to 2006 (0.5%). This disappointment is because of the absence of open interest from public as only 40% of them practice recycling from over 90% who knows about recycling (Fauziah, 2010). However, Ministry of Housing and Local Government (2011) has fixed an objective to expand recycling methods up to 22% by 2020 (Geetha, 2009). In Malaysia, waste disposal through incineration have not been executed from 2002 to 2006, however, it is targeted to be used up to 16.8% in 2020. As of date, five incinerators are yet to be initiated, situated at Pulau Pangkor, Labuan, Pulau Langkawi, Pulan Tioman and Cameron Highlands (Li, 2010). From the target set in 2020, it plainly shows that, Malaysia will practice waste disposal methods of recycling, incineration and sanitary landfill compared to open disposal sites (Agamuthu et al., 2009).

Table 2.3: Methods of waste disposal in Malaysia Adapted from: Agamuthu et al. (2009)

Treatment	Percentage of waste disposed		
	2002	2006	Target 2020
Recycling	5.0	5.5	22.0
Composting	0.0	1.0	8.0
Incineration	0.0	0.0	16.8
Inert landfill	0.0	3.2	9.1
Sanitary landfill	5.0	30.9	44.1
Other disposal sites	90.0	59.4	0.0
Total	100.0	100.0	100.0

2.4 Composting

Composting is a process of converting organic waste into a useful solid product for the use in agriculture by using aerobic biochemical process (Tiquia, 2010). This process is environmental friendly and is suitable for organic waste management (Cerda et al., 2018). Various microbes that differ both temporally and spatially facilitate in the process

including the development of thermophilic temperatures because of naturally produced heat (Atalia et al., 2015). Aerobic bacteria and fungi led the composting process where crude materials are changed into energy and cell biomass and humified organic matter is also created in the process. Composting process can be reflected to be a carbon-based system used as a replacement to landfill (Brown et al., 2008).

During composting process few range of organisms plays an important role to create a complex and fast varying community such as protozoans, microbial organisms (bacteria, actinobacteria, and fungi), Isopoda, Nematoda and fungi. In the phases of composting process, Nematodes and microbial organisms are the main group to be observed due to their universal characteristics (Steel et al., 2013). Another example was stated that main phyla (Actinobacteria, Proteobacteria, Firmicutes and Bacteroidetes) were observed great all the way through sequencing for compost sample. As for now, numerous systems have been progressively emphasized to examine the composition of microbial group and recognize the main aspects prompting microbial group with unique organic limit (Zhao et al., 2017).

Composting process involves various complex chemical reactions and microbiological changes, which comprises hydrolysis, nitrification, humification, proteolysis, ammonification and carbon mineralization. In the first stage of composting process, mesophilic microorganisms begin the process and bloom at temperature range from 10 °C to 40 °C. Then, as the inner temperature of the compost stack surges at temperature range of 40 °C to 80 °C, the microbial action builds as the heat accepting thermophiles conquest (Zibilske, 2005). In the end, mesophilic-restoring stage starts when the temperature diminishes, the substrates become limited and microbial action reduces. Organic fertilizer is the final product of composting which holds basic plant supplements

(Zhou et al., 2016). The quality of the compost is the most vital viewpoint when concerning the assurance of consumers. Most consumers' worry when utilizing food based compost is the content of metal in it which can affect the crops (Hargreaves et al., 2008).

It is very important to carry out the composting process effectively by keeping in mind the end goal, which is to boost the advantages of composting (Fan et al., 2006). Promoting knowledge on the technology to manage organic fraction of MSW is vital because to carry out the technology, a strong foundation of specific parameters like, relative proportions of waste and the bulking agent, air circulation and moisture in the system amid composting and development time to diminish CH_4, N_2O, and NH_3 emission, leachate generation, and possible increase of nasty odors (Amlinger et al., 2008; Edgerton et al., 2009). Besides, advancing microbial growth, developing high temperatures and surging the process speed is also essential (Adhikari et al., 2013). Controlling parameters that affect composting is not only vital for an effective process, but also to reduce insignificant gas emissions and to produce some good quality compost (Barrena et al., 2014).

2.5 Role of environmental parameters during food waste composting processes

2.5.1 Temperature

Temperature is an important and convenient parameter of microbial activities during composting processes as well as to decide its status (Tang et al., 2011). Two temperatures ranges, mesophilic and thermophilic are included in the composting process. For the beginning of the composting process, temperature range of 20 °C to 45 °C is perfect. Mesophilic bacteria (*Enterobacteriaceae*, *Comamonadacea, Pseudomonaceae*, and *Erythrobacteraceae* family) initiates the breaking down of organic matter by using

soluble and readily assimilable compounds like sugar, lipid and amino acids received from the feedstock used for creating compost (Bernal et al., 2009). The following stages, temperature range of 50 °C to 70 °C is ideal when thermophilic organisms attaint.

2.5.2 pH level

During food waste composting processes, pH difference (4.9 to 8.3) is led by the production of short-chain organic acids and ammonia from the feedstock since the early stage of batch composting (Beck-Friis et al., 2001; Conghos et al., 2003). When pH is adjusted, the composting process is improved because various volatile organic compounds (VOCs) and semi-VOCs created throughout composting significantly reduces the microbial movement (Smårs et al., 2002; Komilis et al., 2004). Be it high or low pH, both can cause inhibitory effect on the microbial activities throughout food waste composting stages (Beck-Friis et al., 2001).

2.5.3 Moisture content (MC)

As a standout amongst the other physical parameters during food waste composting, MC shakes the chemical and physical characteristics of waste materials by breaking down the organic matter (Iqbal et al., 2010; Huet et al., 2012). Besides, the MC of the composting blend gives a platform for the passage of liquefied nutrients needed for the metabolic and bodily activities of microorganisms (Liang et al., 2003). For example, in a condition of various MCs (45% to 75%) experimented, it is discovered that for green waste and food waste co-composting 60% is the ideal MC (Kumar et al., 2010).

2.5.4 C/N ratio

The C/N ratio gives carbon and nitrogen source needed for development of microorganisms making it as a vital parameter during composting process (Guo et al.,

2012). When the C/N ratios are high, the speed of composting process decreases because there is abundance of degradable substrate for the microorganisms. However, when C/N ratio is low, ammonia is drained and volatized to discard the extra N from the composting mass. To deliver organic carbon, a bulking agent was included. To get a promising composting outcomes, it is important to distinguish an ideal C/N ratio (25-35) since it is assumed that microbes need 30 parts of C and one unit of N but that rely upon the composting material's characteristics (Adhikari et al., 2008).

2.5.5 Aeration

Aeration rate is viewed as one of the key variables influencing the process and value of compost. The aeration frequency affects volatilization of NH_3 and greenhouse gases (GHG), temperature difference in the composting, microbial activity, and substrate degradation rate (Yuan et al., 2016). High aerations rates prompt high EC values compared with compost manure; however, low aeration rates expand the percentage of total nitrogen (TN), reduction of C/N proportions and long-lasting thermophilic stage. (Rasapoor et al., 2009). Many studies have discussed on the optimal extent of aeration for different composting procedure, for example, Yuan et al. (2016) recorded that maximum N_2O and CH_4 releases happened at a little aeration rate (0.1 L dry kg^{-1} min^{-1}) while maximum NH_3 releases happened at a modest aeration rate (0.2 L dry kg^{-1} min^{-1}).

2.6 Composting Process

Composting is an aerobic microbiological process that is facilitated by bacteria and fungi under controlled aerobic environments to yield compost (Farrell et al., 2009; Partanen et al., 2010). In general composting procedure consists of the following operating stages (Agamuthu, 2000), reception organic waste is collected, weighed and delivered on this passing storage. Segregation at this stage, compostable and non-

compostable wastes are separated because sometimes piles of organic waste are jumbled with plastic bags, paper and other forms of non-compostable waste. Pre-composting, the separated compostable waste is the sent for crushing to break down the huge compost materials to shrink the volume. Composting, organic waste undergoes mesophilic and thermophilic stages where bacteria present in the organic waste will start biodegradation. Additives such as worms, enzyme, bio-catalyst and sewage sludge are able to speed up the process. Environmental parameters like aeration, moisture content, pH value and temperature are kept under closed observation until compost is matured. Finally, the ground compost is sent to packaging area and kept temporarily before marketing out.

2.6.1 Types of Composting

There are few types of composting methods as below (US EPA, 2016):

(a) **Aerated (turned) composting**

Organic waste is formed into rows called windrows. Windrows are turned either manually or mechanically on certain period to provide an ideal condition (temperature, moisture content and oxygen amount) for composting.

(b) ***Vermicomposting***

Organic waste mixed with worms, being digested and become castings. Types of worms generally used in vermicomposting are red worm, branding worm, and tiger worm (Agamuthu, 2000).

(c) **Aerated static pile composting**

Organic waste is formed into piles. Oxygen is blowed into the piles either through layers of lightly piled bulky mediators such as wood chips and tattered newspaper or pipes which placed underneath the piles.

(d) **In-vessels composting**

Organic waste is placed in a container such as drum, silo concreted-lined trench or similar condition. Unlike other composting methods mentioned above, all the factors which can affect the composting results are well-controlled.

2.7 Benefits of Composting

Composting has advantages which comprises of low cost compared with landfill and incineration, volume reduction of biodegradable division in waste and useful for soil remediation (Agamuthu & Faizura, 2005; Bruun et al., 2006; Bogner et al., 2008).

Food waste is bio conversed to compost as substitute fertilizers used for agricultural crops which is of improved quality than commercial inorganic fertilizer by using innovative technologies (Vandecasteele et al., 2016). This process has the advantage of using biomass that or else be landfilled and delivers a balance nutrient in a low-cost improvement. Moreover, compost can impound carbon that can alleviate climate change (Lehmann & Joseph, 2009). Besides, composting allows total hygienization of compost by abolishing pathogenic organisms present in food waste due to the thermophilic settings of the process (Kulikowska, 2016; Pandey et al., 2016).

Moreover, composting has been also effective in reducing moderately persistent organic compounds such as veterinary pharmaceuticals (Li et al., 2015). In addition to

that, composting also has added to the improvisation of the water holding limit of soil and offers better tilts (Mohammad et al., 2012). Furthermore, waste recycling alternative like composting contributes mass reduction of organic waste, differ reliant on authors. Oman (2003) reported mass reduction 20% to 40%, Agamuthu et al. (2005) and Luz et al. (2009) reported mass reduction of 60% and 66.6% respectively.

Due to the increase in environmental, awareness, composting is gaining more popularity as an alternative MSW disposal (Agamuthu et al., 2005; Gabrielle et al., 2005). This because less GHG emissions generated from composting process compared to landfill as the biodegradation process is aerobic.

2.8 Microbial Risk Profiling

The scientific method of determining the connection between introduction to a given danger and the probability of an unfavorable health impact under a characterized set of settings is defined as risk assessment (Jouve & De Nantes, 2002). Being a topic of interest under preliminary risk management, risk assessment has been explained as an illustration of food safety issues and its circumstances (Mok et al., 2014). Thus, risk profiling is carried out which includes the systematic gathering of data expected to settle on a choice on what will be done subsequent and where resources ought to be assigned to more comprehensive scientific assessments.

Microbiological risk assessments have been sectioned into four stages. Firstly, hazard identification, where hazards which might be available in a specific food is identified. Secondly, hazard characterization, where adverse health impact related with the hazard is assessed qualitatively and/or quantitatively. Next, exposure assessment, were the possible ingestion of the hazard and its contact to different sources is assessed qualitatively and/or

quantitatively. At last, risk characterization which is done based on the previous three stages to guess the doubts, chance of happening and seriousness of the identified or possible adverse health effects (Nauta, 2000; Rocourt et al., 2001).

Microbiological dangers constituted by food from compost rely on the type of hazard associated with depending on their pathogenicity, survival and development in foods, and on the possible control methods taken during food production, distribution and preparation. The scope of risk profile was to focus on public health hazards (contamination and outbreak) attributed to compost due to incomplete composting or resistance strain.

2.9 Foodborne pathogens associated with food waste compost

Outbreak of pathogens associated with food waste compost can be originated from the food waste itself or during handling of composting process. The existence of microorganisms in food is common and natural. It is not easy to eliminate them from food or to avoid their entrance, but their destruction to some extend can be done by cooking at high temperature (Akbar & Anal, 2011). Pathogenic microorganisms are microorganisms that are bad and can be hazardous to human health. Foodborne pathogens are a main threat to food safety lately. Food waste is known to contain pathogenic microorganisms like *Salmonella, Listeria, E. coli, Campylobacter, Mycobacteria, Clostridia* and other pathogens that pose significant risk to human health (Sahlström, 2003). The risk of using food waste as compost cannot be neglected because of the presence of pathogens in the food waste itself.

Pathogens are profound to different environmental factors and they might reproduce in the environment under favorable conditions compared to viruses and parasites (Franke-

Whittle & Insam, 2013). Many of these are zoonosis and they can contribute to symptom-free contagions along with severe infections. Food borne disease occurs when the body system takes in contaminated food substances that can either be infectious or toxic to the body system. Normally, in well-operated compost systems, temperatures range 50 °C to 65 °C, where, such temperatures are suitable for the thermal death points of mesophilic pathogens. Pathogens are shattered as the temperature increases during composting. Even though the bacterial existence is not constant, utmost viruses are killed at 70 °C in about 20 minutes (Heydari & Pessarakli, 2010). In Malaysia, it is difficult to estimate prevalence of food borne disease, but is a known fact that people fall sick and die as a result of eating contaminated food (Tweib et al., 2012).

As of fungi, contamination can occur through straight skin contact or inhalation and are mainly detrimental to workers handling plant related waste compared to recycled product. *Aspergillus fumigatus* is recognized as a pathogenic fungus particularly in nose and throat. Mycotoxins is produced by other fungi such as *A. flavus*, *Stachybotrys atra*, and other species of *Fusarium* and *Penicillium* (Reijula & Tuomi, 2003).

2.9.1 *Staphylococcus aureus*

S. aureus is a Gram-positive, catalase and coagulase positive type of bacteria. It has ability to take-charge of human and animal surfaces. *S. aureus* produces enterotoxins which is the major causes of food poison. Globally, *S. aureus* strains have appeared to a greatly resistant to a wide of range of antibiotics.

Morphological appearance of *S. aureus* on plate is a golden yellow color, often with hemolysis on blood agar plates. Staphylococcus is facultative anaerobic organism that range from 0.05 – 1.5 μm in diameter and is characterized with cocci individual, it is non-

motile, non-spore forming, non-sporulating anaerobe that grows by aerobic respiration. Morandi et al. (2007) stated that cells are sphere-like (cocci), they occur either singular as (coccus) or grouped cocci as (diplococci), to form clusters that is grape-like structure (Staphylococci). The cell wall of Staphylococci is not affected by lysozyme but it vulnerable to lysostaphin. It particularly separates the pentaglycin bonds of *Staphylococcus spp* (Ling et al., 2015).

S. aureus is found naturally on human skin, nostril and hair of warm blooded animals. Few percentages (20-30%) of human population (infant and hospitalized patient) are colonized. *S. aureus* has ability of growing within temperatures of 6° to 48 °C, with 30 to 37 °C as the optimal temperature (Cretenet et al., 2011). The pH is also within the range of 5.3 to 7.3, with 4.8 to 9.0 as the optimal pH level. Moreover, the best toxin produced in the presence of oxygen and sodium chloride concentrations (up to 15% NaCl). These are major characteristics that make *S. aureus* to survive in an extensive range of food items (Cretenet et al., 2011; Morandi et al., 2007).

S. aureus is also a catalase positive that can convert hydrogen peroxide (H_2O_2) to water and oxygen, therefore making it an important distinguishing agent in catalase test distinguishing Staphylococci from Enterococci and Streptococci. It increases under favorable situations that, happen in every 15 to 30 minutes. Thus, one cell can end up producing over two million cells in 7 hours and seven million cells after 12 hours in a continuous growing process (Bernthal et al., 2010). It is a known fact that different substances for example, coagulase, hemolysins, nuclease, corrosive phosphatase, lipase, protease, fibrinolysin and enterotoxins are produced for living organisms. These dynamic substances are thought to add to the pathogenicity of living organisms (Karmi, 2013).

2.9.2 *Salmonella*

Salmonella belongs to Gram-negative bacteria, they are non-spore former and facultative anaerobic. Taxonomically, *Salmonella* genus is within the Enterobacteriaceae family, a major cause of food borne disease around the world, including Malaysia (Aguado et al., 2004). Based on diversity of *Salmonella*, over 2500 serovars has been classified, therefore, capable of infecting range of vertebrates, together with human. Though, cases of human infection due to *S. bongori* have been reported (Ranjbar et al., 2011), the species is relatively rare, which makes it non-clinically important compared to *S. enterica* which is responsible for enteric fever while *S. bongori* causes salmonellosis (Fookes et al., 2011).

Food-borne disease occurs when the body system takes in contaminated food substances that can either be infectious or toxic to the body system. This impact has huge effect in the individual wellbeing as general medical issue continues expanding under a few conditions (Loharikar et al., 2012). Recently continuous outbreaks of food borne disease in developing countries as well as developed countries have been a major concern. In Malaysia, it is difficult to estimate prevalence of food borne disease, but is a known fact that people fall sick and die as a result of eating contaminated food. New et al. (2017) showed the alarming increase in food poisoning cases from the year 2012 to 2015 reported in schools with an average of 8000 cases in 2015 (Figure 2.2). Students are recorded to have the highest threat to be affected from food poisoning cases especially in boarding schools because students are restricted to eat the same unclean food from the school's canteen. So far till 2017, 130 students were recorded to be affected by food poisoning cases where Penang recorded 42 students while Johor recorded 99 students (Malaysian Digest, 2017).

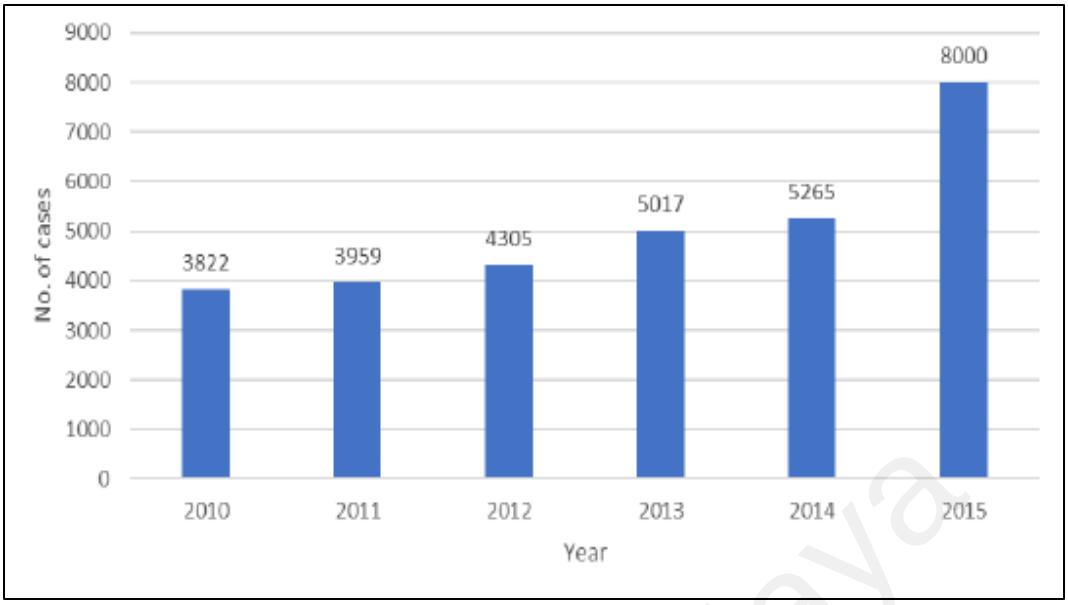

Figure 2.2: Food poisoning cases occurring in schools according to year Adapted from New et al. (2017)

Salmonella are known to survive under any environmental condition and also have an elongated perseverance in various environments (Carrasco et al., 2012). The perseverance of *Salmonella* to adapt in various environments condition has led to the continuous increase in the transmission of *Salmonella* from one environment to another environment (Coburn et al., 2007). *Salmonella* grows within the temperature of 5 °C to 47 °C, and 35 °C to 37 °C for optimal growing temperature. It has been experimented that *Salmonella* grow in several foods between the temperature of 2 °C to 4 °C and 54 °C (Pui et al., 2011b).

In human being diseases such as enteric fever, typhoid fever is caused by *Salmonella* infections. Typhoid fever is transmitted in human by fecal-oral. Also, an exposure to non-typhoid *Salmonella* from food that is contaminated can cause an infection. However, the infection depends on some factors for example consumed bacteria (Hall et al., 2013).

Isolation of *Salmonella* from raw meat, poultry, eggs and dairy products have been reported (Thong et al., 2002). In most cases, *Salmonella* outbreaks are transmitted from source of foods such as fruits, vegetables and so on. The contamination of these foods leads to an outbreak of *Salmonella* (Thong et al., 2002). Similarly, the following issues can also contribute to the outbreak of *Salmonella*; cooking in unhygienic environment, refrigerator, reheating and unclean handling of foods (Gorman & Adley, 2004).

Salmonella diseases at some point happen to be an extreme gastrointestinal sickness such as diarrhea. Diarrhea is as a result of consuming contaminated food substances and it starts between 6-48 hours after the food consumption. Its symptoms and side effects are fever, abdominal pain, fever, chills, nausea, body pain, headache, weight loss and others. (Yang et al., 2016).

Salmonella demonstrated to be fundamental pathogen, influencing the healthiness human being as well as animals on a global scale, thus having a socioeconomic impact. It has been estimated by that 1.3 billion people are infected with this bacterium annually. As indicated by data from the World Health Organization (WHO) estimated that about 21.7 million of infected cases are typhoid fever (Crump et al., 2004).

Recently, in the US, it is estimated that in every year about 40,000 people are infected with *Salmonella* and the frequency is genuinely estimated to be approximately 1.4 million annually with mortality cases of 15,000 and cost the government to spend the sum of approximately 3 billion dollars on victims (Voetsch et al., 2004). Also in Europe as recorded by European Food Safety Authority in 2007 that annually about 151,995 people are affected with *Salmonella* infection (Helwigh & Korsgaard, 2007).

Globally between year 2007 and 2012 survey carried out on *Salmonella* serovars circulation around the world. The results show that 65% of human beings are isolated from enterica serovar Enteritidis. The trend was much more prominent in Europe because about 80% of the cases are reported (Hendriksen et al., 2009). Similarly, *S. enterica* subsp. *enterica* serovar *Typhimurium* with 12% is second type of *Salmonella* isolated from and serovar Newport with about 4% is the third (Majowicz et al., 2010) . The predominance of these serovars could be inferable from their intrusive behavior in animals. From the results, it shows that human being isolation are relatively similar, which is traced and caused by food borne *Salmonella* outbreaks (Langridge et al., 2015).

The major food items that causes this infections are; food items made from milk such as; cheese and ice cream, cold meat, vegetables and fruits (Doyle & Buchanan, 2012). Food contamination occur in several ways in food chain processing, contacts with farm animals such as pets and reptiles with *Salmonella* also causes infections on human (Donado et al., 2014). Water has also known to be medium for transmission of Salmonellosis such as untreated waste water which was used for irrigation farming can contaminate the farm produce (Steele & Odumeru, 2004). The *Salmonella* can easily infect human system through the usage of contaminated water kept in animal reservoirs for either cooking or drinking. But for *S. typhi* and *S. paratyphi* A who are present in animal reservoir, affects human cooking and eating in an environment that is not hygienic (Newell et al., 2010).

Human body resistance to antibiotic is caused when therapeutic and antimicrobial compounds are used in industries for production of animal foods which leads to the presence of *Salmonella*. To avoid the issue of *Salmonella* infections in the both developed and undeveloped countries, there is a need to address the problem posed on food

production (Cooke et al., 2007), substances such as meat, eggs, milk and others. Though different variety of perishable foods such as fruits and vegetables are known to be related with *Salmonella* infections, because they can easily get contaminated (Pui et al., 2011b). *Salmonella* transmission process is in a cyclic form i.e. from the environment to food items, and from food items to human.

The *Salmonella* infection in human are always sporadic, but the case of food borne is not frequent in human (Angulo et al., 2009). Lately, Deng et al. (2012) stated that contaminated peanut butter contained *S. enterica* subsp. enterica serovar Tyhpimurium, which lead to about 741 *Salmonella* infection in human in the US. But in country like Denmark, presently there is a disease outbreak more infectious than *S. enterica* subsp. enterica serovar Typhimurium and yet the source of such outbreak in unknown (Deng et al., 2012).

2.9.3 *Listeria monocytogenes*

L. monocytogenes is a non-spore former, non-capsulated, facultative anaerobic organism belonging to family Listeriaceae within the division Firmicutes (Lester et al., 2007). Recently, the genus *Listeria* is made of 4 clades with varieties of species that comprises of each clade (Den Bakker et al., 2014). The *Listeria* clade comprises of *L. monocytogenes*, *L. welshimeri*, *L. seeligeri*, *L. innocua*, *L. marthii*, *L. ivanovii*. A second clade consists of *L. fleischmannii* together with two new species, *L. aquatica* sp. nov., and *L. floridensis* sp. nov. Third clade comprises of *L. rocourtiae,* and *L. weihenstephanensis*, together with new three species, *L. cornellensis* sp. nov., *L. grandenis* sp. nov., and *L. riparia* sp. nov. Fourth clade consists solely of *L. grayi* (Den Bakker et al., 2014).

Illness caused by *L. innocua, L, grandensis*, and *L.grayi* are very rare (Rapose et al., 2008), prominent species known as true human pathogens are *L.monocytogenes* and *L. ivanovii* (Orsi et al., 2011). *L. invanovii* is more rare than *L. monocytogenes* and predominantly causes disease in ruminant animals (Orsi et al., 2011). However, the first public concerned pathogen is *L. monocytogenes* which was first isolated from rabbits in 1926 and discovered that it exists as saprophyte in the soil and decaying vegetation (Sy et al., 2005).

Based on morphological characteristics, *L. monocytogenes* has the length 1-2 µm and width 0.5 µm, cell appear as short rods with rounded ends (Lester et al., 2007). It is oxidase negative, catalase positive and able to grow at refrigeration temperatures -4.0 ^{o}C and 5 ^{o}C (Giaouris et al., 2014). It has optimum temperature range of between 20 ^{o}C to 25 ^{o}C. The organism possesses peritrichous flagella, which lead to distinctive tumbling motility. However, at temperature 37 ^{o}C, flagellin production is obviously reduced, and motility is limited (Milohanic et al., 2001). When in tryptic soy broth together with yeast extract 0.6% at 30 ^{o}C, with growth permissible pH range recorded from pH 4.5 to 7.0, with minimal growth below pH 4.0 (Saucedo-Reyes et al., 2012). Though the organism grows optimally at water activity (a_w) values >0.97, it is considered to be fairly osmotolerant, can continue to multiply at NaCl concentration as high as 10-12%, and will survive for extended period of time at a_w values as low as 0.83 (Milillo et al., 2012).

Over the years, ready-to-eat foods (RTE) are usually victims in outbreaks of listeriosis. Smoked fish, meat, vegetables are example of foods that get contaminated easily by *L. momocytogenes* (Meloni et al., 2009). In Malaysia, the survey results conducted by Marian et al. (2012) show the occurrence of *L. monocytogenes* in many foods both the raw and RTE foods. Unfortunately, the of food borne listeriosis in Malaysia are yet

unknown and official of food poisoning caused by *L. monocytogenes* in Malaysia (Marian et al., 2012).

In places like North America, Europe, and Japan had reported a large outbreak of listeriosis and the causes of such outbreak were from the following food items raw meat, RTE diary product, and vegetables all consumed (Swaminathan & Gerner-Smidt, 2007). RTE meats can be contaminated in the process of low-level manufacturing stage and *L. monocytogenes* has chances to move to an unsafe level during refrigeration and distribution of RTE products. RTE products pose dangers on human health once the processing facility is contaminated contains *L. monocytogenes* and RTE meats are completely cooked products that do not need to be heat or warm (Swaminathan & Gerner-Smidt, 2007).

The genus *Listeria* consists of *L. monocytogenes*, *L. seeligeri, L. welshimeri, L. ivanovii* and *L. grayi, L. innocua*. The genus *Listeria* is bacteria that causes Listeriosis disease, while the main pathogenic species animals and human is *L. monocytogenes* (Carpentier & Cerf, 2011). The *L. monocytogens* is more severe and surpasses other normal foodborne pathogens, for example, *Clostridium*, *Bacillus* and *Vibrio cholerae* (Liu et al., 2006). Moreover, *L. seeligeri*, *L. welshimeri*, and *L. innocua* species being nonpathogenic was reported by Perrin et al. (2003) to be reason for severe purulent, serious bacteremia and conjunctivitis in humans, suggesting that pathogenicity effects of this agents could result to life-threatening sicknesses.

Being the only species of genus that is of concern for human health, *Listeria monocytogenes* is a ubiquitous bacterium. It causes infection in pregnant women and also affect immuno-compromised of people (Gaul et al., 2012). Severe clinical indicators of

intrusive human listeriosis are septicemia, encephalitis, meningitis, and spontaneous late-term abortion. While sub-clinical indicators are mild influenza-like illness, at times joined with gastroenteritis and additionally visual and cutaneous listeriosis (McLauchlin et al., 2004).

2.9.4 *Escherichia coli*

E. coli is a Gram negative coliform bacteria, facultatively anaerobic and rod-shaped, like *S. enterica*, fits to the Enterobacteriaceae family with recent addition of *Escherichia albertii*, *Escherichia fergusonii, Escherichia vulneris, Escherichia blattae* and *Escherichia hermannii*. Cells of *E. coli* range 2.0µm and 0.25-1.0µm in long and width respectively, with 0.6-0.7 μm^3 of cell volume. *E.coli* is being facultative anaerobic can either be motile by peritrichous flagella or non-motile (Nataro et al., 2011). Serotyping *E. coli* is similar to that of *S. enterica* (O), flagella (H), and capsular (K) antigen profiles can provide sub-classification of the organism; however, only O and H antigens are generally used to define *E. coli* serotypes (MacDonald et al., 2005).

While most *E. coli* exist as commensal part of the gastrointestinal tract of animals and humans (Neil et al., 2011), certain strains have evolved to become pathogenic to hosts (Russo & Johnson 2000). The range in pathogenic mechanisms within the species is diverse, and at least six different pathotypes have been identified to date; these include enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), and diffusely adherent *E. coli* (DAEC). More recently, a large outbreak in Europe involved a strain of *E. coli* O104:H4 that possessed elements that were characteristic of both EAEC and EHEC (Rúgeles et al., 2010). Among the pathotypes, EHECs cause the most severe disease and produce the greatest amount of *E. coli* associated illness in the U.S. Though

strains belonging to several O groups within the EHEC pathotype produce a substantial health burden (Brooks et al., 2005), serotype O157:H7 is the predominantly worldwide source of the debilitating hemolytic uremic syndrome (HUS) associated with EHE. Consequently, since its emergence it has been recognized as an organism of global public health importance (Tarr et al., 2005).

Similar to *S. enterica*, *E. coli* O157:H7 has an optimum growth temperature of 37 °C, though it can survive for up to 9 months at -20 °C, and generally can grow well at temperatures up to 44 °C (Berry & Wells, 2010). Compared to other food borne pathogens, the organism is particularly acid tolerant, and can survive in acidic conditions with a pH as low as 4.0 for up to 56 days. However, the response seems to be dependent on the acidulants and environmental conditions (Biscola et al., 2011). *E. coli* O157:H7 acid tolerance response observed is mediated by the three major acid tolerance mechanisms: a glutamate dependent system, an acid-induced arginine-dependent system, and an acid-induced oxidative system (Lim et al., 2010). The combined action of these systems is especially important for the pathogen's transversal of the stomach during the digestive process for pathogen to endure in acidic foods such as fermented sausage and apple cider (Holck et al., 2011). The pathogen is not particularly salt tolerant, as growth rates noticeable decrease when pathogen is exposed to the 4.5% NaCl, and have been shown to halt at a concentration of ≥8.5% NaCl (Jiang et al., 2002). Though survival of the pathogen is significantly reducing in low a_w foods, and its ideal a_w for growth is 0.95 (Beuchat et al., 2013), its survival for up to 19 weeks at a_w values as low as 0.16 at 5 °C has been documented (Beuchat et al., 2013).

2.9.5 *Bacillus cereus*

Bacillus cereus is a common bacteria of soil and some food strains harmful to humans, Gram-positive, with endospore, motile, aerobic with a rod-shaped like bacterium fitting to family Bacillaceae**.** The genus *Bacillus,* has some significant difference as described in Bergey's Manual of Systematic Bacteriology have been proposed for its classification (Deng et al., 2010). Initially, *Bacillus* species were classified based on only two characters namely aerobic growth and endospore forming nature. However, this resulted in ambiguity, wherein other organisms with unrelated physiology, habitats, ecology and genetic make-up gained place and the level of heterogeneity increased thus making the classification difficult (Deng et al., 2010).

The new methods of analysis have classified the different species into variety of bacteria taxa and have been continuously modified in a dynamic fashion. As a result of this, the group consists of *B. cereus, B. anthracis, B. weihenstephanensis, B mycoides, B. thuringiensis,* and *B. pseudomycoides*. Earlier, these species were considered as distinct from each other, because of their phenotypical differences. Later, studies with molecular biology approaches showed that they have very identical 16S and 23S rRNA sequences, showing that they have deviated from their mutual evolutionary line and be considered as belonging to the same species (Tewari & Abdullah, 2015).

The colonies of *B. cereus* on agar medium have a gloomy or snowy look with irregular and spreading nature. The individual cells are typically large with length 1-3µm and width 1-1.2µm. Motility is with use of peritrichous flagella, often occurring in chains, with DNA mol% G+C of approximately 32-38 (Hyldgaard et al., 2012). The spore formed is central to terminal, ellipsoid or cylindrical that does not extend sporangia. The organism sporulates easily on most media after 2 to 3 days and loses motility during early stages of

sporulation (Pérez-García et al., 2011). L-alanine, glycine or a natural amino acid and purine ribosides are known to prompt germination. The Decimal Reduction Time (D-value) varies approximately from 22 to 30 min at 95°C by moist heat (El-Arabi & Griffiths, 2013). The spores are also resistant to irradiation and desiccants. They have a hydrophobic characteristic and the occurrence of attachments on their surfaces enables them to stick to some forms of exteriors during cleaning and sanitation (Griffiths & Schraft, 2017).

The temperature range for growth is generally between 5 °C and 50 °C. Some strains can develop gradually at 10% sodium chloride concentrations. The least a_w for *B. cereus'* growth is between 0.91 and 0.93. It can also grow above a pH level of 4.4 to 9.3. However, these factors are not completed and are reliant on several aspects as well as the genetic make-up of the strain. It has a condition for amino acids as growing aspects, however vitamins are not very essential, the details of morphological, cultural and biochemical characteristics of *B. cereus* have been well documented in the literature (Griffiths & Schraft, 2017).

Food poisoning caused by *B. cereus* frequently occurs as an effect of spore that survives during cooking or pasteurization process that now germinated and multiplies themselves when food items are inadequately refrigerated. The illness caused by *B. cereus* is under-reported, because both types of manifestations are mild in nature, self-limiting, last less than 24 hours and often does not need medical intervention. Nevertheless, extreme forms of diarrheal type of *B. cereus* have been reported that apart from causing gastrointestinal disorders, it occurs as an infectious ocular pathogen and is known to cause conjunctivitis respiratory tract and wound infections (Guinebretière et al., 2013).

In Japan the emetic type is 10 times more significantly prevalent than the diarrheal type (Granum & Doyle, 2005), while the diarrheal type is predominant in other part of Europe and North America. The percentage of the outbreaks due to *B. cereus* from Japan, North America and Europe vary from 1 to 47% and cases range from 0.7 to 33%. It is approximated annually in US that the *B. cereus* caused about 84,000 cases of food borne diseases at cost of US$43 per person and a total cost of US $36 million. Iceland, Netherlands, Canada, England, Scotland, Wales and Norway have also reported high number of such cases (Iwamoto et al., 2010).

In India, sporadic incidence of *B. cereus* food poisoning has been reported. Various studies have reported the incidence of the organism in foods like milk, milk products, ice-cream, rice spices, vegetables and pulses (Warke et al., 2000). India is being subtropical country that has diverse foods, there is ample chance for majority of the incidences to have gone unnoticed and unreported (Warke et al., 2000).

B. cereus can contaminate wide variety of foods and can live prolonged storage in dried food merchandises. The pattern towards utilization of refrigerated processed foods with lengthy resilience and the growing number of elderly and immune-compromised people doses increase the prominence of *B. cereus* as etiological agent of foodborne disease. Because of the warmth resistance, it is difficult to remove low numbers of spores from foods pasteurization and routine sanitation. Preventing the spores growth and containing the growth of asexual cells could be one of the effective strategies to control the incidence of this organism (McKillip, 2000; Iurlina et al., 2006).

2.9.6 *Clostridium*

The genus comprises of Gram-positive, anaerobic, heat resistant endospore that creates phylogenetical in various organisms. The members of genus *Clostridium* are considered to ally with Gram-positive bacteria that come into existence through evolution (Kim et al., 2012). Nearly 66% Clostridial are mild saprophytes that live in the environment, animals and deteriorating vegetation. The genus consisting of 120 different species, of which 35 are viewed as pathogenic in human and animals. The importance of the genus *Clostridia* is the proteinaceous toxins they produced (Shimizu et al., 2002).

Homology studies using 23S rRNA molecules divided 56 Clostridial species into four main groups. The Group I and II comprises of species that has low G+C content (i.e. 24%-32%). The group III comprises of species with low G+C content that cannot fit into another group. Lastly, the Group IV consists of species that has high G+C content, for example 41%-42% (Guan et al., 2012).

Clostridia produces more toxins than any other bacteria, as a result, above 20 toxins and extracellular enzymes contribute significantly to the virulence of *Clostridium* related species. The presence of virulence genes effects in phenotypic properties such as genetic uncertainty and horizontal gene transfer to non-toxigenic organism leading to dispersion of the toxicgenecity. The potent extracellular toxins produced by the pathogenic *Clostridia* cause cell and tissue to damage, which is a common characteristic of pathogenic *Clostridial* species genus.

Pathogenic *Clostridia* are broadly categorized into three groups, namely; neurotoxic *Clostridia*, histotoxic *Clostridia* and enterotoxic *Clostridia* (Venkataramanan et al., 2013). The classification is based on their host tissue similarity. The neurotoxic *Clostridia*

affect the nervous system. The histotoxic *Clostridia* affect the structural and functional integrity of the host cells. The enterotoxic *Clostridia* affect the gastrointestinal tract (Vignais & Billoud, 2007).

2.10 Bacterial Identification

Bacterial identification which is usually made by cell shape, Gram staining and polymerase chain reaction (PCR) and is very important in various fields. Cell shape and gram staining is commonly used however, they are very time consuming. Whereas, genetic test is expensive and require expertise to run the test. Therefore, these techniques are not ideally suitable for routine identification and alternatives would be welcome for the rapid and low cost identification of microorganisms. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) is becoming a reliable tool for microorganism identification. Despite high initial acquisition costs, this technique can identify bacteria in a few minutes and provide a low cost per sample analysis when compared to conventional methods (Santos et al., 2016).

Numerous investigations have demonstrated that MALDI-TOF MS have shown to outperformed or similar like the conventional methods in time and precision to detect in blood infections in clinical bacteriology (La Scola & Raoult, 2009). Besides, early identification of bacterial hazard that can infect drinking water can be detected using MALDI- TOF MS. In food microbiology, MALDI- TOF MS have been used to identify and group lactic acid bacteria in fermented food, discover bacteria tangled in the decay of milk and pork, detection of bacteria isolated form milk and many more (Barreiro et al., 2010; Nicolaou et al., 2012; Nguyen et al., 2013). Many research has shown that whole cell MALDI-TOF MS have been used as an efficient tool to identify and characterize isolates which originate from specific ecosystems. For example, identification of microbes isolated from sewage sludge, for grouping of bacteria isolated from marine

sponges into different proteotaxanomic groups and for identifying bacteria inhabiting soil contaminated with polychlorinated biphenyl (Singhal et al., 2015).

CHAPTER 3: METHODOLOGY

3.1 Risk profiling

Risk profiling was performed by conducting literature search via online database (Google Scholar, Science Direct and Scopus) for articles related to:

1. Prevalence and epidemiology of foodborne pathogens
2. Foodborne pathogens and risk associated to food waste and compost
3. Survival and persistence of foodborne pathogens against high temperature and moisture

Risk profiling is conducted based on the format of qualitative microbial risk assessment, in which the hazard identification was first performed by listing all the microbial hazards associated with food waste. Then, hazard characterization was conducted to assess the survivability and disease causing ability (dose response) of the identified pathogens. Exposure assessment was the third step where its prevalence in food waste and food waste compost is conducted. Finally, based on the information gathered, the final risk of each pathogen in food waste compost is estimated qualitatively by ranking them as high, medium or low (Table 3.1). For High, a score of 3 is given; medium scored 2; while low scored 1. The sum of scores were tabulated and a sum score of above 8 has a high risk; 6 to 7 has a medium risk; 3 to 5 has a low risk (Table 3.1).

Table 3.1: Risk profiling of pathogen

Score	Risk
9	High
8	High
7	Medium
6	Medium
5	Low
4	Low
3	Low

3.2 Sample collection

Dried grounded package compost (n=3) was collected at University of Malaya Zero Waste Centre and was taken to the laboratory for further analysis. The sample was kept at room temperature and away from direct sunlight exposure.

3.3 Microbial Analysis

Alkaline peptone water (APW) and buffer peptone water (BPW) were used for enhancement of the samples so as to offer an appropriate environment for the bacteria to produce and touch a detectable level for the probable identification. Dilutions of 10^{-1} to 10^{-6} were prepared with the sample by weighing 25g of compost and mixed with 225 ml of APW and BPW broth. The sample was mixed vigorously for 30 minutes in order to homogenize, spread plate method was used for cultivation of bacteria (Bang et al., 2013).

3.3.1 Heterophilic bacteria count, Coliforms and *E. Coli* count and Enterobacteriaceae count

For all this counts, 100 µl of diluted suspension of 10^{-2} to 10^{-6} were pipetted into the media plates (Nutrient agar, CHROMagar^{TM} ECC Chromogenic and Violet Red Bile Glucose Agar) and spread using a sterile hockey steak. Duplicate plates were prepared from each dilution factor 10^{-2} to 10^{-6}. Plates were labeled accordingly with the name of sample, dilution factor and date of plating and then sealed with parafilm® to avoid

contamination, inverted and incubated at 37 ^{O}C for 18-24 hours. The next day, mixed growth on plate was counted and recorded. Purification of pure culture were done by carefully picking colony from mixed plate and sub cultured into new nutrient agar plates and incubated for another 18-24 hours. Then, distinct colony was picked from the plate and stable in agar slant and sealed till when needed.

3.3.2 Fungal count

100 µl of diluted suspension of 10^{-2} to 10^{-6} were pipetted into the media plates (Potato Dextrose Agar, PDA) and spread using a sterile hockey streak. Duplicate plates were prepared from each dilution factor 10^{-2} to 10^{-6}. Plates were labelled accordingly with the name of sample, dilution factor and date of plating and then sealed with parafilm® to avoid contamination, incubated at room tempature for 24-72 hours. After 3 days of incubation, mixture of fungi growth was observed based on colour and apperances, counted and gently picked from the mixture plate to another PDA plates for another 24-72 hours. Pure growth of fungi was picked from plate to slide containing drop of lactophenol blue stain using sterilized inoculating loop. The structure of the mycelium was observed under microscope.

3.3.3 Detection of *Salmonella, Vibrio and Listeria*

For detection, 100 µl of diluted suspension of 10^{-2} to 10^{-6} were pipetted into LB broth for *Salmonella* detection, APW broth for *Vibrio* detection and *Listeria* enrichment broth for *Listeria* detection. The solutions were incubated at 37 ^{O}C for 14 to 18 hours. Next, a loopful of turbid culture was streaked onto the media plate; Xylose Lysine Deoxycholate agar plate, XLD for *Salmonella*; CHROMagar^{TM} *Vibrio* Chromogenic plate for Vibrio and Listeria Identification Agar Base plate, PLACAM for *Listeria*. Duplicate plates were prepared from each dilution factor 10^{-2} to 10^{-6}. Plates were labelled accordingly with the

name of sample, dilution factor and date of plating and then sealed with parafilm® to prevent contamination, inverted and incubated at 37 ^{O}C for 18-24 hours.

3.4 Antimicrobial activity

The Gram positive isolates from food waste compost and the foodborne pathogens (Enteroaggregative *E. coli*, (EAEC-04); Enteropathogenic *E. coli*, (EPEC-09); Enterohemorrhagic *E. coli*, (EHEC-10); Enterotoxigenic *E. coli*, (ETEC-10); Enteroinvasive *E. coli*, (EIEC-12), diffusely stick to *E. coli*, (DAEC-12), B. *cereus*, (BC); *P. mirabilis and Salmonella)* were incubated overnight. The overnight enriched food waste compost isolates and the pathogens were first diluted to reach concentration compatible to 0.5 McFarland standards, which is equivalent to 10^7 to 10^8 CFU/mL. Then, the food waste compost bacterial suspension of the isolates was swabbed onto half of the Mueller Hinton Agar plate (Figure 3.1). The plate was then allowed to dry for 15min on flat surface bench before the pathogen was streaked on another half of the plate (Figure 3.1). The plate was incubated overnight at 37 ^{o}C before it was observed for inhibition of pathogens on the plate. The experiment was conducted in triplicate.

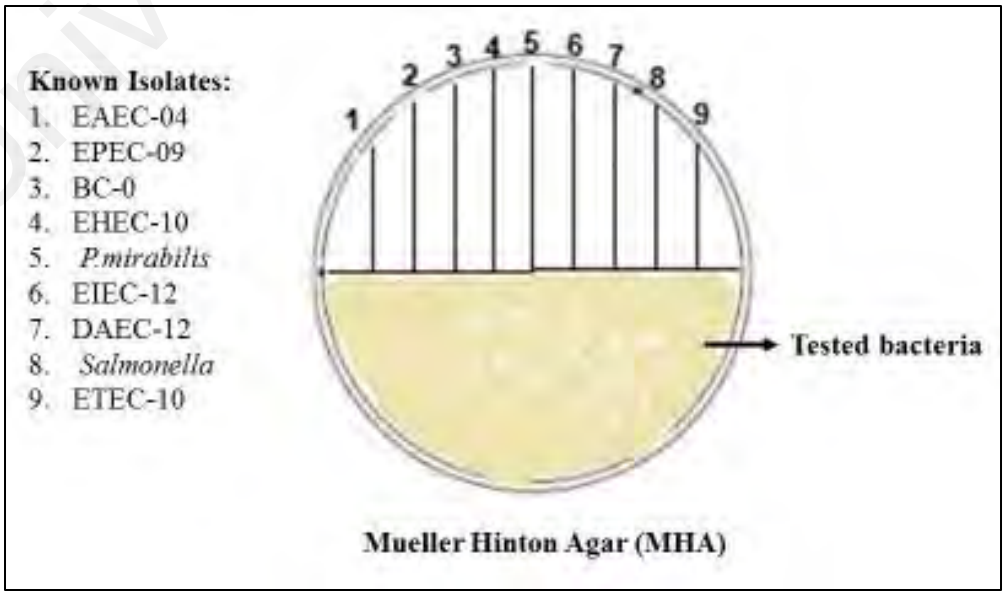

Figure 3.1: Antimicrobial activity

3.5 Bacterial identification

3.5.1 MALDI-TOF approach

Each isolated bacterium was characteracterized by performing Gram staining and microscopy for morphological observaion. Standard method was used for Gram straining and microscopy. For Identification, MALDI-TOF mass spectrometry was utilized for quick identification of all the isolates. Fresh colonies of isolates on Nutrient Agar (Merck, Germany) plates were smeared thinly on the designated wells on the MSP 96 target polished steel BC plate. Subsequently, 1µL of MADI-TOF matrix (with the composition of 10 mg/mL α-cyano-4-hydroxycinnamic acid in 50% acetoonitrile/2.5% trifloroacetic acid) was added to each wells and left for complete desiccastion. Mass spectrometric analysis was performed using a Microflex MALDI-TOF (Bruker Daltonik GmbH, Germany) bench-top mass spectrometer equipped with UV laser at wave length 337 nm coupled with Bruker Flex Control software version 3.3 (Build 108).

Each individual well was bombarded with laser shots and the bacterial MS spectra was analysed in the linear positive ion mode with the mass in range of 2 to 20 kDa using a Bruker MALDI Biotyper Real Time Classification software (Version 3.1, Build 65). The MS spectra was subsequently compared to the available library bacterial MS spectra in the Bruker database and a dedicated assessment system was be calculated to evaluate the accuracy of the identification from the assessment system. A dendogram was constructed using standard MALDI Biotyper MSP creation method (Bruker Daltonics, Germany) to illustrate the graphical distance values between species constructed from their MALDI-TOF reference spectra (Bohme et al., 2011; Pavlovic et al., 2013).

Table 3.2: Score value of MALDI-TOF MS Analysis

Score Value	Description
2.3 – 3.0	Identification is accurate to species level
2.0 – 2.3	Identification is accurate to genus level
1.7 – 2.0	Accuracy of identity is lower than genus
<1.7	No reliable identification/No peak found

3.5.2 16srDNA-sequencing approach

3.5.2.1 Preparation of DNA template for PCR

Boil cell extraction process was used to excerpt crude DNA from bacterial cultures on NA agar plates. Three loopful of distinct pure colonies were suspended in 100 µl of distilled water and vortexed. Then, the suspension was heated at 100 °C for 15 minutes. It was kept at -20 °C for 15min, centrifuged at 800rmp for 60sec, kept back in -20 °C till when needed, 1µl of clear supernatant was used as the DNA template for PCR (Houf et al., 2002).

3.5.2.2 Reaction mixture and cycling condition for amplification 16S rRNA

The total volume of the PCR reaction was 25.0µl that contained, 1× GoTaq™ Green buffer, 1.5Mm $MgCl_2$, 200µl dNTP, 0.5µl of each primer, 1 unit of Taq Polymerase and 1 µl of DNA template (Table 3.2). Cycling conditions of pre-denaturation at 94°C for 7min, 25 cycles of denaturation at 94°C for 2 min, primer annealing at 56°C for 30 min, primer extension at 72°C for 40 min, and a final extension at 72°C for 5min was used for PCR reaction. The products of the reaction were then separated using electrophoresis on 1% agarose gel. Finally, the gels were stained by ethidium bromide (0.5 ug/ml) and destain three times by H_2O and then visualized under UV (Gel Doc™ XR System, BIO-RAD).

Table 3.3: Volume of component used for PCR

Component	Volume (V_1, µl)	10X Reaction (µl)
Buffer	5	50
$MgCl_2$	1.5	15
$dNTP_s$	0.5	5
Primers (27F)	0.5	5
Primers (1492R)	0.5	5
Taq DNA Polymerase	0.1	1
DNA	1	9
ddH_2O	15.9	160
Total Volume	25.0	250

CHAPTER 4: RESULTS

4.1 Microbial Risk Profiling

Hazard identification revealed the prevalence of different food pathogens in variety of food sources. *E. coli* has the highest prevalence ranging from (6% – 93.75%) in salad vegetables and chicken, respectively. Followed by *Clostridium botulinum* (1.7% – 84%) in beef meat and marine fish respectively. *Listeria monocytogens* ranges from (0.32% – 78%) from leafy vegetable to RTE. *Bacillus* sp. ranged (29.33% –72%) from meat and meat product to soil, respectively. *Vibrio* sp. range from (1.7% –55%) from seafood and retail food. *S. aureus* range from salad vegetables and dairy milk range from (4.5% – 47.2%) respectively. *Salmonella* prevalence from eggs and tilapia range from (13.3% – 43.8%).

The pathogens demonstrated variable optimum growth temperature and inactivation temperature that indicate variable potential of survivability of these pathogens during composting process (Table 4.2). *E.coli*, *Salmonella*, *Listeria monocytogens, S. aureus* and *Vibrio* were not detected at average temperature range of 45 ^{o}C to 70 ^{o}C. However from literature, it was confirmed that *Bacillus cereus* and *Clostridium botulinum* were able to survive composting process at temperature range 70 ^{o}C and above.

Table 4.1: Hazard identification

No.	Pathogens	Sources	Prevalence
1	*Listeria monocytogenes*	RTE, lettuce, cabbage, corn, cucumber, potatoes, meat, poultry, seafood, carrot, parsley, watercress and spinach (Marian et al., 2012; Wang et al., 2015; Denis et al., 2016).	Marine fish (10%); Herrera et al. (2006) RTE meat (5.3%); Wang et al. (2015) Leafy vegetables (0.32%); Denis et al. (2016) Raw Vegetables (22.5%); Ponniah et al. (2010) RTE Cereals (78%); Lee et al. (2009) Burgers (33.3%), Minced meat (25%), Sausages (13.3%); Marian et al. (2012) Fermented fish (56%); Hassan et al. (2001)
2	*Stapylococcus aureus*	The udders and teats of cows, unpasteurized milk and cheese, tonsils and skin of pigs, chickens and turkeys often harbor *S. aureus* (Cortimiglia et al., 2016; Boss et al., 2016)	Marine fish (30%); Herrera et al. (2006) Salad vegetable (4.5%); Meldrum et al. (2009) Raw fish and seafood (9%); Boss et al. (2016) Dairy milk (47.2%); Cortimiglia et al. (2016) RTE food (12.5); Yang et al. (2016)
3	*Escherichia coli*	Intestines of man and animal, lettuce, cabbage, corn, cucumber, potatoes, meat, poultry, seafood (Meldrum et al., 2009; Albarri et al., 2017).	Salad vegetable (6%); Meldrum et al. (2009) Aquaculture water (18%); Tajbakhsh et al. (2015) Raw fish and seafood (55%); Boss et al. (2016) Vegetable (62.5%), Meat (56.25%), Chicken (93.75%); Albarri et al. (2017)
4	*Salmonella*	Meat, Eggs and poultry products, celery, watercress, watermelon, lettuce, cabbage, and raw salad vegetables (Pui et al., 2011a; Abatcha et al., 2018)	Chicken Meat (35.83%); Dominguez et al. (2002) Sliced fruit (23.2%); Pui et al. (2011a) Raw vegetable and vegetarian burger patties (28%); Nillian et al. (2011) Catfish (28.1%), Tilapia (43.8%); Budiati et al. (2013) Eggs (13.3%); Tsegaye et al. (2016)

Table 4.1, continued.

			Leafy vegetables (29.5%); Abatcha et al. (2018)
5	*Bacillus* sp.	Soil, decaying organic matter, fresh and marine waters, vegetables and fomites, and the intestinal tract of invertebrates (Chon et al., 2015; Owusu-Kwarteng et al., 2017).	Raw rice (38%); Qureshi et al. (2015) Cooked rice (46%); Qureshi et al. (2015) Salad and sprout (48%); Chon et al. (2015) Soil (72%); Owusu-Kwarteng et al. (2017) Raw milk (47%); Owusu-Kwarteng et al. (2017) Meat and meat products (29.33); Bashir et al. (2017)
6	*Clostridium* sp.	Beef, poultry, gravies, and dried or pre-cooked (Rahimi et al., 2014).	Marine fish (84%); Herrera et al. (2006) Beef meat (1.7%), Buffalo meat (9%), Goat meat (3.3%); Rahimi et al. (2014) Vegetables (30%); Lim et al. (2018)
7	*Vibrio* sp.	Marine and aquatic environments, contaminated seafoods-products including fish, shellfish, oyster, prawn, shrimp, squid and many more fresh water animal (Zhang et al., 2017).	Marine fish (4%); Herrera et al. (2006) Seafood (1.71%); Zhang et al. (2017) Shrimp (20.69%); Jeyasanta et al. (2017) Retail seafood (55%); Vu et al. (2018) Water (26%); Shishir et al. (2018)

Table 4.2: Pathogen characteristics

No.	Pathogens	Optimum temperature	Does high temperature kill	Reported work on survivability in compost
1	*Listeria monocytogenes*	30–37°C	Temperatures above 50 °C is lethal to *L. monocytogenes* (Ryser & Marth, 2007).	-After 3 days of composting at 55 °C, none of these organisms (*E. coli, Salmonella* and *Listeria*) was detectable (Grewal et al., 2006). -During thermophilic stage, lasting existence of seeded *L. monocytogenes* was detected in immature composts collected from only one of four reactors but was never detected during incubation in mature composts (Hassen et al., 2001).
2	*Staphylococcus aureus*	37°C.	*S. aureus* is readily killed during pasteurization or high cooking (Ahmed & Dablool, 2015).	-Increased number recorded at the beginning of composting cycle while, they completely disappeared at the fourth week due to rising of temperature (Hefnawy et al., 2013).
3	*Escherichi coli*	37°C.	Growth inhibited at 45 °C (Hess et al., 2004).	-After 48h of composting at only 45 °C, *E. coli* strain was not detected, though at 25 °C no change in pathogen level was observed (Hess et al., 2004). -*E. coli* was not detected after 48 hours at 45 °C
4	*Salmonella* sp.	35 – 37°C.	Destroyed after being heated to 46.2 °C (Podolak et al., 2010).	- As soon as the temperature reached 60 °C, *Salmonella* vanished completely from compost by the 25th day (Hassen et al., 2001) -At 60 °C and the moisture content between 60–65%, *Salmonella* inactivated within 10h of composting (Ceustermans et al., 2007).

Table 4.2, continued.

				-At temperatures between 60 °C and 70 °C, and within 25h of inoculation no *Salmonellae* could be detected (Jone & Martins, 2003).
5	*Bacillus cereus*	30–40°C.	Destroyed by burning or by autoclaving at a temperature exceeding the boiling point of water, 100 °C (Cléry-Barraud et al., 2004).	-*Bacillus cereus*, was detected after composting for 7 days at temperatures below about 70 °C (Deng et al., 2005). -Temperatures below 100 °C allow the survival of individual spores (Vaitonis & Lukšienė, 2010).
6	*Clostridium botulinum*	35 – 37°C.	Pasteurisation at 70°C for 2min or equivalent) will kill *C. botulinum* bacteria but not its spores (Berger et al., 2010).	-*Clostridium* can survive the composting process (Berger et al., 2010).
7	*Vibrio* sp.	30 and 35°C	Cooking to an internal temperature of 60°C effectively inactivates this organism (Han et al., 2016).	-After 5days of composting at 70°C presence of *vibrio* was not detected (Uddin et al., 2013)

Table 4.3: Hazard characterization and exposure assessment

No.	Pathogens	Diseases caused by pathogenic bacteria	Population at risk	Any cases that it is associated to soil and compost, raw vegetables or farms.
1	*Listeria monocytogenes*	Causes listeriosis, meningitis, encephalitis, meningoencephalitis, central nervous system (CNS) diseases, sepsis, endocarditis, focal infections and gastroenteritis. Miscarriages, stillbirths, preterm labour and even death in new-borns (Maragkoudakis et al., 2009; Jadhav et al., 2012).	Commonly affects babies (abortion or premature babies), pregnant mother and elders with immune system problems (Jadhav et al., 2012.	Case reported associated with corn (Aureli et al., 2000) Case reported associated with ruminant farm (Nightingale et al., 2004) Case reported associated with celery (Gaul et al., 2012)
2	*Staphylococcus aureus*	Commonly affect nostrils, mucous membrane areas like lungs, bronchial, urinary tract, abdomen or intestinal tract (Balasubramaniam et al., 2007; Kluytmans, 2010).	Anyone can develop a *S. aureus* Infection, although certain groups of people are at greater risk, including people with chronic conditions such as diabetes, cancer, vascular disease, eczema, and lung disease (Kluytmans, 2010).	Case reported associated with vermicelli (Huang et al., 2017)
3	*Escherichia coli*	Common diseases are Hemorrhagic colitis, hemolytic uremic syndrome, thrombotic thrombocytopenic purpura (Park et al., 2001; DuPont, 2007; Mor-Mur & Yuste, 2010).	*E. coli* can affect anyone who is exposed to the bacterium but young children and elderly adults have a	Case reported associated with cucumber (Duffell et al., 2003) Case reported associated with lettuce (Söderström et al., 2005)

Table 4.3, continued.

			greater risk of developing a life-threatening form of kidney failure called hemolytic uremic syndrome (Mor-Mur & Yuste, 2010).	Case reported associated with spinach (Wendel et al., 2009) Case reported associated with cookie dough (Neil et al., 2011)
4	*Salmonella* sp.	Mainly associated with salmonellosis infection likes typhoid with symptoms like diarrhoea, fever, headache and abdominal pain. Also causes chronic reactive arthritis (DuPont, 2007)	Children are at the highest risk for Salmonella infection. Young children, older adults, and people with weakened immune systems are the most likely to have severe infections (Farooqui et al., 2009).	Case reported associated with raw almonds (Isaacs et al., 2005) Case reported associated with tomatoes (Greene et al.,2008) Case reported associated with rucola lettuce (Nygård et al., 2008). Case reported associated with drinking water (Farooqui et al., 2009). Case reported associated with raw vegetable (Barton et al., 2011). Case reported associated with ground water (Kovačić et al., 2017).
5	*Bacillus* sp.	Commonly associated with diarrheal and emetic syndrome which causes intoxication with symptoms such as vomiting, dizziness, abdominal pain/ cramps and related to toxic poisoning (Sandra et al., 2012).	Bacillus infection affects young children, older adults, and people with weakened immune individuals (Sandra et al., 2012).	Causative agent of two distinct forms of gastroenteritic disease connected to food-poisoning (Ehling-Schulz et al; 2004).

Table 4.3, continued.

6	*Clostridium botulinum*	People infected with *Clostridium sp*. develop diarrhea and abdominal cramps within 6 to 24 hours (typically 8 to 12 hours). The illness usually begins suddenly and lasts for less than 24 hours. No fever or vomiting, the illness is not passed from one person to another (Su & Liu, 2007; Gambarin et al., 2012).	Everyone is susceptible to food poisoning from *C. botulinum*. The very young and elderly are most at risk of *C. botulinum* infection and can experience more severe symptoms that may last for 1 to 2 weeks. Complications, including dehydration, may occur in severe cases Gambarin et al., 2012).	Case reported associated with carrot **(**CDC, 2006) Case reported associated with chilli sauce **(**CDC, 2006).
7	*Vibrio* sp.	Causes gastroenteritis with symptoms like diarrhea, headache, vomiting, nausea, abdominal cramps and mild fever when raw contaminated, undercooked or post heat-contaminated seafoods are consumed (Feldhusen, 2000).	No at risk groups reported for gastroenteritis**.**	Case reported associated with raw oyster **(**Daniels et al., 2006). Case reported associated with Alaskan oyster **(**McLaughlin et al., 2005). Case reported associated with raw shellfish **(**CDC, 2006). Case reported associated with water **(**Siddiqui et al., 2006).

Table 4.4: Qualitative Risk estimation

No.	Pathogens	Source	Possibility of presence in the source	Chances of surviving the thermal cycle during composting	Severity of disease	Risk
1	*Listeria monocytogenes*	Produce, grain and meats	0.32 – 78% (High)	Low	High	Medium
2	*Staphylococcus aureus*	Dairies, seafoods, meats	4.5-47.2% (Medium)	Medium	Low	Low
3	*Escherichia coli*	Dairies, produce, seafoods and meats	6-93.75% (High)	Low	Medium	Medium
4	*Salmonella*	Produce and meats	13.3-43.8% (Medium)	Low	High	Medium
5	*Bacillus cereus*	Grains and meats	29-72% (High)	High	Low	Medium
6	*Clostridium botulinum*	Produce, seafood and meats	1.7-84% (High)	High	High	High
7	*Vibrio* sp.	Seafoods	1.71-55% (Medium)	Medium	Medium	Medium

4.2 Microbiological profile of mature food waste compost

The food waste composts tested in this study demonstrated high count of heterophilic bacteria (Mean = 2.5×10^4 CFU/g) and fungi (Mean = 3.1×10^4 CFU/g). Although *E.coli,* Enterobacteriaceae and total coliforms were detected in the samples, *Vibrio, Listeria* and *Salmonella* were not detected in the mature food waste compost (Figure 4.1).

Of the 91 isolates collected from the composts, the identification results showed that 35 isolates (38%) were Gram-negative bacteria. Maldi-ToF was only able to identified 21 isolates (23%) of the compost-borne bacteria (Table 4.5). Of these identified bacteria, *Bacillus* sp. was most commonly identified.

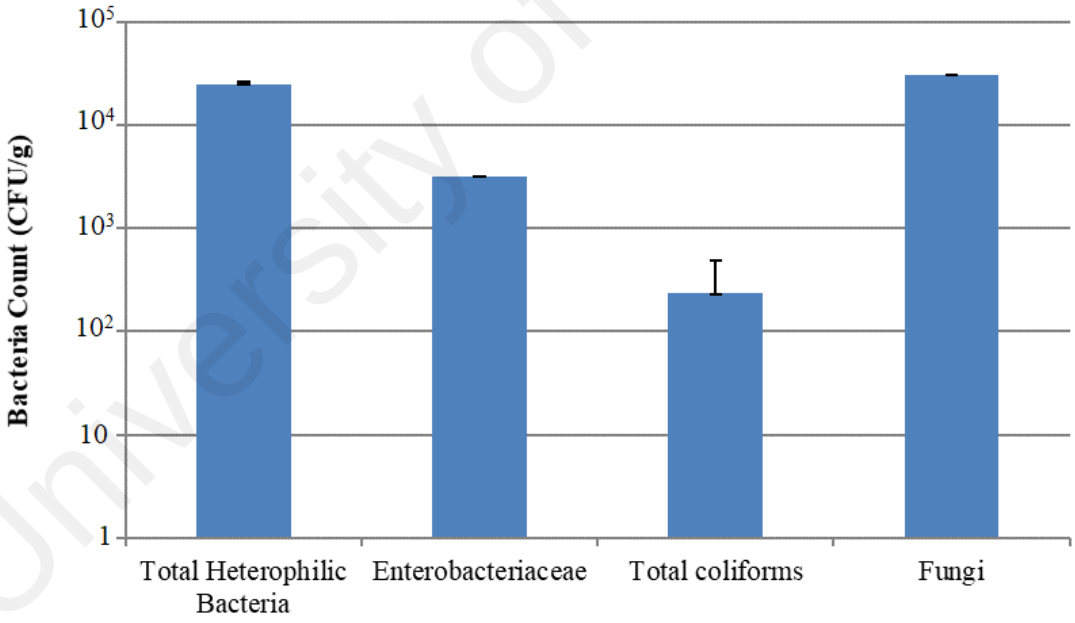

Figure 4.1: Microbiological profile of mature food waste compost

Table 4.5: Microscopy and Biochemical characteristics of the presumptive bacteria isolates

Sample	Serial Number	Media	Gram Stain	MALDI I. D	Score Value
1	1	NA	Rod –ve	*Klebsiella pneumoniae*	2.165
	2	ORI	Rod +ve	NPF	<1.7
	3	ORI	Gram coci	*Staphylococcus haemolyticus*	2.218
	4	ECC	Rod –ve	NPF	<1.7
	5	NA	Coci +ve	NPF	<1.7
	6	VRBG	Coci +ve	NPF	<1.7
	7	NA	Rod +ve	NPF	<1.7
	8	ORI	Coci +ve	NPF	<1.7
	9	VRBG	Rod +ve	*Bacillus licheniformis*	1.953
	10	VRBG	Rod +ve	NPF	<1.7
	11	ECC	Rod –ve	*Citrobacter freundii*	2.193
	12	ORI	Rod –ve	*Klebsiella pneumoniae*	2.653
	13	ECC	Rod –ve	NPF	<1.7
	14	VRBG	Coci –ve	NPF	<1.7
	15	ORI	Rod –ve	*Enterobacter cloacae*	1.976
2	1	NA	Rod -ve	*Klebsiella pneumoniae*	2.407
	2	NA	Rod +ve	NPF	<1.7
	3	NA	Rod +ve	NPF	<1.7
	4	VRBG	Rod +ve	NPF	<1.7
	5	ORI	Rod -ve	NPF	<1.7
	6	ORI	Rod +ve	NPF	<1.7
	7	ECC	Rod -ve	*Eschericha coli*	2.512
	8	VRBG	Rod +ve	NPF	<1.7
	9	VRBG	Rod +ve	NPF	<1.7
	10	NA	Rod -ve	NPF	<1.7
	11	VRBG	Coci -ve	NPF	<1.7
	12	ORI	Coci -ve	NPF	<1.7
	13	ORI	Rod -ve	*Enterobacter aerogenes*	2.236
	14	ECC	Rod +ve	NPF	<1.7
	15	ORI	Coci -ve	NPF	<1.7
	16	VRBG	Rod -ve	NPF	<1.7
	17	ECC	Coci -ve	NPF	<1.7
	18	ORI	Rod +ve,	NPF	<1.7
	19	VRBG	Rod +ve,	NPF	<1.7
3	1	PCA	Rod +ve	NPF	<1.7
	2	PCA	Rod +ve, spore	NPF	<1.7
	3	VRBG	Rod -ve	*Pseudomonas jinjuensis*	2.309
	4	PCA	Rod +ve	NPF	<1.7
	5	PCA	Rod +ve	NPF	<1.7
	6	PCA	Rod +ve	*Bacillus cereus*	1.885

Table 4.5, continued.

7	VRBG	Rod -ve	NPF	<1.7
8	PCA	Rod +ve	NPF	<1.7
9	PCA	Rod +ve	NPF	<1.7
10	PCA	Rod +ve	*Bacillus arsenicus*	1.948
11	PCA	Rod +ve, spore	*Bacillus niacini*	1.739
12	VRBG	Rod -ve	NPF	<1.7
13	ECC	Rod -ve	*Enterobacter cloacae*	2.165
14	ECC	Coci –ve	NPF	<1.7
15	ECC	Coci –ve	NPF	<1.7
16	ECC	Rod +ve	NPF	<1.7
17	ECC	Coci –ve	NPF	<1.7
18	ECC	Rod –ve	NPF	<1.7
19	ECC	Coci –ve	NPF	<1.7
20	VRBG	Rod –ve	NPF	<1.7
21	PCA	Rod +ve, spore	NPF	<1.7
22	PCA	Rod +ve, spore	NPF	<1.7
23	PCA	Rod +ve, spore	*Bacillus cereus*	1.885
24	PCA	Rod +ve	*Bacillus arsenicus*	1.948
25	MAC	Rod +ve, spore	NPF	<1.7
26	MAC	Rod –ve	*Enterobacter cloacae*	2.165
27	NA	Rod +ve	NPF	<1.7
28	NA	Rod +ve, spore	NPF	<1.7
29	NA	Rod +ve	NPF	<1.7
30	NA	Rod –ve	NPF	<1.7
31	VRBG	Rod +ve, spore	*Bacillus cereus*	1.885
32	NA	Rod +ve	*Bacillus luciferensis*	2.127
33	MAC	Rod +ve	NPF	<1.7
34	VRBG	Rod +ve, spore	NPF	<1.7
35	MAC	Rod –ve	NPF	<1.7
36	MAC	Rod +ve	*Bacillus megaterium*	1.749
37	NA	Rod +ve, spore	NPF	<1.7
38	NA	Rod +ve, spore	NPF	<1.7
39	NA	Rod +ve, spore	NPF	<1.7
40	NA	Rod +ve	NPF	<1.7
41	VRBG	Rod +ve, spore	NPF	<1.7
42	VRBG	Rod +ve, spore	NPF	<1.7
43	NA	Rod -ve	*Pseudomonas jinjuensis*	2.309
44	NA	Rod -ve, spore	NPF	<1.7
45	NA	Coci -ve	NPF	<1.7
46	VRBG	Rod +ve, spore	NPF	<1.7
47	NA	Rod +ve, spore	NPF	<1.7

**NPF: No peak found*

4.3 Polymerase Chain Reaction (PCR) results

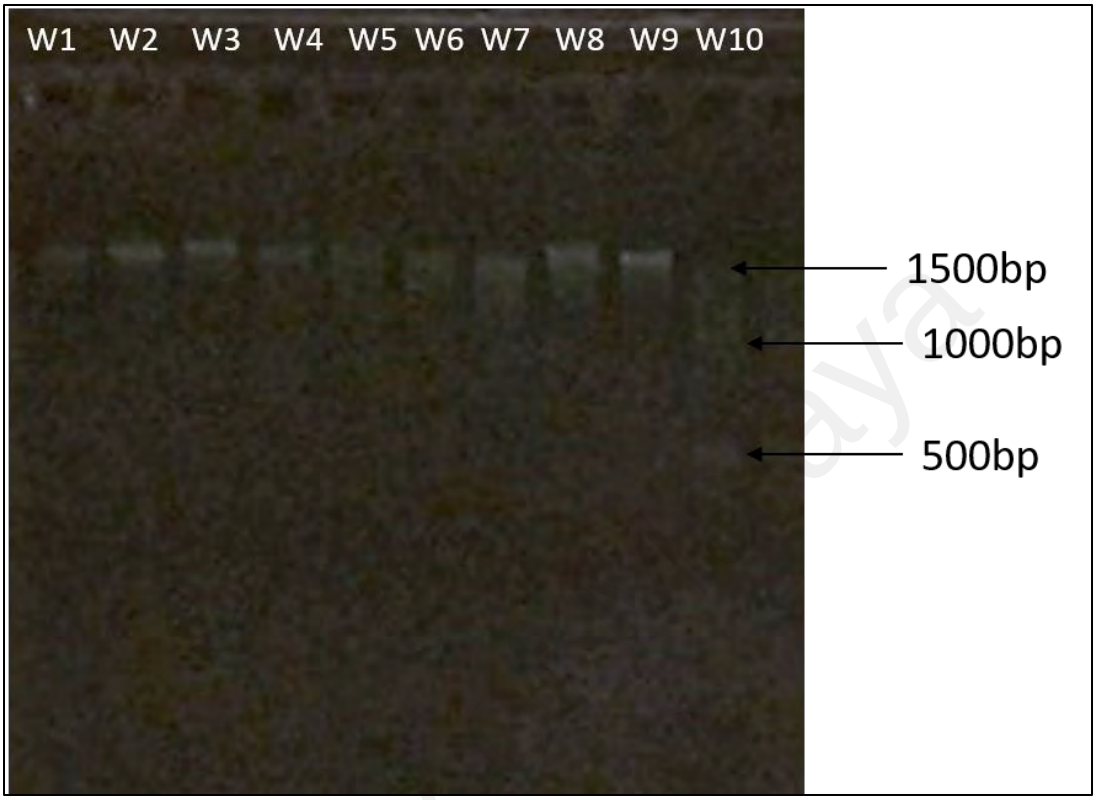

Figure 4.2: Band of PCR for 16S rRNA

Table 4.6: 16S rRNA sequencing results

No	Description	Max score	Total score	Query cover (%)	E-value	Identity (%)	Accession
1	*Enterobacter aerogenes* strain CCFM8313 16S ribosomal RNA gene, partial sequence	2132	2132	89	0.0	99	KJ803870.1
2	*Enterobacter* sp. NCCP-606 gene for 16S ribosomal RNA, partial sequence	1061	1061	100	0.0	97	AB920791.1
3	*Escherichia coli* strain E84-1 16S ribosomal RNA gene, partial sequence	2124	2124	100	0.0	99	KJ477001.1
4	*Klebsiella aerogenes* strain CX-122 16S ribosomal RNA gene, partial sequence	1631	1631	100	0.0	100	MH368434.1
5	*Klebsiella pneumoniae* strain DSM 30104 16S ribosomal RNA gene, partial sequence	2025	2025	100	0.0	99	KX274129.1
6	*Lysinibacillus fusiformis* strain I-12 16S ribosomal RNA gene, partial sequence	1552	1552	100	0.0	100	GQ487630.1

Table 4.6, continued.

7	*Pantoea agglomerans* strain IGCAR-18/07 16S ribosomal RNA gene, partial sequence	745	1393	100	0.0	86	EF523432.1
8	*Shigella dysenteriae* strain CFSAN010956 chromosome, complete genome	1330	9265	84	0.0	98	CP026827.1
9	*Shigella flexneri* strain HBUAS54253 16S ribosomal RNA gene, partial sequence	1622	1622	100	0.0	100	MH817705.1

4.4 Antimicrobial activity of isolated bacteria strains from food waste compost against foodborne pathogens

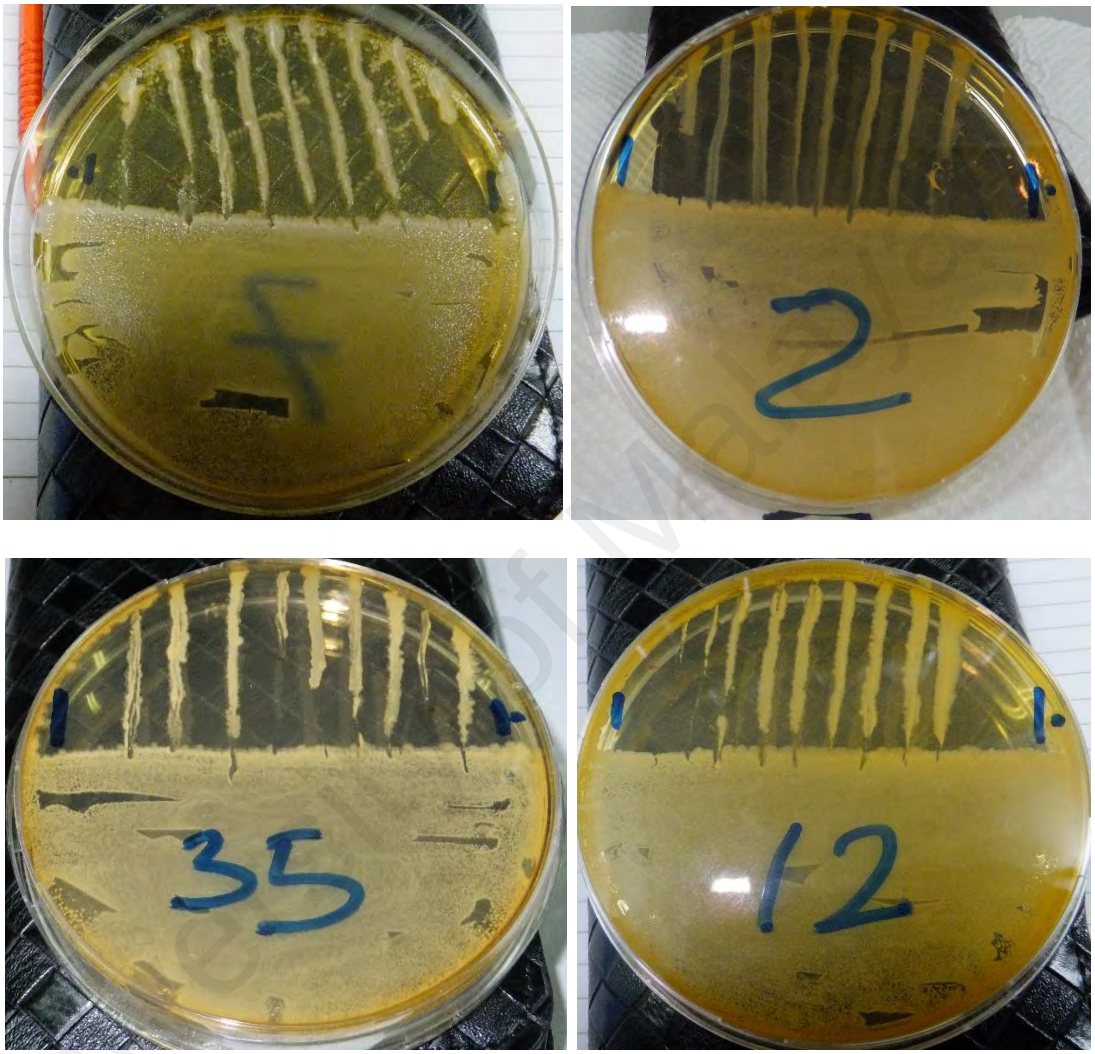

Figure 4.3: Antimicrobial activity plates

All isolates (Table 4.5) were tested to determine the antimicrobial activity pattern of their resistance against nine known pathogen strains (Enteroaggregative *E. coli*, (EAEC-04); Enteropathogenic *E. coli*, (EPEC-09); Enterohemorrhagic *E. coli*, (EHEC-10); Enterotoxigenic *E. coli*, (ETEC-10); Enteroinvasive *E. coli*, (EIEC-12), Diffuse-adhering *E. coli* (DAEC-12), *B. cereus*, (BC); *P. mirabilis* and *Salmonella*. Of all the 91 isolates tested, only nine isolates showed positve activity against the foodborne pathogens tested

(Table 4.7). These nine isolates were identified as *Klebsiella aerogenes, Lysinibacillus fusiformis, Shigella flexneri, Klebsiella pneumoniae, E. coli, Enterobacter aerogenes, Shigella dysenteriae, Enterobacter cloacae* and *Pantoea agglomerans.*

Table 4.7: Antimicrobial Activity test of presumptive bacteria isolates

Isolate from food waste compost		**The average length of inhibiting zone (cm)**								
Code	**Identity**	**EAEC-04**	**EPEC-09**	**EHEC-10**	**ETEC-10**	**EIEC-12**	**DAEC-12**	**BC**	***P. mirabilis***	***Salmonella***
2	*Klebsiella aerogenes*	1.7	-	-	-	-	-	0.8	-	-
3	*Lysinibacillus fusiformis*	-	-	-	-	-	-	-	2.1	-
4	*Pantoea agglomerans*	-	-	-	1.6	-	-	-	-	-
5	*Shigella flexneri*	-	-	-	-	-	-	-	1.9	-
7	*Klebsiella pneumoniae*	1.3	-	-	-	-	-	0.7	-	-
12	*Enterobacter aerogenes*	1.4	-	-	-	-	-	-	-	-
18	*Shigella dysenteriae*	-	-	-	-	0.7	-	-	-	-
20	*Enterobacter cloacae*	-	-	-	-	-	-		1.3	-
35	*E. coli*	-	-	-	-	-	-	0.6	1.8	-

CHAPTER 5: DISCUSSION

In the recent years, fresh food contamination has drawn a boundless attention and interest among public and researchers. Fresh harvest is usually grown in an outdoor environment and is extremely vulnerable to pollution by human pathogens. These pathogens might be existing from agricultural soil, compost as organic fertilizer, handling water, animals surrounding the farms, human labors and many more (Jiang & Dharmasena, 2015). These pathogens from contaminated food waste could be transmitted to the roots and leaves of the plant by dashes from rain water and water systems or settle inside the plant itself through insect nibbles or root uptake (Solomon et al., 2002). Thus, many studies have recorded that fertilizers used for crops from fresh or incompletely compost may result in pathogen contamination and causes outbreak of foodborne diseases (Hanning et al., 2009; Jiang & Shepherd, 2009; Harris et al., 2013).

World Health Organization (2017) estimated that 600 million, or at least one from ten people fall sick and 420,000 deaths occur yearly due to the outbreak of foodborne disease caused by consumption of contaminated food especially fresh food products. Leafy green vegetables and their RTE salads is one of the most consumed fresh produce in regular basis due to their nutritional value which contains enough vitamins, phyto-nutrients and minerals for healthy diet and prevent diseases such as cancer and cardiovascular diseases (Castro-Rosas et al., 2012). However, the increase in production of this product all over the year has been complemented by food safety dangers of cross contamination as they are usually eaten raw (Mir et al., 2018). The cross contamination of these RTE vegetables occurs from various stages from their agricultural farms to their production for consumption. At farm stage, pathogen contamination is caused by the use of polluted water and incomplete composted compost as fertilizer. Whereas, during the production

stage, unhygienic handling, dirty utensils and packaging materials cause further cross-contamination from contaminated produce to other produces (Faour-Klingbeil et al., 2016). The usage of compost as organic fertilizer is undeniably cost effective however, the inadequately treated compost create a major threat of pathogenic contamination for food products (Manyi-Loh et al., 2016).

The compilation of literature search obtained for the qualitative risk estimation (Table 4.4) indicated high prevalence of *C. botulinum* based on its highest possibility of presence in the source (1.7-84%), high chances of surviving the thermal cycle during composting, high severity of disease which categorizes *C. botulinum* as high risk. Several studies have provided data on the geographical distribution pattern, the survival, and the seasonal variability of *C. botulinum* in soils and sediments (Sandler et al., 1993; Lúquez et al., 2007).

The microbiological of matured food waste composts tested in this study showed high count of heterophilic bacteria and fungi. Although Enterobacteriaceae, *E. coli* and total coliforms were detected in the samples, *Salmonella, Listeria monocytogenes* and *Vibrio* sp., were not detected in the matured food waste compost (Figure 4.1). This can be due to the inconsistency in the distribution of moisture content, temperature, aeration, pH and other physical factors during the composting process.

Human pathogens such as *E. coli and Salmonella* are usually present in the intestinal tract of animals and in animal waste used as fertilizer. Whereas, *Listeria monocytogenes* are originated in decaying plants, soil and animal waste, and as a significance contaminator of vegetables growing in farms. Previous studies have reported the survivability of these bacteria in compost which leads to the contamination of fresh

produce. Johannessen et al. (2004) reported the occurance of *E. coli* O157:H7 in compost and slurry used as fertilizer for lettuce farming. Besides, Loncarevic et al. (2005) conducted a survey of organically grown lettuce (n = 179) and reported that *L. monocytogenes* and *E. coli* were found on 2 and 16 samples, respectively, whereas *E. coli* O157:H7 and *Salmonella* were absent. This revealed that when animal compost is used, fresh produce can be contaminated with human pathogens to some extent. *Salmonella* sp. survived in soil samples for 203 to 231 days and in radish and carrot seeds that was sown for 84 and 203 days respectively when grown with compost (You et al., 2006). Similarly, *Salmonella* species have been shown to survive for approximately 200 to 400 days in soil when co-introduced with manure, irrigation water or slurry (Islam et al., 2004).

Ninety-one isolates were recorded from the composts and the results showed that about two-third of the isolates (n=56; 62%) were Gram positive bacteria. Maldi-ToF was only able to identified 21 isolates (23%) of the compost-borne bacteria (Table 4.5). This can be as a result of the bacteria having similar protein content; *Bacillus* sp. is the most commonly identified bacterial genera. This is as a result of its outstanding ability of *Bacillus* sp. to adhere to different conditions. *Bacillus* gains great attention among researchers because it is known to be related not only to outburst of foodborne diseases but also accountable for spoiled food goods. This is due to its nature that contains the largest group of endospore-forming bacteria and has thermophilic nature that grows at temperatures above 55 °C, that is one of the reason why hot compost is a preferred environment for thermophilic *Bacillus* (Nazina et al., 2001). Sung et al. (2002) mentioned that only few heterotrophic spore-forming strains isolated from compost grew above 60 °C from more than 750 that was isolated, and only *Bacillus coagulans* and *Geobacillus stearothermophilus* were isolated at 65 °C.

During composting, many aspects influence the presence of microbial community. However, temperature plays a very significant role during composting process that limits the types of microorganisms, the rate of metabolic activities and species variety. Large variety of mesophilic, thermotolerant and thermophilic aerobic microorganisms, including bacteria, actinomycetes, yeasts and various other fungi have been extensively reported in composts and other self-heating organic materials (Hassen et al., 2001). However, since high count of fungi was obtained from the study, mycotoxin could have also played a significant role in inactivation or inhibition of certain microbes.

From the total isolated bacteria in this work, 10% of the total isolates from compost actually demonstrated antimicrobial activities against selected known pathogens particularly pathogenic *E. coli*. Obviously, the pathogenic microbes estimated so far in composting consist of bacteria, viruses and protozoa. Nevertheless, due to variances in microbial features between and within these groups of organisms, the magnitude to which they survive in the environment or compost depends essentially on the characteristics of the particular organism, the source and chemical composition of manure pH, dry matter, temperature, oxygen, microbial competition and moisture of these materials (Hess et al., 2004).

The presence of anti-pathogen bacteria in compost also plays a significant role in pathogen survival in the compost. In a well-mixed compost to which the input of new nutrients is high, individuals with similar nutritional requirements, such as members of the same population, will be in competition for acquisition of these nutrients as they become depleted by the growing population. This led to production of antimicrobial compound as mechanism to inhibit or kill other surrounding bacteria to compete for

nutrient and becomes too dominant. Such pathogen can be further explored for artificial inoculation into compost to enhance inactivation of foodborne pathogens in compost.

CHAPTER 6: CONCLUSION

In conclusion, this study showed that improperly composted food waste could pose a significant public health risk if the compost is used for food crops farming. Although the thermophilic stage during the composting has been thought to be the major inactivation mechanisms of pathogenic microorganisms, the survival of pathogens during the composting process could be very complex and involves numerous physicochemical factors as well as the antagonistic effect of other microorganisms present in the compost. This study found that the mature food waste compost is microbiologically active with an average count of heterophilic bacteria at 2.5×10^4 CFU/g and fungi count at 3.1×10^4 CFU/g. Nonetheless, the food waste composts tested were also found to contain 1.6×10^4 CFU/g of Enterobacteriaceae and 1.1×10^4 CFU/g of total coliforms, indicating failure to inactivate all facal related bacteria during food waste composting process. However, *Vibrio, Listeria* and *Salmonella* were not detected in the mature food waste composts tested in this work. The public health risk of the food waste compost produced is considerably low. Other than the thermophilic phase of composting, the presence of bacteria that has antagonistic activity against foodborne pathogens could contribute to the inactivation of these pathogens in the food waste compost. These beneficial bacteria could be further explored for application to enhance the inactivation rate of pathogenic bacteria during food waste composting.

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