**PUNJAB AGRICULTURAL UNIVERSITY, LUDHIANA**

**Synopsis of dissertation problem of Post-Graduate student: Ph.D**

Name of the Student: BHAVISH SOOD Admission No. : L-2012-BS-72-D

Major Subject: MICROBIOLOGY Minor Subject : BIOCHEMISTRY

Major Advisor: Dr. (Mrs.) Param Pal Sahota

1. **Title**

**DEVELOPMENT OF DIAGNOSTIC PROTOCOL FOR ASSESSMENT OF MICROBIOLOGICAL QUALITY OF FRESH VEGETABLES**

1. **Introduction**

Fresh produce is popular worldwide because it is recognized as an important source of nutrients, vitamins and fibre for humans. Vegetables are an important part of the daily diet and average family in Punjab takes about 388.8g vegetables as daily dietary intake (Bains and Shruti 2007). In urban areas, almost 38 per cent of vegetables intake is consumed as raw, whereas in rural areas 15 per cent of the vegetables are consumed raw. However, the quality of vegetables in terms of bacterial load is a matter of concern. It has been well established that food-borne bacterial pathogens use plants as vectors between animal hosts, all the while following the life cycle script of plant-associated bacteria. Similar to phytobacteria, *Salmonella*, pathogenic *Escherichia* *coli*, and cross-domain pathogens have a foothold in agricultural production areas. The commonality of environmental contamination translates to contact with plants. Absence of kill steps against human pathogens for fresh produce, arrival on plants leads to persistence and the risk of human illness.

Since most fresh produce receives minimal processing and is often eaten raw, pathogen contamination can represent serious risk. Further, cutting, slicing or peeling cause tissue damage which releases nutrients and facilitates growth of microorganisms (Harris *et al* 2003).

The occurrence of foodborne illness from contaminated fresh produce challenged the belief that such disease was linked to the consumption of foods of animal origin, including meat, poultry, eggs, and milk. Early epidemiological investigations of produce as a source of infection were triggered mostly by the increased isolation of a rare species or serovar of enteric pathogens from clinical patients; thus, outbreaks from common types of pathogens may have remained undetected (Tauxe 1997). Since the early 1990s, awareness of the potential of fresh produce to cause foodborne disease has increased, and reported outbreaks associated with this commodity have grown steadily (Sivapalasingam 2004). Several studies have demonstrated the presence of foodborne pathogenic bacteria on crops grown in soil to which naturally or artificially contaminated manure was applied (Solomon and Matthews 2006).

The use of improperly composted manure or the feces from free roaming domestic or wild animals in the fields, enhance the risk of microbial contamination of crops. Additionally, poor hygiene practices by field-workers and a lack of on-site sanitation facilities may result in produce-associated outbreaks, particularly enteric illness such as shigellosis, which is easily contracted from human feces because of the low infectious dose of the causal agent, *Shigella* (Montville and Matthews 2005). Crop management practices such as irrigation and application of pesticides with contaminated water also are considered as primary sources of inoculum in the field. This is of particular concern for production of fruits and vegetables in areas where the supply of fresh water is scarce, and where water reclaimed from effluents increasingly serves for agricultural purposes. *E. coli* and *S. enterica* survive well in water sediments (Gerba and McLeod 1976; Hendricks 1971) seasonal flooding of fields with overflowing stream water adds to the risk factors of potential crop contamination. The emergence of outbreaks of human diseases linked to the contamination of produce is likely one of the most important problems that face horticultural production in the current century.

Epidemics of foodborne disease are not only a threat to public health but also erode consumer confidence in the causal food product and thus, impact the economic viability of the industry. A recent report by the Centers for Disease Control and Prevention, USA which revealed that contaminated produce caused 46% of the individual cases of foodborne illness in the United States between 1998 and 2008 confirmed that the risk of acquiring infections from produce is high and persisting despite increased awareness and prevention measures taken by producers and processors (Painter *et al* 2013).

Traditional biochemical and immunochemical methods for the detection of microorganisms in food have been supplemented by a number of DNA based methods during the last decade (Olsen 1995). Multiplex PCR based detection technique save time and minimize the expense on detection of food borne pathogens (Bottero *et al* 2004).

**2.1 Knowledge gaps:**

1. Database regarding food borne pathogens in raw vegetables is lacking
2. Safe, effective, and economical biochemical and DNA based detection kits for food borne pathogens need to be devised.

**2.2 Objectives:**

The present study is planned with the following objectives:

1. To undertake Epidemiological Surveillance of routinely consumed raw vegetables.
2. Biochemical and molecular characterization of the isolates and preparation of antibiogram.
3. Distribution of the virulence factors among the food borne pathogens.
4. Development of rapid and sensitive kit for testing quality of food based on biochemical properties and multiplex PCR detection.

**3. Expected new knowledge**

The studies will provide detailed information on the:

1. Prevalence of food borne pathogens on fresh vegetables.
2. Distribution of the virulence factors among the food borne pathogens.
3. Standardization of the detection method for food pathogens.

**4. Review of literature**

Fresh vegetables are an essential part of the diet of people around the world and their consumption is increasing rapidly due to their health benefits and changes in people lifestyles (Huxley *et al* 2004; Ajlouni *et al* 2006). The trend to consume fresh produce including vegetables has grown tremendously over the last few decades (López-Gálvez *et al* 2009). However, along with the increase in fresh vegetable consumption, concerns about the safety of consumers have risen, as presence of spoilage bacteria, yeasts and molds and pathogens is common in these foods (Zhang and Farber 1996; Seymour *et al* 2002).

More than 90 percent of the cases of food poisoning each year are caused by *Staphylococcus aureus*, *Salmonella*, *Clostridium perfringens*, *Campylobacter*, *Listeria monocytogenes*, *Vibrio parahaemolyticus*, *Bacillus cereus*, and Entero-pathogenic *Escherichia coli*, *Proteus*. Johnston *et al* (2005) studied the prevalence of selected pathogens in 398 produce samples (leafy greens, herbs, and cantaloupe) collected from the southern United States were checked through production and the packing shed and assayed by enumerative tests in accordance with U. S. Food and Drug Administration and found that for all leafy greens and herbs, geometric mean indicator levels ranged from 4.5 to 6.2 log CFU/g (aerobic plate count); less than 1 to 4.3 log CFU/g (coliforms and *Enterococcus*); and less than 1 to 1.5 log CFU/g (*E. coli*). However, for cilantro and parsley, total coliform levels increased during the packing process. For cantaloupe, microbial levels significantly increased from field through packing, with ranges of 6.4 to 7.0 log CFU/g (aerobic plate count); 2.1 to 4.3 log CFU/g (coliforms); 3.5 to 5.2 log CFU/g (*Enterococcus*); and less than 1 to 2.5 log CFU/g (*E. coli*). The prevalence of pathogens for all samples was 0, 0, and 0.7% (3 of 398) for *L. monocytogenes, E. coli* O157:H7, and *Salmonella,* respectively. This study demonstrates that each step from production to consumption may affect the microbial load of produce and reinforces government recommendations for ensuring a high-quality product.

The microbiological quality of ready-to-use (RTU) vegetables, including chopped lettuce, salad mix, carrot sticks, cauliflower florets, sliced celery, coleslaw mix, broccoli florets, and sliced green peppers was determined before and after processing and found up to a 1-log decrease in aerobic colony counts after processing with the exception of green peppers. These counts increased to preprocessing levels after 4 days of storage at both 4 and 10°C. Increased levels of *Listeria monocytogenes* in RTU vegetables were associated with temperature abuse. Levels of >100 MPN/g for *L. monocytogenes* were detected in 8 of 120 (6.7%) samples stored at l0°C but not in 175 samples stored at 4°C after 7 days. Overall, *L. monocytogenes* was detected in 13 of 120 (10.8%). *E. coli* was detected in 2 of the 120 (1.7%) processed RTU vegetables after day 7 of storage at 10°C and 1 of the 65 (1.5%) unprocessed vegetables from the same batches of vegetables used for processing (Odumeru *et al* 1997).

Eni *et al* (2010) examined the microbial quality and safety of fruits (sliced and intact) and vegetables (intact) in Nigeria and identified 9 bacteria belonging to 8 genera. These included *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Klebsiella* spp., *Salmonella* spp., actinomycetes, Pseudomonas spp. and *E. coli*. Viswanathan and Kaur (2001) tested the microbial quality of 120 produce samples consisting of whole fresh vegetables, fresh-cut fruits and sprouts collected from street vendors in India. They isolated *P. aeruginosa*, non-pathogenic *E. coli*, *Staphylococcus aureus*, *Enterobacter* spp. and *Salmonella* spp., from 77 (64.2%), 47 (39.2%), 70 (58.3%), 55 (45.8%) and 34 (28.3%) of the samples, respectively.

The number of outbreaks caused by foodborne pathogens associated with fresh produce consumption for 1973 through 1997 reported to the Centers for Disease Control and Prevention. A total of 190 produce-associated outbreaks were reported, associated with 16,058 illnesses, 598 hospitalizations, and eight deaths for 1973 through 1997. Among produce-associated outbreaks, the food items most frequently implicated included salad, lettuce, juice, melon, sprouts, and berries. Among 103 (54%) produce-associated outbreaks with a known pathogen, 62 (60%) were caused by bacterial pathogens, of which 30 (48%) were caused by *Salmonella.* During the study period, *Cyclospora* and *Escherichia coli* O157:H7 were newly recognized as causes of foodborne illness.

Enterotoxigenic *E. coli* is a common cause of travelers’diarrhea, an illness sometimes experienced when visiting developing countries. Raw vegetables are thought to be a common cause of travelers’diarrhea. A prospective study of 73 physicians and 48 family members attending a conference in Mexico City in 1974 revealed that enterotoxigenic *E. coli* was the most common cause of illness (Merson *et al* 1976). Fifty nine participants became ill from eating salads containing raw vegetables.

There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to Salmonella (Bhunia 2008). Outbreaks of salmonellosis have been linked to a wide variety of fresh fruits and vegetables including apple, cantaloupe, alfalfa sprout, mango, lettuce, cilantro, unpasteurized orange juice, tomato, melon, celery and parsley (Pui *et al* 2011b)

Minimally processed vegetables and sprouts are often contaminated with enterotoxigenic strains of Staphylococcus aureus. Of 345 examined samples, 40 samples (11.6%) were contaminated with S. aureus. A total of 25 enterotoxigenic S. aureus strains were biotyped and their resistance to antibiotics was examined. Most isolated strains produced Staphylococcal enterotoxin A (SEA) (n=23) followed by Staphylococcal enterotoxin I (SEI) and Staphylococcal enterotoxin G (SEG) and mainly belonged to the human biotype (88%) (Youn *et al* 2010).

*Campylobacter jejuni* and *Campylobacter coliare* a leading cause of bacterial enteritis. While consumption of contaminated food of animal origin, particularly poultry, is largely responsible for infection, Campylobacter enteritis has also been associated with lettuce or salads. Some strains of C. jejuni produce heat labile enterotoxin (CJT) (Ruiz-Palacios *et al* 1983). The symptoms of the disease are diarrhea, sometimes bloody, abdominal pain, occasionally fever and vomiting.

Outbreaks of *E. coli*, *L. monocytogenes* and *Salmonella* and *Bacillus cereus* have been linked to the consumption of contaminated raw salad vegetables (Zhuang *et al* 1995; Beuchat 1996), raw tomatoes (Beuchat 1996) and raw vegetable seed sprouts (Beuchat 1996), respectively. A variety of fresh fruits and vegetables have been used as vehicles for these pathogens in foodborne outbreaks (Bryan 1988; Beuchat 1996).

Yersiniosis due to infection with the bacterium *Yersinia enterocolitica* is the frequently reported zoonotic gastrointestinal disease after campylobacteriosis and salmonellosis in many developed countries, especially in temperate (Rahman *et al* 2011). The high prevalence of gastrointestinal illness including fatal cases due to yersiniosis is also observed in many developing countries like Bangladesh (Butler 1984), Iraq (Kanan and Abdulla 2009), Iran (Soltan-Dallal and Moezardalan 2004) and Nigeria (Okwori 2009), which indicates major underlying food safety problems in low- and middle-income countries. Worldwide, infection with *Y. enterocolitica* occurs most often in infants and young children with common symptoms like fever, abdominal pain, and diarrhea, which is often bloody.

*Aeromonas* spp. were first considered as possible causative agents of human gastroenteritis more than 30 years ago (Lautrop 1961).The presence of *Aeromonas* in drinking water, fresh and saline waters, brackish water and sewage has been demonstrated on a global scale. Cytotoxic strains have been isolated from a wide range of sea foods, meats and poultry as well as from seed sprouts, lettuce or salad greens, mixed raw vegetables, parsley, and carrots.

Zhou *et al* (2013) developed Multiplex-PCR method for simultaneous detection of six pathogens associated with food poisoning outbreaks, including *Salmonella enterica*, *Escherichia coliO157:H7*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Shigella* spp., and *Campylobacter jejuni* using GeXP analyzer with the detection limit of the GeXP-PCR assay to be 420 CFU/mL for *Salmonella*, 93 CFU/mL for *E. coli O157:H7*, 310 CFU/mL for *L. monocytogenes*, 270 CFU/mL for *S. aureus*, 85 CFU/mL for *Shigella* spp. and 66 CFU/mL for *C. Jejuni*. For each of the six pathogens, GeXP-PCR assay achieved a sensitivity of 101-102 CFU/mL for both a single bacterial target and when all the six pre-mixed bacterial targets were present.

**IPR search:** IPR search on the topic under investigation was carried out using WIPO,USPTO, IPAIRS version 2.0 and no relevant material has been found with respect to this research problem.

**5. Technical programme**

**Experiment No.1**

1. **Name of the experiment:** Epidemiological surveillance for food borne pathogens and database generation for the quality of freshly consumed vegetables.
2. **Location:** Department of Microbiology, PAU, Ludhiana
3. **Methodology:** The fresh produce vegetables (carrot, raddish, cucumber, cabbage, tomato, spinach, long melon) from vegetables growing belt of Ludhiana and Malerkotla will be collected into sterile poly bags, stored in ice and transported to the laboratory for analysis immediately and/or stored at 40C for upto 2 days until use. The isolates will be identified using enrichment techniques.
4. **Observations to be recorded:** Samples will be analysed for
5. ***Escherichia coli***
6. ***Aeromonas hydrophila***
7. ***Yersinia enterocolitica***
8. ***Salmonella spp.***
9. ***Shigella spp.***
10. ***Staphylococcus aureus***
11. ***Campylobacter jejuni***
12. ***Listeria monocytogenes***
13. ***Klebsiella spp.***
14. ***Vibrio spp.***
15. ***Enterobacter sakazakii***
16. ***Erwinia spp.***
17. ***Clostridium perfringens***
18. ***Bacillus cereus***

Screening and prevalence of region specific pathogens amongst these isolates will be further studied.

1. **Statistical analysis:** NIL

**Experiment No.2**

**Name of the experiment:** Biochemical and Molecular characterization of Food borne pathogens.

1. **Biochemical characterization**

**Location:** Department of Microbiology, PAU, Ludhiana

**Methodology:** Isolates characterized on the basis of biochemical, serological, antibiogram susceptibility and haemolytic activity.

**Observations to be recorded:** Following observations will be recorded

1. **Biochemical activities (Carbohydrate utilization, indole, Methyl red, Voges Proskauer’s, Citrate utilization, ONPG decarboxylase and Esculin Hydrolysis)**
2. **Serological tests**
3. **Antibiogram (Broad Spectrum Antibiotics)**

**Statistical analysis:** Nil

1. **Molecular characterization**

**Location:** Department of Plant pathology, PAU, Ludhiana

**Methodology:** Molecular characterization of indigenous isolates will be carried out on the basis of virulence genes as listed below:

1. ***Escherichia coli*** *(stx1,stx2,cdt,cdtB,cnf1,univcnf,cvaC,east1, ehxA, hlyA,eltA,estII,estI)*
2. ***Aeromonas hydrophila*** *(alt,act,ast,hlyA,ail, aerA)*
3. ***Yersinia enterocolitica*** *(ystA,ystB,ystC,ail,yadA,inv,virF)*
4. ***Shigella spp.*** *(ipaBCD, ipaH,invE,ipaR)*
5. ***Salmonella spp.*** *(**sipC,hilA,invA**)*
6. ***Clostridium perfringens*** *(cpb2,cpe,etx,netB,tpeL**)*
7. ***Vibrio spp.*** *(tcpA, toxR, nanH**)*
8. ***Bacillus cereus*** *(**cer A, cerB, hblCDA,nheABC,cytK,entKM**)*
9. ***Cronobacter sakazaki*** *(nanAKTR)*
10. ***Klebsiella spp.*** *(Capsular K1,K2 identification by magA, k2A)*
11. ***Campylobacter jejuni*** *(cdt, iam, flaA,flab)*
12. ***Listeria monocytogenes*** *(prfA,plcA,actA,inlA,inlB,hylA,iap)*
13. ***Staphylococcus aureus*** *(spa,,sek,aroA)*

**Observations to be recorded:** PCR amplification profile for individual virulence genes from respective pathogens will be generated. The amplification data will be analyzed using NTSYS software. Furthermore, amplification products will be cloned, sequenced and compared with the sequence of reference isolates. Multiple sequence alignment will be done using BLASTn and CLUSTAL OMEGA online software resources.

**Statistical analysis:** Nil

**Experiment No. 3**

1. **Name of the experiment:** Development of effective reliable detection kit for testing quality of food based on biochemical properties and Multiplex PCR
2. **Biochemical based testing kit**
3. **Multiplex PCR for prevalent pathogens**
4. **Location:** Department of Microbiology & Department of Plant pathology, PAU, Ludhiana
5. **Methodology:** Biochemical based testing kit will be developed for presumptive inference of food borne pathogens. The sequence will be used to design PCR primers for multiplex PCR kits**.**
6. **Observations to be recorded:** Efficacy of kit and assay procedure will be determined by validating the kits.
7. **Statistical analysis:** Standard error will be analyzed.
   * 1. **SCHEDULE WORK-FLOW DIAGRAM**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S. No** | **ACTIVITY** | **SEM-II** | | | | | | **SEM-III** | | | | | | **SEM-IV** | | | | | | **SEM-V** | | | | | | **SEM-VI** | | | | | |
| **J** | **A** | **S** | **O** | **N** | **D** | **J** | **F** | **M** | **A** | **M** | **J** | **J** | **A** | **S** | **O** | **N** | **D** | **J** | **F** | **M** | **A** | **M** | **J** | **J** | **A** | **S** | **O** | **N** | **D** |
| **1** | **Submission of synopsis** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **2** | **Epidemiological surveillance for food borne pathogens and database generation for the quality of freshly consumed vegetables.** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **3** | **Biochemical and Molecular characterization of Food borne pathogens** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **4** | **Development of effective reliable detection kit for testing quality of food based on biochemical properties and Multiplex PCR** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **5** | **Thesis writing** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **6** | **Thesis seminar** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **7** | **Rough thesis submission** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **8** | **Final submission** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

**7. Statistician**

**Consent of Statistician:**

**Dr. Mohammed Javed**

**(Associate Professor)**

**References**

# Ajlouni S, Sibrani H, Premier R and Tomkins B (2006) Ultrasonication and fresh produce (cos lettuce) preservation. *J Food Sci* 71 (2): 62-68.

# Bains K and Shruti (2007) Analysis of various vegetable preparations for calcium, iron and zinc intake of Punjabi and urban rural families. *IJNPR* 6 (2): 106-10.

Beuchat L R (1996) Pathogenic microorganisms associated with fresh produce. *J Food Prot* **59:** 204–16.

Bhunia A K (2008) Foodborne microbial pathogens: Mechanisms and pathogenesis. *Food Sci Nut* **8**: 276.

Bottero M T, Dalmasso A, Soglia D, Rosati S, Decastelli L and Civera T (2004) Development of multiplex PCR assay for the identification of pathogenic gene of *E. coli* in milk and milk products*. Mol Cell Probes* **18**: 283-88.

Butler T, Islam M and Islam M R (1984) Isolation of *Yersinia enterocolitica* and *Y. intermedia* from fatal cases of diarrhoeal illness in Bangladesh *Transactions of the Royal Society of Tropical Medicine and Hygiene* **78(4)**: 449–50.

Eni A O, Oluwawemitan I A and Solomon O U (2010) Microbial quality of fruits and vegetables sold in Sango Ota, Nigeria. *Afr J Food Sci* **4**: 291-96.

GerbaC Pand McLeod J S (1976) Effect of sediments on the survival of Escherichia coli in marine waters. *Appl Environ Microbiol* **32**(1): 114–120.

Harris L J, Farber J N, Beuchat L R, Parish M E, Suslow T V, Garrett E H and Busta F F (2003) Outbreaks associated with fresh produce: incidence, growth and survival of pathogens in fresh and fresh-cut produce. *Comp Rev Food Sci Food Safety* **2**: 78-141.

**Hendricks C W** (1971) Increased recovery rate of salmonellae from stream bottom sediments versus surface waters. *Appl Microbiol* **21:** 379-80.

# Huxley R R, Lean M, Crozier A, John J H and Neil H A W (2004) Effect of dietary advice to increase fruit and vegetable consumption on plasmaflavonol concentrations: results from a randomized controlled intervention trial. *J Epidemiol Commun Health* 58: 288-89.

# Johnston L M, Jaykus, L A, Moll D, Martinez M C, Anciso J, Mora B and Moe C L (2005) A Field Study of the Microbiological Quality of Fresh Produce *J Food Prot* 68 (9): 1840-47.

Kanan T A and Abdulla Z A (2009) Isolation of *Yersinia spp.* from cases of diarrhoea in Iraqi infants and children. *East Mediterr Health* **15** (2): 276–84.

Lautrop H (1961) *Aeromonas hydrophila* isolated from human faeces and its possible pathological significance. *Acta Pathologica Microbilogica Scandinavica* **51**: 299–301.

López-Gálvez F, Allende A, Selma M V and Gil M I (2009) Prevention of Escherichia colicross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. *Int J Food Microbiol* **133** (1-2):167-171.

Merson M H, Morris G K, Sack D A, Wells J E, Feeley J C, Sack R B, Creech W B, Kapikian A Z, Gangarosa E J (1976) Travelers’diarrhea in Mexico. *N Engl J Med* **294**: 1299-305.

Montville T J, Matthews K R (2005) *Food microbiology: An introduction.* Mossel, D A and Van P A Netten (Eds.) Pp- 607-25 ASM Press, Washington D.C.

# Odumeru J A, Mitchell S J, Alves D M, Lynch J A, Yee A J, Wang S L, Styliadis S and Farber J M (1997) Assessment of the Microbiological Quality of Ready-To-Use Vegetables for Health-Care Food Services. *J Food Protect* 60 (8): 954-60.

Okwori A E J, Martínez P O, Fredriksson-Ahomaa M, Agina S E and Korkeala H (2009) Pathogenic Yersinia enterocolitica 2/O:9 and Yersinia pseudotuberculosis 1/O:1 strains isolated from human and non-human sources in the Plateau State of Nigeria. *Food Microbiol* **26** (8): 872–75.

Olsen J E, Aabo S, Hill W, Notermans S, Wernars K, Granum P E, Popovic T, Rasmussen H N and Olsvik (1995) Probes and Polymerase chain reaction for detection of food-borne bacterial pathogens. *Int J Food Microbiol* **28**: 1-78.

Painter J A, Hoekstra R M and Ayers T, et al. (2013) Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008*. Emerg Infect Dis* **19**:407–15.

Pui C F, Wong W C, Chai L C, Nillian E, Ghazali F M, Cheah Y K, Nakaguchi Y, Nishibuchi M and Radu S (2011b) Simultaneous detection of *Salmonella spp.,* *Salmonella typhi* and *Salmonella typhimurium* in sliced fruits using multiplex PCR. *Food Control* **22**: 337-42.

Rahman A, Tania S B, Stonsaovapak S and Ananchaipattana C (2011*) Yersinia enterocolitica*: Epidemiological Studies and Outbreaks. *Journal of Pathogens* DOI:10.4061/2011/239391.

Ruiz-Palacios G M, Lopez-Vidal Y, Lopez-Vidal A B, Torres J and Rubino S (1983) *Systemic and local immune response in experimental campylobacter infection. In Campylobacter II*. A. D. Pearson, M. B. Skirrow, B. Rowe, J. R. Davies & D. M. Jones (eds) Pp. 115-16 London: Health Laboratory Service.

Seymour I J, Burfoot D, Smith R L, Cox L A and Lockwood A (2002) Ultrasound decontamination of minimally processed fruits and vegetables. *Int J Food Sci Technol* **37:** 547-57.

# Sivapalasingam S, Friedman C R, Cohen L and Tauxe R V(2004) Fresh Produce: A Growing Cause of Outbreaks of Foodborne Illness in the United States, 1973 through 1997 *J Food Protect* 67(10): 2342-53.

Solomon E B and Matthews K R (2006) Interaction of live and dead Eschrichia coli O157:H7 and fluorescent microspheres with lettuce tissue suggests bacterial processes do not mediate adherence. *Let Appl Microbial* **42** (2): 88-93.

Soltan-Dallal M M and Moezardalan K (2004) Frequency of Yersinia species infection in paediatric acute diarrhoea in Tehran. *East Mediterr Health* **10** (1-2): 152–58.

Tauxe R V (1997) Emeging foodborne diseases: an evolving public health challenge. *Emerg Infest Dis* **3** (4): 425-34.

Viswanathan P and Kaur R ( 2001) Prevalence and growth of pathogens on salad vegetables, fruits and sprouts. *Int J Hyg Environ Health* **203**: 205-13.

Youn JH, Hwang SY, Kim SH, et al.: 2010, mecA gene transferability and antibiogram of zoonotic *Staphylococcus intermedius* from animals, staff and the environment in animal hospitals in Korea. *J Microbiol Biotechnol* **20**:425–32.

Zhang S and Farber J M (1996) The effects of various disinfectants against Listeria monocytogeneson freshcut vegetables. *Food Microbiol* **13**: 311-21.

Zhou B, Xiao J, Liu S, Yang J, Wang Y, Nie F, Zhou Q, Li Y and Zhao G (2013) Simultaneous detection of six food-borne pathogens by multiplex PCR with a GeXP analyzer. *Food Control* **32**: 198-204.

Zhuang R Y and Beuchat L R (1996) Effectiveness of trisodium phosphate for killing *Salmonella* monterideoon tomatoes. *Lett Appl Microbiol* **22**: 93–100.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Signature of the student**

**Advisory Committee**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Name** | **Designation and Department** | **Signature** |
| 1. | Dr.(Mrs) Param Pal Sahota  (Major Advisor) | Sr. Microbiologist  Department of Microbiology |  |
| 2. | Dr. (Mrs) Maninder Arora | Sr. Food Microbiologist  Department of Microbiology |  |
| 3. | Dr. (Mrs.) Manjeet Kaur Sangha | Biochemist  Department of Biochemistry |  |
| 4. | [**Dr. Mandeep Singh Hunjan**](http://web.pau.edu/coa/index.php?_act=manageDepartments&DO=viewFacultyData&intFacultyID=409&intDepTitleID=80&intLinkID=10) | Assistant Plant Bacteriologist  **Department of Plant Pathology** |  |
| 5. | Dr. (Mrs.) S.K. Gosal  (Dean PGS Nominee) | Professor  Department of Microbiology |  |

Forward five copies to the Dean, Post-graduate studies for the approval by the synopsis approval committee.

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Head of the Department

Memo No. \_\_\_\_\_\_\_\_\_

Dated: \_\_\_\_\_\_\_\_\_\_\_\_\_\_

Dean

Postgraduate Studies

PAU, Ludhiana