

**PUNJAB AGRICULTURAL UNIVERSITY, LUDHIANA**  
**Synopsis of Thesis Problem of Postgraduate Student: Integrated M.Sc. (Hons.)**

Name of the Student: KAMALPREET KAUR  
Major Subject : Microbiology  
Major Advisor : Dr. (Mrs.) Param Pal Sahota

Admission No. : L-2011-BS-12-IM  
Minor Subject: Biochemistry

**1. Title**

PREVALENCE OF *AEROMONAS HYDROPHILA* IN SALAD VEGETABLES

**2. 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, *Aeromonas*, *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 the 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 present serious health 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). Besides the health risk associated with plant inhabiting, water carried pathogens such as *Aeromonas* have also gained importance in recent years.

*Aeromonas* species are widely distributed in aquatic environment, including raw and processed drinking water (Yigal *et al* 2012), and have been frequently isolated from various food products such as fish and shellfish, raw meat, vegetables and raw milk (Maria Elena *et al.*, 2013). Motile aeromonads are considered as emerging food borne pathogen because it was shown that some *Aeromonas* isolates can produce different virulence factors, not only at optimal growth temperature, but also at refrigeration temperature. *Aeromonas hydrophila* is commonly involved in causing human infections such as septicemia, gastroenteritis and cellulitis, wound sepsis with necrosis, gangrene, as well as pneumonia and traveler's diarrhea normally resulting from handling and consumption of contaminated food (Hawraa Natiq *et al* 2013). *A. hydrophila* secretes several extracellular proteins including enterotoxin, hemolysin and aerolysin that are associated with the bacterial virulence factors (Puttalingama *et al* 2013).

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). Fresh-cut fruits and vegetables are no longer considered low risk in terms of food safety. Recently, a number of outbreaks have been traced to fresh-cut fruits and vegetables that were processed under less than sanitary conditions. It is well known that disinfection is one of the most critical processing steps in fresh-cut vegetable production, affecting the quality, safety and shelf-life of the end product. Washing is designed to remove dirt, pesticide residues and microorganisms responsible for quality loss.

In a study conducted in Tamil Nadu, India, on the virulent strains of motile *Aeromonas* from commercial food products, a total of 389 food samples were aseptically collected throughout the year and processed for the isolation and identification of motile *Aeromonas* by performing array of morphological, biochemical and phenotypical tests, along with their virulence determination of polymerase chain reaction. A total of 72 (18.50%) tentative isolates of *Aeromonas* were identified as *A. hydrophila* 38 (52.77%). In duplex PCR, extracellular haemolysin gene (*ahh1*) was detected from 55.26% *A. hydrophila*, whereas *A. hydrophila* aerolysin gene (*aerA*) gene was detected along with the *ahh1* genes in 68.42% isolates of *A. hydrophila*. Virulent strains of motile *A. hydrophila* in human consumable food and food products impending a high risk of foodborne illness (Arunava Das *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. Multiplex PCR

based detection technique save time and minimize the expense on detection of food borne pathogens (Bottero *et al* 2004).

So, objective of this study is molecular characterization of indigenous isolates of *Aeromonas hydrophila* from salad vegetables and use of chemical disinfectants for public health appraisal.

### **Knowledge gaps**

Database regarding the prevalence of virulent strains *Aeromonas hydrophila* in salad vegetables is lacking.

### **Objectives**

The present study is planned with the following objectives:

- To undertake epidemiological Surveillance of *Aeromonas* strains in salad vegetables.
- Biochemical and molecular characterization of the *Aeromonas hydrophila*.

### **3. Expected new knowledge**

The studies will provide detailed information on the:

Prevalence and distribution of virulence factors of *Aeromonas hydrophila* in fresh vegetables.

### **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).

Food is biological in nature, it is capable of supporting the growth of microorganisms and food borne diseases result from the ingestion of contaminated foods and food products (Guyader and Atmar 2008). More than 250 different types of viruses, bacteria, parasites, toxins, metals, and prions are associated with foodborne diseases in humans. The infections range from mild gastroenteritis to life threatening neurologic, hepatic, and renal syndromes caused by either toxin from the “disease-causing” microbe, or by the human body’s reaction to the microbe itself (Teplitski *et al* 2009).

According to study conducted on raw vegetables in Sowthwest Ethiopia, *Aeromonas hydrophila* was isolated from Tomato (9.3%), Cabbage (8.7%), Carrot (4.5%), Lettuce (9.9%), Green pepper (12.6%) i.e. a total of six bacterial genera were identified out of which *Aeromonas hydrophila* constitute 9.3% (Duggassa *et al* 2014).

*Aeromonas hydrophila* secretes several extracellular proteins including enterotoxin, hemolysin and aerolysin that are associated with the bacterial virulence factors (Puttalingamma *et al* 2013). Isolation of *A. hydrophila* from water and food sources, and the increasing resistance of this

organism to antibiotics and chlorination in water, presents a significant threat to public health (Chang *et al* 2008). Toxins produced by this organism are generally active on the cell surface, causing pore formation and disruption of plasma membrane that subsequently leads to cell lysis.

Members of the *Aeromonas* genus are widely prevalent in the aquatic environment and are frequently isolated from various foods, mainly seafood meat, milk and vegetables (Ottativini *et al* 2011). The number of motile mesophilic *Aeromonas spp.* organisms in foods varies from  $<10^2$  cfu/g to  $10^5$  cfu/g (Neyts *et al* 2000). This is also confirmed by (Palumbo *et al* 1985), demonstrating that *Aeromonas spp.* counts in lamb, veal, pork and minced beef ranged from  $10^2$  to  $10^5$  cfu/g (Penchev *et al* 1996) enumerated the counts of *Aeromonas spp.* in foods and found variations from  $1 \times 10^2$  to  $5 \times 10^5$  cfu/g. By the 7th day of storage at 5°C, the counts of aeromonads increased by 1–3 log or 10–1000 times. Schuman *et al* (1997) isolated *Aeromonas hydrophila* from a variety of foods (red meat, poultry meat, eggs and raw milk) stored in the fridge.

On 12 May 2012, over 200 college students with acute diarrhoea were reported to the Guizhou Center for Disease Control and Prevention. A confirmed case also had a positive *Aeromonas hydrophila* culture from a stool sample. A retrospective study of 902 students compared attack rates (AR) by dining place, meals. Environmental investigation showed that vegetables were washed in polluted water from a tank close to the sewage ditch, then left at 30°C for two hours before serving. The *Escherichia coli* count of the tank was well above the standard for drinking water. This outbreak of *Aeromonas hydrophila* was most probably caused by salad ingredients washed in contaminated tank water (Zhang *et al* 2012).

These microbes are usually associated with an aquatic environment, but they are commonly isolated from different terrestrial ecosystems, such as food, invertebrates, plants and animals (Janda and Abbott 2010). The interest in this genus (and in its members as human pathogens) has grown over the past two decades because of the world-wide distribution of aeromonads, the occurrence of antibiotic resistance and the ability of some strains to survive safety treatments (Khajanchi *et al* 2010). Many studies have detected the presence of different *Aeromonas* species in various foods and vegetables (Palumbo *et al* 1985), and their prevalence in such products has been well characterized worldwide in recent years. Dhiraputra *et al* (2005) examined the bacterial contamination of vegetables served in hospitals in Bangkok, Thailand. They detected *Aeromonas* in 14 (3.5%) of 403 fresh vegetable samples (including lettuce, parsley, celery and tomato) before being washed and in 17 (4.3%) of 396 ready-to-serve vegetable samples. These findings support the view that food rather than water might be the source of aeromonads associated with outbreaks occurring in hospitals and in the community.

Conventional identification of *Aeromonas spp* is based on biochemical methods is challenged by the heterogeneous nature of the species. Virulence associated genes have been targeted for the detection of potentially pathogenic *Aeromonas hydrophila* by PCR assays that include either

cytolysin or haemolysin or both of these genes (Wang *et al* 1996). Yousr *et al*, reported a rapid mPCR which can simultaneously detect pathogenic *Aeromonas hydrophila* from fish targeting aerolysin and 16S rDNA genes. Several studies revealed that these organisms can produce exotoxins, including  $\alpha$  and  $\beta$  -hemolysins and cytolytic enterotoxin (Sen and Rodgers 2004). Broad methods have been reported, including, tissue cell culture assays, immunoassays, DNA probes and animal models that detect identification of presence of virulence factors in pathogenic *A. hydrophila* .

#### 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

- i. **Name of the experiment:** Surveillance for *Aeromonas hydrophila* and database generation for the occurrence in salad vegetables.
- ii. **Location:** Department of Microbiology, PAU, Ludhiana.
- iii. **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, and analyzed immediately. The isolates will be identified using enrichment techniques and Vegetable Testing Kit.
- iv. **Observations to be recorded:** Samples will be analyzed for occurrence of *Aeromonas hydrophila*. Screening and prevalence of region specific pathogen amongst these isolates will be further studied.
- v. **Statistical analysis:** SD

#### Experiment No. 2

- i. **Name of the experiment:** Characterization of *Aeromonas hydrophila* and studies on virulence factors.
- ii. **Location:** Department of Microbiology, PAU, Ludhiana
- iii. **Methodology: 1. Biochemical characterization:** Characterize *Aeromonas hydrophila* on the basis of biochemical, antibiogram activity.

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

- Biochemical activities (Carbohydrate utilization, indole, Methyl red, Voges Proskauer's, Citrate utilization, ONPG decarboxylase and Esculin Hydrolysis),
2. Antibiogram (Broad Spectrum Antibiotics)
3. Determination of virulence factors by different methods:

Total polysaccharides (Dubois *et al.*, 1956), Total protein content (Lowry *et al.*, 1951), Alginate production (Mathee *et al.*, 1999), Lipopolysaccharide production (Morrison and Leive *et al.*, 1975), Cell surface hydrophobicity (Perez *et al.*, 1998), Swimming swarming and twitching motility (Rasid *et*

*al.*, 2000), Protease production (Visca *et al.*, 1992), Elastase activity(Visca *et al.*, 1992), Pyrazinamidase activity(Simonava *et al.*,2007), Siderophore production(Arnou 1937), Hemolysin production(Linkish and vogt, 1972), Ramnolipid production(Koch *et al.*, 1991)

**iv. Statistical analysis:** Nil

#### **Experiment No. 3**

**i. Name of the experiment :** Molecular characterization of *Aeromonas hydrophila*.

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

**iii. Methodology:** Molecular characterization of pathogenic isolates will be carried out on the basis of virulence genes as listed below *Aeromonas hydrophila* (*alt,act,ast,hlyA,ail aerA, aspA,ahpB, lip and tap*).

**iv. Observations to be recorded:** PCR amplification profile for individual virulence genes from different isolates of *Aeromonas hydrophila* will be generated. The amplification data will be analyzed using DARWin 5.1 software.

**v. Statistical analysis:** Nil

#### **Experiment No. 4**

**i. Name of the experiment :** To test the bacteriocidal properties of disinfectants against *Aeromonas* strains.

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

**iii. Methodology:** Effectiveness of chlorinated water (different concentrations of sodium hypochlorite) and peracetic acid (peracetic acid, hydrogen peroxide acetic acid) will be determined. 25g of sample(whole, surface and macerate) will be rinsed in chlorinated water of varied degree of concentration i.e. 30, 50, 75 and 100ppm for varied time period i.e. 2, 5, 10 mins and different concentration of peracetic acid(1-5%) at different time period (1-10min). The samples will then be evaluated for log reduction aeromonads load using enrichment culture techniques described in Experiment no. 1.

**iv. Observations to be recorded:** Colony forming units per ml will be recorded and organoleptic characteristic will be determined and correlation will be carried out with untreated samples as control.

**v. Statistical analysis:** CD5% using ANOVA



## 6. Schedule work flow diagram

S. N o.	A cti vi ty	Se m es te r II	Se m es te r III	Semester IV															
J		F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	
1.	S yn op si s pr ep ar ati on & su b m is si on				*	*													



2.	E pi de m io lo gi cal su rv ill en ce fo r A. h y dr o p hi la an d da ta ba se ge ne ra ti on fo r th e qu ali ty of sa la d ve ge ta bl es					*	*	*	*	*	*							
----	--	--	--	--	--	---	---	---	---	---	---	--	--	--	--	--	--	--

3.	Bi oc he m ic al & m ol ec ul ar ch ar ac te ri za ti on of <i>A. h y dr o p hi la</i>							*	*	*	*	*	*	*				
4.	P ol y m er as e ch ai n re ac ti on										*	*	*	*	*	*		
5.	T he si s w rit ti ng														*	*	*	

6.	T h e s i s s e m i n a r																	*
7.	R o u g h t h e s i s s u e s u b m i s s i o n																	*
8.	F i n a l T h e s i s s u e s u b m i s s i o n																	*

JFM.....D refer to name of the month

7. Collaborations, if any: nil

## 8. References

- Arnou L E(1937) Calorimetric detection of the components of 3,4- dihydroxyphenylalanine-tyrosine mixtures. *J Biol Chem* **118**: 531-37.
- Arunav D, M E Sindhuja, Ashi R, Mkarthikeyan, C R Santhos and G S Murgesan (2013) Diagnosis of virulent strains of motile *Aeromonas* from commercial food . *IntJ Cur Micobl Ap Sci* **2(11)**: 300-06
- Ajlouni S, Sibrani H, Premier R and Tomkins B (2006) Ultrasonication and fresh produce (cos lettuce) preservation. *J Food Sci* **71(2)**:62-68.
- Alemmayehu D, Keetema B and Tschasige K (2014)Microbiological quality and safety of some selected vegetables sold in Jimma town, Southwestern Ehiopia. *Afr J Env Sci Tech* **8(11)**:653-63.
- 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.
- 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 genes of *E.coli* in milk and milk products. *Mol Cell Probes* **18**: 283-88.
- Dhiraputra C, Tiensasitorn C, Techachaiwiwat W, Jirapanakorn N, Kachintorn K and Danchaivijitr S (2005) Bacterial contamination of vegetables served in hospitals. *J Med Assoc Thai* **88(10)**: S42-8.
- Dugassa A, Bacha K, and Ketama T (2015). Microbiological quality and safety of some selected vegetables sold in Jimma town, Southwestern Ethiopia. *African Journal of Environmental Science and Technology* **8(11)**: 633-653.
- Dubois M, Gilles K, Hamilton J, Rebers P and Smith F (1956) Calometric method for determination of sugars and related substances. *Analyical Chemistry* **28(3)**: 350-56.
- 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.
- Fadl A A, Galindo C L, Sha J, Zhang F, Garner H R, Wang H Q, Chopra A K (2007) Global transcriptional responses of wild-type *Aeromonas hydrophila* and its virulence-deficient mutant in a murine model of infection. *Microbial Pathogenesis*. **42(5-6)**:193-203.
- Guyader F S, Le Saux J C, Ambert-Balay K, Krol J, Serais O, Parnaudeau S and Atmar R L (2008). Aichi virus, norovirus, astrovirus, enterovirus, and rotavirus involved in clinical cases from a French oyster-related gastroenteritis outbreak. *Journal of Clinical Microbiology* **46(12)**: 4011-17.
- Harris L J, Farber J N, Beuchat L R, Parish m E, Sulow T V, Garrett E H and Busta F F (2003) Outbreaks associated with fresh produce. *Comp Rev Food Sci Food Safety* **2**:78-141.
- Hawraa N K and Al-Ammar M H (2013) Study of some virulence factors of *Aeromonas hydrophila* isolated from clinical samples (Iraq). *Int J Sci Engg Investigation* **2**:113-21.
- 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 plasma flavonol concentrations: results from a

- randomized controlled intervention trial. *J Epidemiol Commun Health* **58**: 288-89.
- Izumi H (1999) Electrolyzed water as a Disinfectant for fresh-cut vegetables. *J Food Sci* **64**(2).
- Janda J M, Abbott S L (2010) The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev* **23**: 35–73.
- 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.
- Khajanchi B K, Fadl A A, Borchardt M A, Berg R L, Horneman A J, Stemper M E and Chopra A K (2010). Distribution of virulence factors and molecular fingerprinting of *Aeromonas* species isolates from water and clinical samples: suggestive evidence of water-to-human transmission. *Applied and environmental microbiology* **76**(7): 2313-25.
- Koch KA, Kappeli O, Fiechter A, Reiser J (1991) Hydrocarbon assimilation and biosurfactant production in *Pseudomonas aeruginosa* mutants. *J Bacteriol* **173** :4212- 4219.
- Linkish P G and Vogt W (1972) Direct hemolysin activity of phospholipase. *A Biochem Biophys Acta* **270**: 241-50.
- López-Gálvez F, Allende A, Selma M V and Gil M I (2009) Prevention of *Escherichia coli* cross-contamination by different commercial sanitizers during washing of fresh-cut lettuce. *Int J Food Microbiol* **133**(1-2):167-71.
- Lowry O H, Rosebrough N J, Farr A L and Randall R J(1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265-75.
- Maria E M, Luca F, Filomena M, Enrico N and Barbara C (2013) *Aeromonas* spp.: ubiquitous or specialized bugs? *SOAM* **10**.
- Maria I G, Maria V S, Francisco L G and Ana A (2009) Fresh-cut product sanitation and wash water disinfection: Problems and solution. *Int J Food Microbiol*.
- Mathee K, Ciofu O, Stenberg C, Lindum P W, Campbell J I, Jenson P and Kharazmi A (1999) Mucoid conversion of *Pseudomonas aeruginosa* by hydrogen peroxide: a mechanism for virulence activation in the cystic fibrosis lung. *Microbiology* **145**(6): 1349-57.
- Morrison D C and Leive L (1975) Fractions of lipopolysaccharide from from *Escherichia coli* O111:B4 prepared by two extraction procedures. *J of Biological Chem* **250**(8): 2911-19.
- Painter J A, Hoekstra R M and Ayers T (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.
- Palumbo S A, and Buchanan R L (1988). Factors affecting growth or survival of *Aeromonas hydrophila* in foods. *Journal of Food Safety* **9**(1): 37-51.
- Perez P F, Minnaard Y, Disalvo E A and Antoni G L (1998) Surface properties of Bifidobacterial strains of human origin. *Appl Env Microbiol* **64**(1):21-26.
- Puttalingamma V, Niveditha S P and Batra H (2013) A multiplex PCR for detection of haemolytic *aeromonas hydrophila* from vegetable sources in Karnataka, India. *RRST* **5**(3):19-23.
- Qian Z, Guo-Qing S, Guang-Peng T, Zhi-Tin Z, Guang-Hai Y and Guang Z (2012) A foodborne outbreak of *Aeromonas hydrophila* in a college, Xingyi City, Guizhou, China, 2012. *WPSAR*

3(4):38-42.

- Rashid M H and Kornberg A (2000) Inorganic polyphosphate is needed for swimming, swarming and twitching motilities of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **25**: 4885-90.
- Schuman J D, Shledon B W, Foegeding PM(1997) Thermal Resistance of *Aeromonas hydrophila* in Liquid Whole Egg. *J. Food Prot* **60**: 231-36
- Sen K and Rodger M (2004) Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. *J Appl Microbiol* **97**(5):1077-86.
- 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.
- Soren P, Al-Shuweli S, Seval Y, Johan J N and Katharina E P (2014) Identification of Clinical *Aeromonas* species by *rpoB* and *gyrB* Sequencing and Development of a Multiplex PCR Method for Detection of *Aeromonas hydrophila*, *A. caviae*, *A. veronii* and *A. media*. *J Clin Microbiol* **53**:653-56.
- Solomon E B and Matthews K R (2006) Interaction of live and dead *Escherichia coli* O157.H7 and fluorescent microsphere with lettuce tissue suggests bacterial processes do not mediate adherence. *Let Appl Microbiol* **42**(2): 88-93.
- Tauxe R V (1997) Emerging foodborne diseases : an evolving public health challenge. *Emerg Infect is* **3**(4): 425-34.
- Visca P, Charini F, Mansi A, Vetriani C, Serino L and Orsi N (1992) Virulence determinants in *Pseudomonas aeruginosa* strains from urinary tract infections. *Epidemiology and infect* **108**(2): 232-36.
- Wang G, Tyler K D, Munro C K and Johnson W M (1996) Characterization of cytotoxic, hemolytic *Aeromonas caviae* clinical isolates and their identification by determining presence of a unique hemolysin gene. *Journal of Clinical microbiology* **34**(12): 3203-05.
- Yigal S, Shifra, KenDror, Irina V, Dvora B, Ido I and Malka H (2012) A Molecular Study on the Prevalence and Virulence Potential of *Aeromonas* spp. Recovered from Patients Suffering from Diarrhea in Israel. *Plos One* **7**(2).
- Yousr A H, Napis S, Rusul G R A and Son R (2007) Detection of Aerolysin gene in *Aeromonas* spp. Isolated from Environmental and Shellfish Sources by Polymerase Chain Reaction. *Asean Food J* **14**(2): 115-22.
- Zhang S and Faber J M (1996) The effects of various disinfectants against *Listeria monocytogenes* freshcut vegetables. *Food Microbiol* **13**: 311-21.

\_\_\_\_\_  
Signature of the student

**ADVISORY COMMITTEE**

	<b>Name</b>	<b>Designation</b>	<b>Department</b>	<b>Signature</b>
Major Advisor	Dr. (Mrs.) Param Pal Sahota	Sr. Microbiologist	Microbiology	
Member	Dr. Mandeep Singh Hunjan	Assistant Plant Bacteriologist	Plant Pathology	
Member	Dr. (Mrs.) Rachna D Bhardwaj	Assistant Biochemist	Biochemistry	
Member	Dr. Madhurama Gangwar	Senior Microbiologist	Microbiology	
Nominee of Dean PGS	Dr. (Mrs.) Veena Khanna	Sr. Microbiologist	Plant Breeding and Genetics	

Forwarded five copies to the Dean, Postgraduate Studies, for approval by the Synopsis Approval Committee.

\_\_\_\_\_  
(Head of the Department)

Memo No : \_\_\_\_\_

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

Dean

Postgraduate Studies

P.A.U., Ludhiana