

ISOLATION OF *YERSINIA* AND OTHER POTENTIAL PATHOGENIC BACTERIA FROM PIG FECES AND MAHA OYA STREAM WATER, SRI LANKA

S.J. Medagangoda¹, C.L. Abayasekara^{1*}, R. Holley² and G. Blank²

¹*Department of Botany, Faculty of Science, University of Peradeniya*

²*Department of Food Science, Faculty of Agriculture and Food Science, University of Manitoba, Winnipeg, Canada*

Introduction

Yersinia enterocolitica is a rod shaped, Gram negative, facultative anaerobic, non spore-forming bacterium which belongs to the family Enterobacteriaceae. It is an important food and water borne pathogen causing the disease yersiniosis in humans. The bacterium can be transmitted by contaminated water and food including meat, meat products, fresh vegetables and dairy products. Pigs are considered to be the major animal reservoir of *Y. enterocolitica* (Bowman *et al.*, 2007). The organism is present in tonsils of the pig, and to a lesser degree in their intestines and feces (Nesbakken *et al.*, 2006). The presence of *Y. enterocolitica* has been reported in river water at 16 °C and in ground water at 30 °C (Guan and Holley, 2003). The psychrotrophic nature of the bacterium can be related to its abundance in temperate countries such as Canada, USA and Europe. A limited number of cases have been reported in tropical countries.

As there were no records of the presence of *Y. enterocolitica* in Sri Lanka, the current study was attempted to isolate *Y. enterocolitica* from feces of pigs from the piggery at Uda Peradeniya and from water samples of the stream Maha Oya that flows through the University of

Peradeniya, to which run off water from the piggery is directed. Other pathogens were recovered using methods for *Yersinia* isolation, and their identities are also reported here.

Materials and Methods

Water samples (n=8 near piggery and n=8, 1 km down stream of Maha Oya) and pig feces samples from the piggery at Uda Peradeniya (n=8 mature pigs and n=8 young pigs) were collected during four sampling trials over nine months (from June, 2008 to February, 2009) covering both wet and dry seasons. They were subjected to four different treatments, viz. a) direct plating on Cefsulodin Irgasan Novobiocin (CIN) agar and Mac Conkey (MAC) agar, b) alkaline treatment, c) Irgasan Ticarcillin Potassium chlorate (ITC) enrichment, and d) cold enrichment, where (b), (c) and (d) were followed by plating on CIN medium. Typical colonies of *Y. enterocolitica* on CIN medium (1-2 mm diameter, deep red centers surrounded by a transparent border) and on MAC (1-2 mm diameter, flat, colorless or pale pink colored) were sub-cultured on tryptone soy agar (TSA) medium in order to obtain pure isolates for further identification. Three preliminary tests namely, Gram test, oxidase and catalase tests were performed for each isolate. The

Table 1. Number of isolates exhibiting characters similar to *Yersinia* spp.

sample	No. of isolates showing culture characters similar to <i>Y. enterocolitica</i>	No. of isolates presumed positive for <i>Yersinia</i> spp. in all 3 preliminary tests	No. of isolates believed positive for <i>Y. enterocolitica</i> by bile esculin, LAIA, and urea agar tests
FM	47	19	03
FY	70	41	06
WP	86	53	05
WS	60	28	03
Total	263	141	17

FM – Fecal samples from mature pigs, FY – Fecal samples from young pigs

WP – Water samples near piggery, WS – Water samples from downstream

cultures that were Gram negative, oxidase negative and catalase positive were further tested with three confirmatory biochemical tests namely, bile esculin, lysine arginine iron agar (LAIA) and urea agar. Subsequently, the isolates exhibiting biochemical characters similar to *Y. enterocolitica* (bile esculin negative, LAIA positive and urea agar positive) were identified using the API 20E system.

Results

From a total of 263 isolates, 141 isolates showed characters similar to *Yersinia* spp. in the three preliminary tests, 17 isolates were positive for *Yersinia* spp. in all three confirmatory tests (Table 1).

Nine different species of bacteria were identified after performing the API 20 E test for 15 of the 17 isolates that were believed *Yersinia* positive. *Yersinia kristensenii*, *Proteus mirabilis*, *Morganella morganii*, *Citrobacter freundii*, *Citrobacter braakii*, and *Enterobacter sakazakii*

were identified from water samples, while *Citrobacter freundii*, *Citrobacter koseri*, *Providencia stuartii*, *Providencia rettgerii* came from pig feces. *Yersinia kristensenii* was isolated from water from Maha Oya, subjected to cold enrichment. The identification of the latter organism was confirmed by the Ontario Ministry of Health in Toronto, Canada.

Discussion and Conclusion

Although *Y. enterocolitica* was not isolated in the current study, another species, *Yersinia kristensenii*, was isolated from water samples from Maha Oya. To our knowledge this is the first report of the presence of the genus *Yersinia* in Sri Lanka. It is recorded that *Y. kristensenii* is an opportunistic -pathogen which has sometimes been implicated in human disease and it is most often isolated from the environment. Although it has been reported to cause diarrhea, urinary infections and bacteremia in humans, its pathogenicity is uncertain (Loftus *et al.*, 2002). It was formerly included as a single species with *Y.*

enterocolitica, along with two other groups, *Y. intermedia* and *Y. frederiksenii* that are now considered to be four distinct hybridization groups (Bercovier *et al.*, 1980).

Use of enrichment methods prior to sample plating is important as it improves recovery of the bacterium from the sample by allowing the organism to grow to detectable levels. The isolation of *Y. kristensenii* in the current study occurred when water samples were subjected to cold enrichment.

Recommendations for further research would include the consideration of the age of pigs and sampling of pig tonsils, as the prevalence of *Y. enterocolitica* is high in tonsils of pigs at the age of 60-80 days. Further, the study should be extended to other regions of the country, probably where pig farming is predominant.

Other bacterial genera identified in the current study are important opportunistic pathogens capable of causing a wide variety of nosocomial infections. Among them *Enterobacter sakazakii* has been implicated in outbreaks causing meningitis or enteritis especially in infants younger than 3 to 4 months of age (Foods-info.net, 2009). Occurrence of such pathogenic bacteria in the stream water is a serious health concern, as the people living along the stream bank use the water for bathing, washing clothes and sometimes even for drinking. The direct discharge of excreta and wastewater from animal farms and houses into the stream cause water pollution, unnecessarily increasing the risk that pathogenic bacteria will compromise human

health, particularly that of infants and the elderly.

Acknowledgement

Funding by the Association of Commonwealth Universities (Grant No: 2006/07 SRDF-2006-34) is gratefully acknowledged.

References

- Bercovier, H., Brenner, D. J., Ursing, J., Steigerwalt, A. G., Fanning, G. R., Alonso, J. M., Carter, G. P., and Mollaret, H. H. (1980). Characterization of *Yersinia enterocolitica sensu stricto*. *Current Microbiology*, 4: 201-206.
- Bowman, A. S., Glendening, C., Wittum, T. E., LeJeune, J. T., Stich, R. W., and Funk, T. A. (2007). Prevalence of *Yersinia enterocolitica* in different phases of production on swine farms. *Journal of Food Protection*, 70 (1): 11-16.
- Foods-info.net (2009). *Enterobacter sakazakii*. Wageningen University, The Netherlands .<http://www.foods-info.net/uk/qa/sakazakii>. Htm. accessed on 7th July 2009.
- Guan, T. Y., and Holley, R. A. (2003). Pathogen survival in swine manure environments and transmission of human enteric illness. A Review. *Journal of Environmental Quality*, 32: 383-392.
- Loftus, C.G., Harewood, G.C., Cockerill, F.R., and Murray, J.A. (2002). Clinical features of patients with novel *Yersinia* species. *Digestive Diseases and Sciences*, 47: 2805-2810.
- Nesbakken, T., Iversen, T., Eckner, K., and Lium, B. (2006). Testing of pathogenic *Yersinia enterocolitica* in pig herds based on the natural dynamic infection. *International Journal of Food Microbiology*, 111: 99-104.