JOURNAL OF INTERNATIONAL ACADEMIC RESEARCH FOR MULTIDISCIPLINARY
Impact Factor 1.393, ISSN: 2320-5083, Volume 2, Issue 6, July 2014

ISOLATION, IDENTIFICATION, AND MOLECULAR DETECTION OF PANTOEA AGGLOMERANS FROM NUTS IN COMMERCIAL MARKETS IN AL SAMAWA CITY

LAITH A.H ALOBAIDI*

*Dept. of Biology, College of Science, Al Muthanna University 550, Samawa-Iraq

ABSTRACT

Microbiological and molecular study has been carried on to perform isolation, identification and PCR detection of Pantoea agglomerans strain isolated from consumed nuts stored in Al Samawa markets. Ten samples of each salted and unsalted (almondand pistachio) were collected from five different markets in Samawa city, Iraq. Our results showed that there are five different bacterial species isolates from salted and unsalted samples, which are Escherichia coli, Pantoea spp, Enterobacter cloacae, Klebsiella spp, and Pseudomonas aeurogenosa. Specific isolation and identification were done for Pantoea spp. Out of the 8 Pantoea spp isolates, 6 (75%) were PCR positive for P. agglomerans using a specific target repA gene (780 bp). The current study provide for the first time molecular detection of P. agglomeransfrom nuts, which may be a powerful tool for food safety.

KEYWORDS: Nuts, Pantoea agglomerans, PCR, Bacterial Contamination

INTRODUCTION

Nuts is an potential source of supplementary protein for human food. They contain an important amount of protein and fat and their products have wide acceptance as food throughout the world (Day 2013).Consumption of nuts with a reduced risk of heart disease. Although it is known that nut consumption can lower serum cholesterol, it has been hypothesized that nut consumption can have additional beneficial effects on health (Allen 2008). However, negative impact on human health can be observed due to improper storage and bacterial contamination of nuts (Jenkins et al. 2008; Nareen 2013).

Enterobacteriaceae are the significant causes of serious infection, and many of the most important members of this family are becoming increasingly resistant to currently available antimicrobials(Grimont and Grimont2005).Pantoea agglomerans is one of the gram negative aerobic bacillus belong the family Enterobacteriaceae.It is considered to be an opportunistic pathogen of humans and animals. The most common infections caused by P. agglomerans is septic arthritis or synovitis (Kratz et al. 2003), ostitis (Laporte et al.2002), polymicrobial peritonitis (Lau et al. 2005), peritonitis (Lim et al. 2006), and sepsis after rotavirus

gastroenteritis (Cicchetti et al.2006). Many cases reported P. agglomerans infections (Cruz et al. 2007). However, until present there are no easy and reliable tests to determine whether any particular strain of P. agglomerans has pathogenic or non-pathogenic characteristics (Wright and Beer 2006).

Therefore, it was found of interest, in this first study to investigate, identify, and molecular detection ofPantoea agglomerans bacterium from various nuts stored in commercial markets in Al Samawa city.

MATERIALS AND METHODS

A. Sampling:

Samples of various nuts (shelled and unshelled, salted and unsalted) were collected from different stores in Al Samawa market in 5 replicates. The nuts include almonds (Pruns amygdalus) and pistachio-nuts (Pistacia vera). Any sample unit submitted for laboratory testing, contains at least 100 g of product(Smith and Arends 1976).

B. Bacterial isolation:

For specific isolation of Pantoea spp from samples, the nuts were surface sterilized with 70% ethanol (Merck Co.) and were opened in a laminar flow cabinet. Samples were individually finely ground in a common household blender and the powder kept tightly packed in a new study bags. Pantoea spp was isolated according to Mullane et al. 2006. We prepared 3 Erlenmeyer flask each of sterile distilled water (pre-warmed to 45ºC) at 9, 90 and 900 ml containing 1, 10 and 100 g of powdered nuts (almonds and pistachios), respectively. After the powder was completely mixed and dissolved in distilled water, it was incubated at 37ºC for 18-24 h. Following the incubation, 10 ml of each sample was added to 90 ml of Enterobacteriaceae enrichment (EE) broth medium and placed at 37ºC for 18-24 h. After incubation, a loopful of the enrichment culture was streaked onto duplicate violet red bile glucose agar (VRBGA) plates and cultured at 37ºC for 18-24 h. A total of 4 suspicious colonies were picked from each VRBGA plate and pure culture was performed. For detection of non-lactose fermenting isolates, presumptive colonies were streaked onto MacConkey agar and incubated at 37ºC for 72 h. The API-20E biochemical kit system (Bio-Mérieux) and manual biochemical tests were used to identify the organism according to the manufacturer instruction (De Champs et al. 2000). For long term storage, the purified isolates were saved in tryptic soy broth (TSB) with 20% glycerol (Merck Co.) at -20ºC

C. Total DNA extraction:

Bacterial strains were grown on tryptone-casein-soy agar for 24 h at 30°C. DNA was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI).

D. Detection ofPantoea agglomeransby PCR

PCR was performed to detect P. agglomerans among all other Pantoea spp., using repA (780 bp) as a gene biomarker with the oligonucleotide primers rep-F 5-TTGTGGGGGACATAAATTAACC-3 and rep-R5-AGGGCCATAGTGAGGAAGGT-3. The primers design from National Centre of Biotechnology Information (NCBI), which are flaks the complete coding region of repA gene in P. agglomerans. The amplifications was carried out in a final volume of 25µl containing 10X reaction buffer (10mM Tris-HCl, 50mM KCl,1.5mM, MgCl), 0.2mM of each dNTPs, 2.5pmol/µl of each primer, 2 units of Taq polymerase and approximately 50 ng of genomic DNA(Applied Biosystems). Reaction was run for 34 cycles, each consisting of 1 min. at 95 °C, 45 sec. at 53 °C, and 2 min. at72 °C, with initial denaturation of 5 min. After PCR amplification, five µl of the PCR product was run in 1% (w/v) agarose gel, stained with ethidium bromide and visualized under a UV transilluminator.

RESULTS AND DISCUSSION

A. Bacterial isolates:

Our results showed that different type of bacteria were isolate from study samples and for both shelled and unshelled, salted and unsalted Table (1). Out of the 8Pantoea sppisolates, 6 (75%) samples were positive for P. agglomerans. The Gram staining showed Gram negative rods. On VRBGA agar purple/pink colored colonies, and on MacConkey agar convex, smooth, punctuate, umbilicated lactose fermenting glistening colonies were grown Fig (1). The Pantoea spp was characterized biochemically by API 20E tests. All eight isolates (five from each nut samples) were typical of the genus Pantoea. they were urease negative; lysine and ornithine were not decarboxylated; and H_2S was not produced from thiosulfat. All strains used glucose, mannitol, rhamnose, saccharose, arabinose, and amygdalin as substrates Fig (1). According to the API 20E system database, it was not possible to identify any isolate at the species level. However, the current database includes only Pantoea spp.

Table (1) The bacterial isolates from Pistachios and almonds. Pistachios[1] : the sample is unsalted and non-encysted. Pistachios[2]:The sample is salted and non-encysted. Almonds[1'2'3]: The samples are unsalted and non-encysted. Almonds 4: The sample is salted and non-encysted.

Type of sample	Place of collection	Type of isolates
Pistachios[1]	Samawa	*Pantoea spp* and *Escherichia coli*
pistachios[2]	Samawa	*Escherichia coli* and *Klebsiella spp*
Almonds[1]	Samawa	*Enterobacter cloacae*
Almonds[2]	Samawa	*Klebsiella spp* and *Escherichia coli*
Almonds[3]	Samawa	*Escherichia coli, Pantoea spp, and Pseudomonas aeurogenosa*
Almonds[4]	Samawa	*Klebsiella spp*

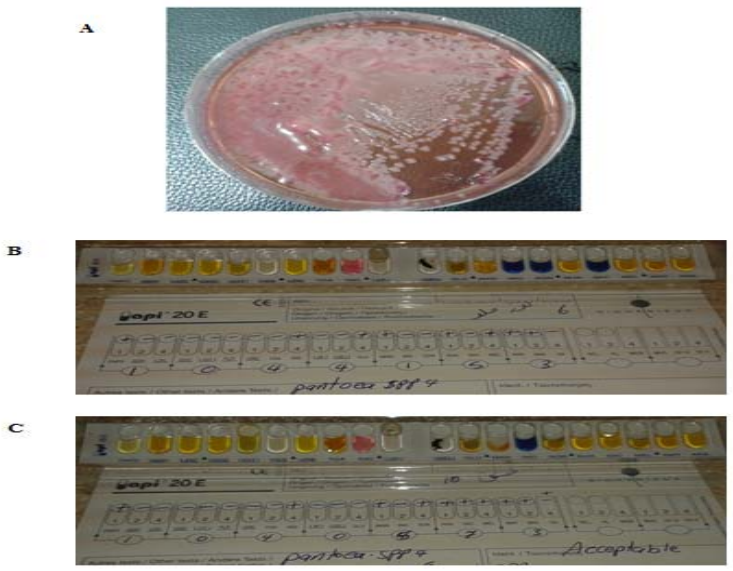

Fig (1) Identification of Pantoea spp. by culturing and API 20E system. (A): The growth of Pantoea spp on MacConkey agar. (B): Bacterial isolates from unsalted pistachios. (C): bacterial isolates from unsalted almonds.

B. Molecular detection of P. agglomerans:

In order to specifically detect P. agglomeranson molecular level, specific primers were used to identify this strain from Pantoea group. It appeared from our results that from eight isolates, six of them were positive to repA by PCRand detect 780 bp bands on agarose gel electrophoresis Fig (2).

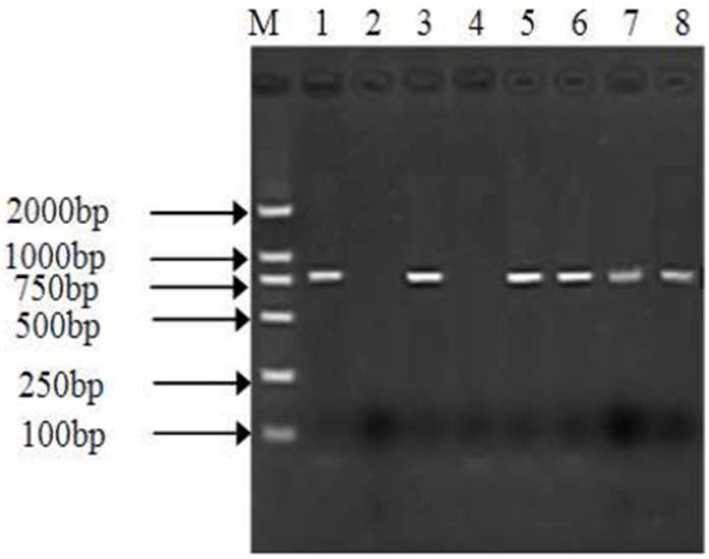

Fig (2) Detection of repA gene by PCR. Lanes 1, 3, 5, 6, 7, 8 represent positive isolates with bands (780 bp) in size for repA. Lanes 2, 4 are negatives. Lane M represent DNA marker (100-2000 bp).

Nuts potential source of supplementary protein and minerals for human food. However, improper storage can cause severe diseases due to bacterial contaminations. The results from present study showed that there are five different bacterial isolates from shelled and unshelled, salted and unsalted nuts. These results confirm the work of King eal. 1970, who reported that microorganisms associated with commercially shelled nuts were numerous and varied.One of the bacterial species found in food is Pantoea agglomerans. It is a new nomenclature is not yet widely in use (Sanders and Sanders 1997). It is an opportunistic pathogen and, when introduced into the organs of humans or other animals, may cause severe and occasionally fatal infections. Since clinical reports involving P. agglomerans are typically of polymicrobial nature, confirmed virulence of P. agglomerans is difficult to reveal. Infections caused by this organism often involve patients that are already affected by diseases of other origin, and isolates are rarely conserved for confirmatory analysis (Rezzonico et al. 2009). P. agglomerans is ubiquitous in nature and it has been isolated from a wide variety of

ecological niches and from different kinds of specimens from humans and animals (Ewing and Fife 1972). Previous studies isolated P. Agglomerans isolated from powdered infant formula in developed and developing countries through the world (Muytjens et al. 1988; Fauziah et al. 2008; Songzhe et al. 2011).

It is clear from our data that almonds and pistachios were highly contaminated with microorganisms. This support the finding reported that due to the extremely high fat and low water content of nuts, this productsare quite refractory to spoilage by bacteria and mould (Rastogi and Raghavarao 2007; Nareen 2013).

To our knowledge, there is no previous report on the isolation and identification of P. agglomerans from nuts in Iraq. Meanwhile, a bacteriumP. agglomerans was detected and confirmed by PCR using repA. This gene was chosen because it is importance virulence gene responsible for bacterial plasmid replication and it is most suitable for the design of the identification method (Basu et al. 2002) . Previous study had been successfully detect repA (Weinthal et al. 2007).

CONCLUSION

It had been successfully isolate and biochemically identified five different bacterial species from nuts (almonds and pistachios) in commercial markets in Al-Samawa city. Moreover, the pantoes spp was further analyzed to detect and confirm the occurrence of P. agglomeransin isolates samples by PCR using a gene(repA) as a biomarker. Further studies on molecular level are necessary to clarify the role of P. agglomeransin pathogenesis.

ACKNOWLEDGMENTS

Author would like to thanks Biology Department, Al Muthanna University for the necessary support to complete this work.

REFERENCES

1. Allen LH. 2008. Priority areas for research on the intake, composition, and health effects of tree nuts and peanuts. J Nutr, 138: 1763S-1765S.
2. Basu A, Chawla-Sarkar M, Chakrabarti S, Das Gupta SK. 2002. Origin binding activity of the Mycobacterial plasmid pAL5000 replication protein RepB is stimulated through interactions with host factors and coupled expression of repA. J Bacteriol, 184: 2204-2214.
3. Cicchetti R, Iacobini M, Midolla F, PapoffP , Mancuso M, Moretti C. 2006. Pantoea agglom-eranssepsis after rotavirus gastroenteritis. Pediatr. Infect. Dis J., 25: 280–281.
4. Cruz AT, Cazacu AC, Allen CH. 2007. Pantoea agglomerans; a plant pathogen causing human disease. J. Clin. Microbiol, 45: 1989–1992.
5. Day LI. 2013. Proteins from land plants – Potential resources for human nutrition and food security. Trends in Food Science & Technology, 32: 25–42.

6. De Champs C, Le Seaux S, Dubost JJ, Boisgard S, Sauvezie B, Sirot J. 2000. Solation of Pantoea agglomerans in two cases of septic monoarthritis after plant thorn and wood sliver injuries. J Clin Microbiol, 38: 460-461.
7. Ewing WH, Fife MA. 1972. Enterobacter agglomerans (Beijerinck) comb. nov. (the Herbicola-Lathyri Bacteria). Int J Syst Bacteriol, 22: 4-11.
8. Fauziah T, Norrakiah AS, Uma Priya K, Norizan J. 2008. Detection of Cronobacter (Enterobacter) sakazakiiand Enterobacteriaceae in Powdered Infant Formula and Children's Milk. Proceeding of the Seminar on Food Biotechnology: Perspectives, Challenges and opportunities, 352-360.
9. Grimont PA, Grimont F: Genus Pantoea. Manual of systematic bacteriology. Volume 2. 2nd edition.New York: Proteobacteria SpringerVerlag: 713-720, 2005.
10. Jenkins DJ, Kendall, CW, Marchie A, Josse AR, Nguyen TH, Faulkner DA, Lapsley KG, Blumberg J. 2008. Almonds reduce biomarkers of lipid peroxidation in older hyperlipidemic subjects. Journal of Nutrition, 138: 908-913.
11. Kratz A, Greenberg D, Barki Y, Cohen E, Lifshitz M. 2003. Pantoea agglomerans as a cause of septic arthritis after palm tree thorn injury; case report and literature review. Arch. Dis. Child, 88: 542–544.
12. Laporte C, Demachy MC, Thevenin-Lemoine C. 2002. Ostéite tibiale à Pantoea agglomerans au décours d'une fracture ouverte stade IIIB de jambe. Rev. Chir. Orthop. Réparatrice Appar. Mot, 88: 625–627.
13. Lau KK, Ault BH, Jones DP. 2005. Polymicrobial peritonitis including Pantoea agglomerans from teething on a catheter**.** South Med J, 98**:** 580-581.
14. Lim PS, Chen SL, Tsai CY, Pai MA. 2006. Pantoea peritonitis in a patient receiving chronic ambulatory peritoneal dialysis. Nephrology, 11: 97-99.
15. Mullane NR, Murray J, Drudy D, Prentice N, Whyte P, Wall P, Parton A, Fanning S. 2006. Detection of Enterobacter sakazakii in Dried Infant Milk Formula by Cationic-Magnetic-Bead Capture. Appl. Environ. Microbiol,72: 6325-6330.
16. Muytjens HL, Roelofs-Willemse H, Jaspar GH. 1988. Quality of powdered substitutes for breast milk with regard to members of the family Enterobacteriaceae. J Clin Microbio, 26: 743-746.
17. Nareen Q.F. 2013. Evaluation of Fungal Flora and Mycotoxin in Some Important Nut Products in Erbil Local Markets. Research Journal of Environmental and Earth Sciences, 5: 330-336.
18. Rastogi NK, Rachavarao KS. 2007. Opportunities and Challenges in High Pressure Processing of Foods. Critical Reviews in Food Science and Nutrition, 47: 69-112.
19. Rezzonico F, Smits TH, Montesinos E, Frey JE, Duffy B. 2009. Genotypic comparison of Pantoea agglomerans plant and clinical strains. BMC Microbiol 9: 204.
20. Sanders WE, Sanders CC. 1997. Enterobacter spp: pathogens poised to flourish at the turn of the century. Clin Microbiol Rev, 10: 220-241.
21. Smith FR, Arends RE. 1976. Remel Microbiology Products. Instructions for Use of MacConkey Agar. Microbiological Methods for Foods, 51: 614-619.
22. Songzhe FU, Gao J, Liu Y, Chen H. 2011. Isolation of Cronobacter spp isolates from infant formulas and their survival in the production process of infant formula. Czech J Food Sci, 29: 391-399.
23. Weinthal DM, Barash I, Panijel M, Valinsky L, Gaba V, Manulis-Sasson S. 2007. Distribution and replication of the pathogenicity plasmid pPATH in diverse populations of the gall-forming bacterium Pantoea agglomerans.Appl Environ Microbiol, 73: 7552-7561.
24. Wright SA, Beer SV. 2006. Pantoea agglomerans, a biocontrol agent and ubiquitous microorganism. Proc. 1st Int. Symposium. On Biological Control of Bacterial Plant Diseases. Mitteilungen ausder Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem, 408: 334–338.