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Survey of antibiotic resistance of *Pseudomonas* isolated from fresh cut red chicory (*Cichorium intybus* L., Asteraceae)

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Summary

The present work was carried out to investigate the safety aspects of minimally processed red chicory (*Cichorium intybus* L., family Asteraceae) regarding the antibiotic resistance of *Pseudomonas* Migula, 1894 populations (Bacteria Pseudomonadaceae). The *Pseudomonas* strains tested for their characteristics were previously isolated from experimental monovarietal salads prepared with two different processes, a classic ready-to-eat (RTE) process and a process with entire leaves, and stored under refrigeration (4 °C) up to 15 days from production. Due to their dominance over the microbial community, *Pseudomonas* strains were better characterised for their undesirable features that might be exerted on the health of consumers. To this purpose, the 21 dominant *Pseudomonas* strains were subjected to the antimicrobial resistance test, applying the antibiotics commonly administered to combat intestinal bacteria responsible for human infections. The results revealed a certain sensibility to almost all the antibiotics diffused among these bacteria, with the exception of ampicillin, amoxicillin, and chloramphenicol. If, on one hand, this study has found that, generally, *Pseudomonas* are not related to the dissemination of the majority of antibiotics used for hospitalized patients, on the other hand, it poses the attention to this microbial group regarding ampicillin and amoxicillin.

Key Words

ready-to-eat; red chicory; *Pseudomonas*; antibiotic-resistance.

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Introduction

In the last decades, there has been the affirmation of a healthy life style of consumers in several countries. For this reason, vegetables became essential components of a healthy diet, thanks to their nutrients and micronutrients. They are a source of a wide variety of minerals, vitamins, and phytochemicals with antioxidant activity (Siriamornpun et al., 2012; Tango et al., 2018).

The high attention of consumers towards the composition of foods and the awareness of the risk factors represented by several foods of animal origin, determined an increasing demand for vegetables, especially those available in a ready-to-eat (RTE) form that require no further treatment before consumption (Maffei et al., 2016). This phenomenon determines a continuous increase of the request of vegetables (Chakraborty & Chattopadhyay, 2018).

In order to obtain RTE products, leafy vegetables are minimally processed through selection, prewashing, cutting, washing, drying, packaging, and cold storage (Codex Alimentarius Commission, 2003; Kennedy & Wall, 2007). However, due to the large consumption, RTE vegetables may represent a public health issue because they can be a vehicle for the transmission of bacterial, parasitic, and viral pathogens (Abadias et al., 2008). The contamination of fresh cut products can take place during the pre- and post-harvest operations due to the contact with contaminated irrigation water, soil, manure of wild and domestic animals, following a cross-contamina-

tion in any point of the production process (Berger et al., 2010; Harris et al., 2003; Park et al., 2012; Verhoeff-Bakkenes et al., 2011), and during the administration to the final consumers. Furthermore, the refrigerated temperature storage does not stop the growth of pathogenic and/or spoilage micro-organisms (Aguado et al., 2004; Gleeson & O'Beirne, 2005).

Pseudomonads (Bacteria Pseudomonadaceae) are commonly associated to the spoilage of RTE salads (Nguyen-the and Prunier, 1989) and may represent up to 90% of the microbial community of fresh vegetables (Garg et al., 1990; Zagory, 1999; Wulfkuehler et al., 2015). In a previous work (Alfonzo et al., 2018), *Pseudomonas* Migula, 1894 represented the dominant group of red chicory subjected to cut and uncut processes, with *P. fluorescens*, *P. endophytica*, *P. grimontii,* and *P. poae* as being the most frequently isolated species.

Antimicrobial resistant bacteria represent a public health issue, since their emergence, due to the antimicrobial agents used for clinical therapeutic use occurred worldwide (ECDC/EFSA/EMA 2015). In this work, the effect of the antibiotic commonly used against human pathogens was tested on *Pseudomonas* spp. from fresh cut *Cichorium intybus* L.

Material and Methods

The *Pseudomonas* strains used in this study belonged to the culture collection of the Agricultural Laboratory - Department of Agricultural, Food and Forest Science (University of Palermo, Italy). The strains were: *Pseudomonas azotoformans* (Ac. No. KY939740); *Pseudomonas beatica* (Ac. No. KY939741); *Pseudomonas brenneri* (Ac. No. KY939742); *Pseudomonas endophytica* (Ac. No. KY939743 – KY939744); *Pseudomonas extremaustralis* (Ac. No. KY939745); *Pseudomonas fluorescens* (Ac. No. KY939746 – KY939748); *Pseudomonas grimontii* (Ac. No. KY939749 – KY939750); *Pseudomonas helleri* (Ac. No. KY939751); *Pseudomonas libanensis* (Ac. No. KY939752); *Pseudomonas marginalis* (Ac. No. KY939753); *Pseudomonas poae* (Ac. No. KY939754 – KY939755); *Pseudomonas psychrophila* (Ac. No. KY939756); *Pseudomonas simiae* (Ac. No. KY939757); *Pseudomonas trivialis* (Ac. No. KY939758); *Pseudomonas viridiflava* (Ac. No. KY939759); *Pseudomonas yamanorum* (Ac. No. KY939760). All strains were reactivated in Luria Bertani broth (Oxoid) incubated overnight at 25 °C.

The assay for the antimicrobial susceptibility test was carried out according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015) that, except *Pseudomonas aeruginosa*, are described for species other than *Pseudomonas*. The tests were performed applying the disk diffusion method. Bacterial inoculums were obtained from colonies developed overnight on PAB, suspended in Ringer's solution (5 ml) to a cell density of 0.5 McFarland standard and swabbed for confluent growth onto Mueller Hinton agar (Oxoid).

The following antibiotics at the concentrations generally used for the treatment of human and animal infections were tested: high-level gentamicin (GN—120 µg); high-level streptomycin (STR—300 µg); amoxicillin/clavulanic acid (AMC—30 µg); ampicillin (AMP—10 µg); chloramphenicol (C—30 µg); ciprofloxacin (CIP—5 µg); levofloxacin (LEV—5 µg); tetracycline (TE—30 µg). All antimicrobial compounds were purchased from Oxoid.

The effects of the antibiotics were evaluated after 18 hours of incubation at 37 °C, by measuring the inhibition halos according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2015). The antibiotics quinupristin/dalfopristin (QD—15 µg) was also included in the assay since Gram negative bacteria, such as *Pseudomonas* spp., are inherently resistant to this antibiotics because of cell wall impermeability (Nadler et al., 1999).

Results and Conclusions

Due to their dominance over prokaryotic and eukaryotic microorganisms found in red chicory samples by Alfonzo et al. (2018), *Pseudomonas* were deeply characterized for their resistance to the common antibiotics used for the treatment of infections.

The results of the resistance of the 21 *Pseudomonas* strains to the antibiotics tested is reported in Table 1. Only *P. viridiflava* 4G477 was susceptible to ampicillin. A low resistance was registered towards chloramphenicol and amoxicillin that inhibited 14 and five strains, respectively. Some strain-dependent behaviours were also observed, since only the strain 4G558 was inhibited by amoxicillin within the species *P. poae*. All strains were highly susceptible to gentamicin, streptomycin, ciprofloxacin, levofloxacin, and tetracycline with the diameter of the inhibition areas ranging between 16.0 to 36.0 mm. The most sensitive strain was *P. viridiflava* 4G477, inhibited by all antibiotics.

The epiphytic and endophytic microbial communities of plants play different roles. Basically, within

bacteria, some populations are plant pathogens, some can act as plant growth promoters and some others are responsible for human diseases (Jackson et al., 2013). The operation of cutting leafy vegetables stimulates the growth of several microorganisms (Steele, 2004) as a consequence of the higher and direct availability of nutrients generated by the damaged cells.

The involvement of pseudomonads in the deterioration of RTE salads is well known (Nguyen-the & Prunier, 1989). However, to our knowledge, no previous work characterised this dominating population of minimally processed red chicory for their antibiotic resistance. In the recent years, the rapid emergence of resistant bacteria is occurring across the globe, endangering the efficacy of antibiotics (Ventola, 2015). These bacteria present in foods can be a possible intermediate vehicle for the transmission of multidrug resistances to the human intestinal tract (Gaglio et al., 2016; Jamet et al., 2012). Several initiatives have been launched worldwide to address the biosafety concerns of starter cultures and probiotics (Mathur & Singh, 2005), but very little is known about the role of saprophytic microorganisms in the dissemination of antibiotic resistance characters. With this in mind, the dominant strains isolated from the red chicory processed in this study were evaluated for their resistance to the antibiotics commonly administered to combat intestinal bacteria responsible for human infections. In particular, eight antibiotics generally used to test the resistance of enterococci (CLSI, 2015), ubiquitous in several foods and human gut (Lebreton et al., 2014), were used. Basically, the majority of the dominant *Pseudomonas* strains were susceptible to the action of the antibiotics.

In conclusion, *Pseudomonas* do not represent a risk of antibiotic resistance genes related to the consumption of fresh cut red chicory. However, their resistance to ampicillin and amoxicillin should be better considered in RTE salads.

Strains	Antibiotics[a]								Quality control
	Aminoglycosides		Penicillins		Phenicols	Fluoroquinolones		Tetra-cyclines	Strepto-gramins
	GN	STR	AMP	AMC	C	CIP	LEV	T	QD
P. azotoformans 4G504	18.5 ± 0.5	22.5 ± 0.5	0	0	10.0 ± 0	24.0 ± 0	30.5 ± 0.5	29.0 ± 1.0	0
P. baetica 4G537	18.5 ± 0.5	19.0 ± 0	0	0	0	25.5 ± 0.5	24.5 ± 0.5	20.5 ± 0.5	0
P. brenneri 4G513	23.0 ± 1.0	27.5 ± 0.5	0	0	20.5 ± 0.5	30.0 ± 0	34.0 ± 1.0	28.5 ± 1.5	0
P. endophytica 4G414	21.0 ± 1.0	25.5 ± 1.5	0	16.0 ± 0	14.0 ± 0	24.0 ± 0	22.5 ± 0.5	30.0 ± 1.0	0
P. endophytica 4G764	18.0 ± 0	21.0 ± 0	0	17.0 ± 1.0	12.5 ± 0.5	24.0 ± 1.0	23.5 ± 0.5	28.5 ± 1.5	0
P. extremaustralis 4G619	20.0 ± 1.0	24.0 ± 1.0	0	0	9.5 ± 0.5	22.0 ± 0	23.0 ± 0	24.0 ± 1.0	0
P. fluorescens 4G628	18.0 ± 0	20.0 ± 1.0	0	0	0	24.0 ± 1.0	26.0 ± 1.0	25.0 ± 0	0
P. fluorescens 4G1034	18.0 ± 1.0	20.0 ± 0	0	0	0	25.0 ± 0	22.5 ± 0.5	23.0 ± 0	0
P. fluorescens 4G1237	22.5 ± 0.5	27.0 ± 1.0	0	0	0	30.0 ± 1.0	30.0 ± 1.0	26.5 ± 0.5	0
P. grimontii 4G769	18.5 ± 1.5	26.5 ± 0.5	0	0	12.5 ± 0.5	30.0 ± 1.0	30.5 ± 0.5	28.5 ± 0.5	0
P. grimontii 4G483	18.0 ± 1.0	27.0 ± 1.0	0	0	11.5 ± 0.5	30.0 ± 1.0	30.0 ± 1.0	30.0 ± 1.0	0
P. helleri 4G893	10.5 ± 0.5	16.0 ± 0	0	11.5 ± 0.5	10.5 ± 0.5	23.0 ± 0	20.0 ± 0	23.0 ± 1.0	0
P. libanensis 4G787	20.0 ± 1.0	18.0 ± 0	0	0	0	27.5 ± 0.5	29.5 ± 0.5	25.5 ± 0.5	0
P. marginalis 4G518	20.5 ± 1.5	2.65 ± 0.05	0	0	14.0 ± 2.0	28.5 ± 1.5	32.0 ± 1.0	31.5 ± 1.5	0
P. poae 4G558	20.0 ± 0	24.0 ± 0	0	8.0 ± 0	0	30.5 ± 0.5	34.0 ± 1.0	30.0 ± 1.0	0
P. poae 4G1030	18.0 ± 1.0	23.5 ± 0.5	0	0	8.0 ± 0	28.5 ± 0.5	28.0 ± 0	27.5 ± 0.5	0
P. psychrophila 4G793	16.5 ± 0.5	19.0 ± 1.0	0	12.5 ± 0.5	18.5 ± 0.5	22.5 ± 0.5	22.5 ± 0.5	36.0 ± 2.0	0
P. simiae 4G1010	18.0 ± 1.0	24.5 ± 0.5	0	0	0	26.0 ± 2.0	28.5 ± 1.5	24.5 ± 1.5	0
P. trivialis 4G921	20.0 ± 1.0	25.5 ± 0.5	0	0	12.5 ± 0.5	23.0 ± 1.0	26.0 ± 0	25.5 ± 0.5	0
P. viridiflava 4G477	24.0 ± 0	30.5 ± 0.5	7.5 ± 0.5	21.0 ± 0	15.5 ± 1.5	35.5 ± 1.5	33.0 ± 2.0	28.5 ± 1.5	0
P. yamanorum 4G531	19.0 ± 1.0	23.0 ± 0	0	0	10.0 ± 0	27.5 ± 0.5	26.0 ± 1.0	25.5 ± 0.5	0

Table 1. Antimicrobial resistance of *Pseudomonas* strains collected from cut and uncut red chicory samples during storage. GN: gentamicin; STR: streptomycin; AMP: ampicillin; AMC: amoxicillin; C: chloramphenicol; CIP: ciprofloxacin; LEV: levofloxacin; T: tetracycline; QD: quinupristin-dalfopristin.

References

Abadias M., Usall J., Anguera M., Solsona C. & Viñas I., 2008. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology*, 123: 121–129. DOI: 10.1016/j.ijfoodmicro.2007.12.013

Aguado V., Vitas A.I. & García-Jalón I., 2004. Characterization of *Listeria monocytogenes* and *Listeria innocua* from a vegetable processing plant by RAPD and REA. *International Journal of Food Microbiology*, 90: 341–347. DOI: 10.1016/S0168-1605(03)00313-1

Alfonzo A., Gaglio R., Miceli A., Francesca N., Di Gerlando R., Moschetti G. & Settanni L., 2018. Shelf life evaluation of fresh-cut red chicory subjected to different minimal processes. *Food Microbiology*, 73: 298–304. DOI: 10.1016/j.fm.2018.02.008

Berger C.N., Sodha S.V., Shaw R.K., Grififn P.M., Pink D., Hand P. & Frankel G., 2010. Fresh fruits and vegetables as vehicles for the transmission of human pathogens. *Environmental Microbiology*, 12: 2385–2397. DOI: 10.1111/j.1462-2920.2010.02297.x

Chakraborty I. & Chattopadhyay A., 2018. Pre- and post-harvest losses in vegetables. In: Singh B., Singh S. & Koley T.K. (Eds.), Advances in Postharvest Technologies of Vegetable Crops, CRC Press Taylor & Francis.

Clinical and Laboratory Standards Institute, 2015. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement (M100-S25). Clinical and Laboratory Standards Institute, Wayne, PA.

Codex Alimentarius Commission, 2003. Code of hygienic practice for fresh fruits and vegetables (CAC/RCP 53-2003). Available at: http://www.codexalimentarius.net/download/standards/10200/cxp_053e.pdf. Accessed 21.01.2018.

ECDC/EFSA/EMA, 2005. First joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food- producing animals. EFSA Journal, 13: 4006, 114 pp.

Gaglio R., Couto N., Marques C., Lopes M.D.F.S., Moschetti G., Pomba C., & Settanni L., 2016. Evaluation of antimicrobial resistance and virulence of enterococci from equipment surfaces, raw materials, and traditional cheeses. *International Journal of Food Microbiology*, 236: 107–114. DOI: 10.1016/j.ijfoodmicro.2016.07.020

Garg N., Churey J.J. & Splittstoesser D.F., 1990. Effect of processing conditions on the microflora of fresh-cut vegetables. *Journal of Food Protection*, 53: 701–703. DOI: 10.4315/0362-028X-53.8.701

Gleeson E. & O'Beirne D., 2005. Effects of process severity on survival and growth of *Escherichia coli* and *Listeria innocua* on minimally processed vegetables. *Food Control*, 16, 677–685. DOI: 10.1016/j.foodcont.2004.06.004

Harris L.J., Farber J.N., Beuchat L.R., Parish M.E., Suslow T.V., Garrett E.H. & Busta F.F., 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. *Comprehensive Review in Food Science and Food Safety*, 2: 78–141. DOI: 10.1111/j.1541-4337.2003.tb00031.x

Jackson C.R., Randolph K.C., Osborn S.L. & Tyler H.L., 2013. Culture dependent and independent analysis of bacterial communities associated with commercial salad leaf vegetables. *BMC Microbiology*, 13: 1–12. DOI: 10.1186/1471-2180-13-274

Jamet E., Akary E., Poisson M.A., Chamba J.F., Bertrand X. & Serror P., 2012. Prevalence and characterization of antibiotic resistant *Enterococcus faecalis* in French cheeses. *Food Microbiology*, 31: 191–198. DOI: 10.1016/j.fm.2012.03.009

Kennedy J. & Wall P., 2007. Food safety challenges. In Storrs M., Devoluy M.-C. & Cruveiller P. (Eds.), Food safety handbook: Microbiological challenges. BioMérieux Education, France, pp. 8–19.

Lebreton F., Willems R.J. & Gilmore M.S., 2014. *Enterococcus* diversity, origins in nature, and gut colonization. In: Gilmore M.S., Clewell D.B., Ike Y. & Shankar N. (Eds.), Enterococci From Commensals to Leading Causes of Drug Resistant Infection. Massachusetts Eye and Ear Infirmary, Boston, pp. 5–63.

Maffei D.F., Alvarenga V.O., Sant'Ana A.S. & Franco B.D., 2016. Assessing the effect of washing practices employed in Brazilian processing plants on the quality of ready-to-eat vegetables. *LWT Food Science and Technology*, 69: 474–481. DOI: 10.1016/j.lwt.2016.02.001

Mathur S. & Singh R., 2005. Antibiotic resistance in food lactic acid bacteria-a review. *International Journal of Food Microbiology*, 105: 281–295. DOI: 10.1016/j.ijfoodmicro.2005.03.008

Nadler H., Dowzicky M.J., Feger C., Pease M.R. & Prokocimer P., 1999. Quinupristin/dalfopristin: a novel selective-spectrum antibiotic for the treatment of multi-resistant and other gram-positive pathogens. *Clinical Microbiology Newsletters*, 21: 103–112. DOI: 10.1016/S0196-4399(00)80041-0

Nguyen-The C. & Prunier J.P., 1989. Involvement of pseudomonads in deterioration or 'ready-to-use' salads. *International Journal of Food Science and Technology*, 24: 47–58. DOI: 10.1111/j.1365-2621.1989.tb00618.x

Park S., Szonyi B., Gautam R., Nightingale K., Anciso J. & Ivanek R., 2012. Risk factors for microbial contamination in fruits and vegetables at the preharvest level. *Journal of Food Protection*, 75: 2055–2081. DOI: 10.4315/0362-028X.JFP-12-160

Siriamornpun S., Kaisoon O. & Meeso N., 2012. Changes in colour, antioxidant activities and carotenoids (lycopene, beta-carotene, lutein) of marigold flower (*Tagetes erecta* L.) resulting from different drying processes. *Journal of Functional Foods*, 4: 757–766. DOI: 10.1016/j.jff.2012.05.002

Steele R., 2004. Understanding and measuring the shelf-life of food. Sawston.

Tango C.N., Wei S., Khan I., Hussain M.S., Kounkeu P.F.N., Park J.-H. & Kim S.-H., OH D.H., 2018. Microbiological quality and safety of fresh fruits and vegetables at retail levels in Korea. *Journal of Food Science*, 83: 386–392. DOI: 10.1111/1750-3841.13992

Ventola C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacological Therapy*, 40: 277–283.

Verhoeff-Bakkenes L., Jansen H.A., In 'T Veld P.H., Beumer R.R., Zwietering M.H. & Van Leusden F.M., 2011. Consumption of raw vegetables and fruits: a risk factor for *Campylobacter* infections. *International Journal of Food Microbiology*, 144: 406–412. DOI: 10.1016/j.ijfoodmicro.2010.10.027

Wulfkuehler S., Dietz J., Schmidt H., Weiss A. & Carle R., 2015. Quality of fresh-cut radicchio cv. Rosso di Chioggia (*Cichorium intybus* L. var. *foliosum* Hegi) as affected by water jet cutting and different washing procedures. *European Food Research and Technology*, 240: 159–172. DOI: 10.1007/s00217-014-2317-6

Zagory D., 1999. Effects of post-processing handling and packaging on microbial populations. *Postharvest Biology and Technology*, 15: 313–321. DOI: 10.1016/S0925-5214(98)00093-3