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Phenotypic and genotypic characterization of *Pseudomonas* spp. present in spoiled poultry fillets sold in retail settings

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ABSTRACT

The objective of this study was to characterize and compare the phenotypic and genotypic diversity of most frequent *Pseudomonas* associated with spoilage at 4 °C in skinless marinated poultry breast fillets. We selected four or five prevalent *Pseudomonas* isolated 2 days after expiration date from 11 poultry samples with different production dates. Most *Pseudomonas* were *Pseudomonas fragi* (n = 24) and *Pseudomonas fluorescens* (n = 14). Phenotypical tests showed that *P. fluorescens* had proteolytic, lipolytic and lecithinase activities while *P. fragi* produced mainly proteolytic enzymes. Genotypic characterization showed a high number of RAPD types (≥85% similarity); nevertheless, some isolates from different samples had similar RAPD types. 14 *P. fluorescens* were grouped into 13 RAPD types, 3 *Pseudomonas lundensis* into 2 RAPD types and 24 *P. fragi* into 13 RAPD types.

The findings revealed a high level of phenotypic and genotypic variability between and within *Pseudomonas* species isolated from poultry. In addition, some strains with similar RAPD types showed intra strain variability, despite not having the same phenotypic profile.

This study has illustrated biological variability of *Pseudomonas* microbiota present in spoiled poultry fillets. Such variability may have strong importance across individual processors and spoilage and should be considered for risk assessments and when developing HACCP plans.

1. Introduction

Poultry is the second most important meat consumed worldwide, and it is expected that by 2022 it will be positioned first (OECD-FAO, 2013). During production, the presence of different processes and manipulation in food plants, can lead to contamination by pathogens and microorganisms that cause spoilage (Figueroa, Troncoso, López, Rivas, & Toro, 2009; Vihavainen & Björkroth, 2010).

During distribution, sale and before consumption, the organoleptic properties of poultry can be affected by the proliferation of microorganisms that cause spoilage (Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). Early spoilage of poultry can lead to product losses (Bruckner, Albrecht, Petersen, & Kreyenschmidt, 2012). For example, in 2010 it was estimated that approximately 21% of poultry was lost at the consumer- and retail-level in USA (Buzby, Wells, & Hyman, 2014). Food spoilage is wasteful. It affects that company's name, consumer confidence and has a negative economic impact for both companies and consumers.

Refrigerated chicken meat is a highly perishable food, with high water activity, relatively neutral pH and plenty of nutrients, making it an excellent milieu for psychrophilic bacterial proliferation (Vihavainen & Björkroth, 2010). The spoilage microbiota of refrigerated poultry is heterogeneous and mostly inherent to the production environment (Vihavainen & Björkroth, 2010), where the worldwide dominant microorganism is *Pseudomonas* spp. This microorganism is not currently included in HACCP plans because its presence is not a causative agent of human illnesses. However, *Pseudomonas* spp. is the major organism that dictate the quality and shelf life of poultry meat (Arnaut-Rollier, De Zutter, & Van Hoof, 1999), which is especially important in a globalized world, where foods can travel long distances to reach foreign markets (Maxwell & Slater, 2003). The presence of *Pseudomonas* spp. in chicken is also negative because it is recognized as a key factor in generating a microaerophilic environment that helps the aerobic survival of *Campylobacter jejuni*, an important enteric pathogen (Hilbert, Scherwitzel, Paulsen, & Szostak, 2010).

Poultry spoilage is associated with *Pseudomonas* that have proteolytic, lipolytic, saccharolytic and biosurfactant abilities.

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These contribute to an increase in the availability of nutrients, which help *Pseudomonas* spp. to survive and grow (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Mellor, Bentley, & Dykes, 2011; Nychas et al., 2008). Better survival increases the probability of food spoilage, especially if the cold chain is broken between distribution and consumption (Doulgeraki, Ercolini, Villani, & Nychas, 2012).

The dominant *Pseudomonas* group is different in fresh poultry compared to refrigerated meat (Arnaut-Rollier et al., 1999). Although, many species can be present in spoiled chicken, studies reveal that *Pseudomonas fluorescens*, *Pseudomonas fragi*, *Pseudomonas lundensis*, and *Pseudomonas putida*, are commonly involved in spoilage of refrigerated poultry (Arnaut-Rollier et al., 1999; Bruckner et al., 2012; Geornaras, Kunene, Von Holy, & Hastings, 1999; Hanning, Jarquin, O'Leary, & Slavik, 2009).

Recent studies have shown large biological variability responses between and within bacterial species (Aguirre & Koutsoumanis, 2016; Aguirre, Pin, Rodríguez, & García de Fernando, 2009; Lianou & Koutsoumanis, 2011), which can be phenotypic and genotypic (Foweraker, Laughton, Brown, & Bilton, 2005; Stringer et al., 2013). Lianou and Koutsoumanis (2011) indicate that biological variability should be included in quantitative risk assessment and in strain selection for exploitation in challenge studies.

Genetic studies confirm a great diversity of strains of *Pseudomonas fluorescens* in fresh chicken (Geornaras et al., 1999). Nevertheless, data of diversity of strains linked to spoilage are scarce in the literature and therefore, it is mostly unknown how homogeneous or diverse the spoilage genotypes are during the shelf life of poultry products. Hence, it is important to know the *Pseudomonas* spoilage species diversity and its strain variability to plan effective strategies to extend the shelf life of poultry, either controlling its initial contamination, or retarding its growth in poultry meat, allowing the product to reach distant markets.

The main objective of this study was to characterize and compare phenotypic and genotypic diversity of most prevalent *Pseudomonas* strains associated with spoilage at 4 °C of skinless marinated poultry breast fillets from the same producer obtained in a retail setting.

2. Materials and methods

2.1. Frequency of Pseudomonas spp. in spoiled poultry

Poultry samples were randomly purchased in trays of skinless marinated (15%) poultry breast fillets packaged in permeable oxygen film from one producer. Brine consist in a mix of water, sodium polyphosphates, salt, carrageenan, guar gum, xanthan gum and maltodextrin with pH 6.5. The poultry carcasses were brine injected post chilling and prior to the bucking and breast fillets in the slaughterhouse.

The fillets were obtained on the first day on sale at a local supermarket from a shelf storage at 4 °C (277.15 K). The sample was immediately transported under refrigeration at 4 °C to the Microbiology and Probiotics Lab at the Institute of Nutrition and Food Technology (INTA) (Santiago, Chile) and stored in a refrigerator (CFL 633, Haier, China) at 4 °C. Samples were refrigerated until two days after the expiration date indicated by the poultry producer. This process was repeated eleven times and the batch number, purchase date, expiration date and the total storage time at 4 °C of each sample was recorded (Table 1).

The presence of *Pseudomonas* spp. and the total viable counts (TVC) in poultry samples was assessed at the end of the storage time. Briefly, the whole tray content was transferred to a sterile sampling bag with 200 mL of peptone water 0.1% (PW) and manually shaken for 2 min (Zhang, Ye, Xu, Zhou, & Cao, 2012). The obtained rinse liquid was serially diluted (1:10) in PW and 100 μL plated in duplicate.

Pseudomonas enumeration was conducted in *Pseudomonas* agar base with Cetrimide Fusidin Cephaloridine supplement (CFC; Oxoid, UK) and TVC in plate count agar (PCA, Merck, Germany). Both cultures were incubated at 22 °C for 5 days (Arnaut-Rollier et al., 1999). Bacterial counts were reported as Log_{10} colony forming units per gram (log CFU/g).

2.2. Selection and molecular identification of prevalent Pseudomonas species

Four or five most frequent different colonies, to the naked eye, by color, texture, brightness or viscosity were picked from CFC agar from each sample. Then selected colonies were transferred to Nutrient agar (NA, Oxoid, UK; 24–48 h at 22 ± 2 °C). *Pseudomonas* spp., identified as Gram negative bacillus, oxidase positive and non-glucose fermenter, (Kiska & Gilligan, 2003), were then submitted to two molecular identification tests as described below.

Initially we used a Multiplex PCR amplification (Ercolini et al., 2007) to perform simultaneous differentiation of *Pseudomonas fragi*, *P. lundensis*, and *P. putida*. Those colonies that gave negative responses in this multiplex PCR, were tested with a specific PCR for *P. fluorescens* as proposed by Scarpellini, Franzetti, and Galli (2004).

Briefly, template DNA was prepared from *Pseudomonas* spp. isolates that were suspended in 100 μL PCR sterile water ($OD_{600} = 0.15$; performed in a spectrophotometer WPA Biowave II, Biochrom, UK) and boiled for 15 min.

For Multiplex PCR 20 μL mixture consisted of 1 μL of crude DNA, 1× of GoTaq Green Master mix (Promega, USA), 0.2 μM of each forward primer (CarAfraF, 5′-CGT CAG CAC CGA AAA AGC C-3′; CarAputF, 5′-ATG CTG GTT GCY CGT GGC-3′; CarAlunF, 5′-TGT GGC GAT TGC AGG CAT T-3′) and 0.6 μM of reverse primer (CarAR, 5′-TGA TGR CCS AGG CAG ATR CC-3′).

For specific *P. fluorescens* PCR, 20 μL mixture consisted of 1 μL of crude DNA, 1× of GoTaq Green Master mix and 0.5 μM of each primer (16SPfluF, 5′-TGC ATT CAA AAC TGA CTG-3′; 16SPR, 5′-AAT CAC ACC GTG GTA ACC G-3′).

Both PCR reactions were performed in a thermal cycler (MultiGene™ OptiMax, Labnet international Inc., USA), with the following thermal profile: 1 min at 94 °C; 30 cycles consisting of 94 °C for 30 s, 60 °C for Multiplex PCR or 56 °C for *P. fluorescens* PCR for 30 s, 72 °C for 1 min; a final extension of 72 °C for 7 min; and final cooling at 4 °C.

The PCR products of these tests were run in 2% agarose electrophoresis gels (150 V for 45 min; electrophoresis cam MGU-202T, C.B.S. Scientific, USA; power supply PowerPac 1000, Bio-Rad, USA). A 100 bp ladder mix (AccuRuler 100 bp DNA RTU Ladder, Maestrogen, USA) was included as a size marker.

2.3. Phenotypic characterization of Pseudomonas

Phenotypical tests performed for each *Pseudomonas* isolate included:

1) Biosurfactant production with drop collapse method (Bodour & Miller-Maier, 1998; Youssef et al., 2004)
2) Proteolytic enzyme production (NA plates containing 10% skim milk, Colun, Chile) (Arnaut-Rollier et al., 1999)
3) Lipase production (peptone agar 1% tween 80) (Arnaut-Rollier et al., 1999);
4) Lecithinase production (NA plus 5% v/v egg-yolk emulsion, Liofilchem s. r.l., Italy) (Franzetti & Scarpellini, 2007)
5) Growth at 37 °C for 48 h on NA plates.

Table 1
Information on samples.

Sample No	Batch number	Purchase date	Expiration date	Analysis date	Storage time 4 °C (days)	CFC count (Log cfu/g)	TVC (Log cfu/g)
1	14141000	10-oct	18-oct	20-oct	10	8.5	8.6
2	14241000	16-octss	25-oct	27-oct	11	8.2	8.6
3	14244000	20-oct	28-oct	30-oct	10	8.2	8.5
4	14245000	21-oct	29-oct	31-oct	10	8.4	8.8
5	14341000	22-oct	01-nov	03-nov	12	8.5	8.8
6	14343000	24-oct	03-nov	05-nov	12	8.6	8.8
7	14346000	28-oct	06-nov	08-nov	11	8.2	8.6
8	14441000	30-oct	08-nov	10-nov	11	8.3	8.7
9	14443000	03-nov	10-nov	12-nov	9	8.3	8.6
10	14641000	05-nov	15-nov	17-nov	12	8.1	8.7
11	14643000	07-nov	17-nov	19-nov	12	8.2	8.6

2.4. Genotypic characterization of Pseudomonas

DNA from each *Pseudomonas* isolate was then genotyped through random amplification of polymorphic DNA (RAPD), according to Aslam and Service (2008) with a few modifications. Briefly, *Pseudomonas* spp. DNA templates were prepared from isolates suspended in 100 μL PCR sterile water and boiled for 15 min. 20 μL PCR mixture consisted of 1 μL of crude DNA, 1× of GoTaq Green Master mix (Promega, USA) and 0,5 μM of oligonucleotide (5′-AGC GGG CCA A-3′) was used. Sterile water and DNA from *Pseudomonas fluorescens* ATCC 13525 were used as negative and positive controls, respectively.

PCR amplification was performed in a thermal cycler (MultiGene™ OptiMax, Labnet international Inc., USA) according to the following protocol: 1 cycle: 5 min each at 94, 40 and 72 °C; 30 cycles: 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C; 1 cycle: 5 min at 94 °C, 1 min at 50 °C and 2 min at 72 °C; and a final extension for 7 min at 72 °C.

Amplified DNA fragments were separated on 1.8% agarose gel (70 V for 1.5 h; electrophoresis cam MGU-202T, C.B.S. Scientific, USA; power supply PowerPac 1000, Bio-Rad, USA). A MassRuler DNA ladder mix (Thermo Fisher Scientific Inc, USA) was included as size marker.

The experiments were done under controlled conditions, in triplicate and conducted with the same inputs (from the same batches).

2.5. Statistical analysis

Pseudomonas spp. counts and TVC were compared using analysis of variance with LS means using Infostat® software (Argentina). Statistically significant differences were reported at $P < 0.05$.

For genotypic comparison of *Pseudomonas* isolated, RAPD fingerprinting were analyzed with BioNumerics software (Applied Maths, Belgium). Similarities between the DNA patterns of *Pseudomonas* based on band positions were determined using the Dice similarity coefficient. A dendrogram was constructed using unweighted pair group method using arithmetic averages (UPGMA) to reflect similarities in the matrix. *Pseudomonas* isolates were grouped into RAPD types which were considered genetically similar (≥85% Dice similarity) based on DNA patterns (Aslam & Service, 2008).

3. Results and discussion

3.1. Frequency of Pseudomonas spp. in spoiled poultry

The final *Pseudomonas* spp. counts in spoiled skinless marinated poultry breast fillets after 11 days at 4 °C (Table 1), was 8.32 ± 0.15 log CFU/g, compared with TVC 8.67 ± 0.10 CFU/g ($P < 0.05$). The result confirms that *Pseudomonas* spp. is the dominant microorganism in spoiled fillets sampled from a retail setting. This is in agreement with other studies that find *Pseudomonas* species as the major spoilage bacteria on poultry meat stored aerobically under refrigeration conditions in retail settings (Ercolini, Russo, Torrieri, Masi, & Villani, 2006; Mead, 2004; Oakley et al., 2013; Russell, Fletcher, & Cox, 1995).

3.2. Molecular identification of prevalent Pseudomonas species

From each of the 11 spoilage fillet samples, we selected four or five most frequent colonies that were macroscopically different. In total, 46 prevalent colonies were accumulated from CFC agar, of which 42 (91%) were identified as *Pseudomonas* spp.

PCR species identification tests determined that, from 42 *Pseudomonas* spp. isolates, 24 (57%) were *P. fragi*, 14 (33%) *P. fluorescens*, 3 (7%) *P. lundensis* and 1 (2%) was not recognized by the primers used. This is in agreement with the *Pseudomonas* species found in 3 or 8 days spoiled chicken by Arnaut-Rollier et al. (1999) but not with Bruckner et al. (2012), who reported about 90% of *Pseudomonas* spp isolates from 1 or 6 days spoiled poultry and pork were *P. putida*.

Liang et al. (2012) established that bacterial diversity and spoilage-related microbiota associated with freshly prepared chicken products under aerobic conditions at 4 °C varied by manufacturer. Thus, data from each producer should trace which *Pseudomonas* species prevails in their industry.

In general, psychrotrophic *Pseudomonas* present in poultry meats has an environmental origin, which can explain the different species reported in the literature. They can be introduced at the abattoir by the chickens (feathers, feet), also through vectors and human personnel. Some species can persist for a long time in abattoirs as biofilms, as stated by Liang et al. (2012).

In meat processing the use of brine solution can also explain the variability in spoilage microbiota. Our fillets samples were brine injected in contrast with those used by Arnaut-Rollier et al. (1999) and Bruckner et al. (2012). Brines in poultry meat is used to improve flavor, tenderness and product shelf life (Alvarado & McKee, 2007), but simultaneously, it enhances the moisture of meat. The brine injection process resulted in an increase in bacterial contamination (Bohaychuk & Greer, 2003) and brine chill systems can be a major site of contamination with spoilage microorganisms (Cerveny, Meyer, & Hall, 2009).

3.3. Phenotypic characterization of prevalent Pseudomonas spp.

Biosurfactant activity was not detected in any of the tested isolates using the drop collapse method with Pennzoil® or mineral

oil, neither when they were incubated in mineral salts nor in medium E with agitation at 25 °C until 14 days as proposed by others (Bodour & Miller-Maier, 1998; Youssef et al., 2004). This is different to results reported by Mellor et al. (2011), who found that 72% of *Pseudomonas* spp. isolated from spoiled chicken thigh skin have biosurfactant activity. This disparity in the results can be due to differences in the type of the chicken meat used. The samples we tested had low fat (skinless marinated poultry breast fillets); in contrast, Mellor et al. (2011) studied chicken skin, which is well known for its high lipid level. The response of bacteria to different food nutrients (poultry fillets and skin) depends mainly on the selective pressure exerted by the environment and the lifestyle of the organism (Cases, de Lorenzo, & Ouzounis, 2003). In addition, these differences could partially be explained by pH differences; the breast muscle has a pH between 5.7 and 5.9 compared with a pH value 6.4–6.7 for the skin (Barnes & Impey, 1968; Theron & Lues, 2010).

We isolated 24 strains of *P. fragi* in this study and one showed a negative phenotypic profile to all phenotypic tests (1/24) (Fig. 1A). The remaining strains had three different enzymatic profiles: most of them were proteolytic without lecithinase production ability (14/24), followed by non-proteolytic without lecithinase production ability (7/24) or proteolytic and lecithinase positive ability (2/24). None of them were lipolytic and have a limited replication at 37 °C confirming its environmental origin (Fig. 1A). Our results are in contrast with previous reports that established most *P. fragi*, isolated from different food matrices, have protease and lipase activity and around half are lecithinase positive (Franzetti & Scarpellini, 2007). This can be explained due to the intrinsic intra species variability previously mentioned (Lianou & Koutsoumanis, 2011). Regardless of the proteolytic and lipolytic capacities shown *in vitro*, different types of *P. fragi* can release odors suggesting the liberation of active volatile molecules that play a clear role that contribute to spoilage in poultry meats (Ercolini et al., 2010).

On the other hand, in the present study, *P. fluorescens* appears to have more versatile metabolic capabilities than *P. fragi* (Fig. 1). All isolates were proteolytic and lecithinase producers and were lipolytic (8/14) or not (3/14) with limited grown at 37 °C (Fig. 1B). Only three isolates did not grow at 37 °C and were either lipolytic (2/14) or not (1/14) (Fig. 1B). Franzetti and Scarpellini (2007) confirmed that *P. fluorescens* have a positive lecithinase reaction, but also reports that most of the isolates have variable proteolytic activity with no or little lipolytic capabilities. This discrepancy in enzymatic abilities between different strains of *P. fluorescens* could be explained also by a probable intra species variability response (Lianou & Koutsoumanis, 2011).

The three *P. lundensis* isolated in this study showed different phenotypic profiles (Fig. 1C). Two of them had evident growth capabilities at 37 °C, but they differed in their proteolytic activities. The third strain was proteolytic, but with limited ability to grow at 37 °C (Fig. 1C). It has been described that environmental isolates of *Pseudomonas* have an optimal temperature growth range of 25–30 °C and are not virulent to human cells, but certain strains such as *P. fluorescens* have a higher permissive growth range, up to 37 °C, and show increased virulence for human cells (Scales, Dickson, LiPuma, & Huffnagle, 2014). Considering that two *P. lundensis* isolates found in this work grew at 37 °C, further studies should be conducted to evaluate if these strains have some pathogenic potential.

3.4. Pseudomonas genotypic characterization

In this study, 42 prevalent *Pseudomonas* (24 *P. fragi*, 14 *P fluorescens*, 3 *P. lundensis* and 1 unidentified specie) isolates obtained from 11 poultry fillets were characterized by genotype and compared using the RAPD method (Fig. 1).

P. fragi isolates were grouped into 13 RAPD types (≥85% similarity) (Fig. 1A), from which eight (61%) consisted of isolates shared in two or more sampling times, whereas the remaining five types were unique to one sample (Fig. 1A). This can be explained because certain strains of *P. fragi* can produce an exopolymer that confer them the primary capacity to form biofilms (Sasahara & Zottola, 1993); therefore, some *P. fragi* exopolymer-producing strains could be established in the abattoir or processing plant and produce cross-contamination among different batches of poultry fillets (Masák, Čejková, Schreiberová, & Řezanka, 2014).

Fourteen isolates of *P. fluorescens* tested generated 12 RAPD types with ≥85% similarity (Fig. 1B); 10 (71%) isolates had an unique RAPD type, 2 (14%) isolates from different poultry fillets samples had the same RAPD types, and 2 (14%) isolates from the same sample were genetically similar (Fig. 1B).

Geornaras et al. (1999) established a great diversity of *P. fluorescens,* but isolated from fresh chicken samples taken the same day as slaughtering and also from the environmental source of abattoirs.

Three *P. lundensis* from different poultry fillets samples showed two RAPD types (Fig. 1C); suggesting that certain *P. lundensis* can persist through biofilm, similar to *P. fragi*, as mentioned above (Sasahara & Zottola, 1993).

The results presented here showed intra species diversity of RAPD types related to *Pseudomonas* (De Jonghe et al., 2011; Ercolini et al., 2007) and this result persists in spoilage strains, providing evidence that these genetic types could belong to different ecological niches. These *Pseudomonas* RAPD types may represent novel diversity currently undescribed by cultivation of poultry spoilage; their associations remain an interesting and important topic for further research.

Most available literature described bacterial diversity in fresh meats, meaning not spoiled products. We speculate that the storage time should select one particular species and a few spoilage strains. On the contrary, this study showed that the most frequent spoilage species remained *Pseudomonas fragi*, *fluorescens* and *lundensis* at the end of shelf life (10–12 days) and revealed a high level of phenotypic and genotypic variability between and within prevalent *Pseudomonas* species. Several studies (Aguirre & Koutsoumanis, 2016; Lianou & Koutsoumanis, 2011; Métris, Le Marc, Elfwing, Ballagi, & Baranyi, 2005; Nauta, 2002) have suggested that, quantitative microbial risk assessment, HACCP plans and mathematical survival and growth models should include this biological variability to achieve the food safety objective and reduce the food losses due to spoilage products.

Our findings reveal the importance of knowing the prevalent *Pseudomonas* clones in each poultry slaughterhouse, and whether they persist short and long term. We suggest that *Pseudomonas* strains might have different kinetics behavior and that they could have a variable resistance to sanitizer. Future research should consider these topics.

Finally this study has illustrated biological variability of *Pseudomonas* microbiota present in spoiled poultry fillets. Such variability may have strong importance across individual processors and spoilage and should be considered for risk assessments and when developing HACCP plans to prolong the shelf life of poultry.

4. Conclusions

In this study we characterize and compare phenotypic and genotypic diversity of most frequent *Pseudomonas* isolates associated with spoilage at 4 °C of skinless marinated poultry breast fillet samples with different production dates.

Prevalent *Pseudomonas* species, *P. fragi*, *P. fluorescens* and

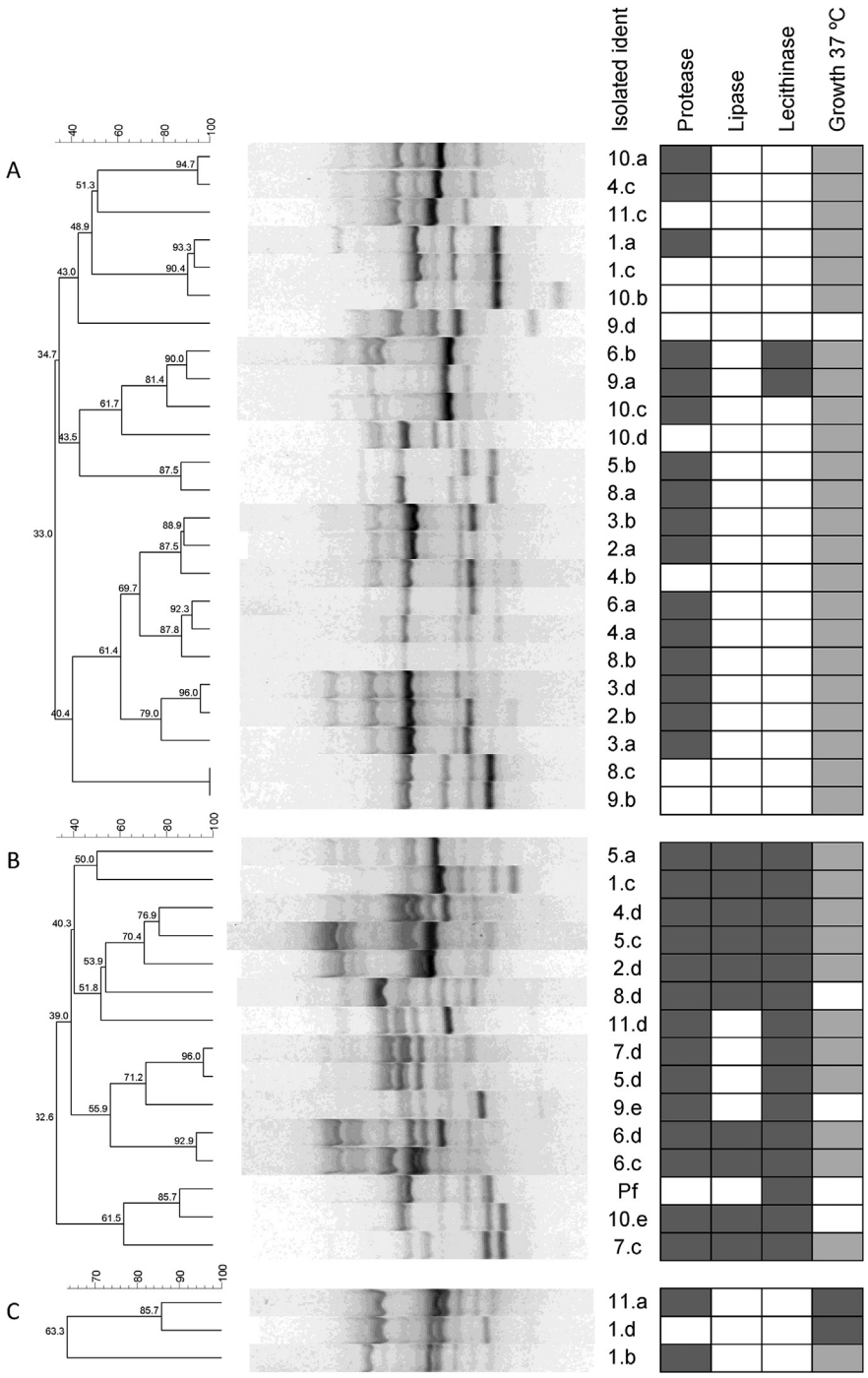

Fig. 1. RAPD fingerprinting plus phenotypic characteristics. A: *P. fragi*; B: *P. fluorescens*; C: *P. lundensis*. Isolated identification: the number represents the sample origin, whereas the letter represents the isolate within the sample. Dark Gray = evident; White = absence; Light Gray = limited. Pf = *P. fluorescens* ATCC 13525, control in all assays.

P. lundensis, demonstrated a high level of phenotypic and genotypic variability between and within the species. In addition, we observed intra strain variability in RAPD genotyping, with one RAPD type having a different phenotypic profile.

Multiple *Pseudomonas* strains could be synergistically associated with provoking poultry spoilage. The high phenotypic and genotypic diversity of *Pseudomonas* present at different poultry fillets samples from the same producers, but packaged in different days indicates various sources of contamination. The presence of *Pseudomonas* isolates from different poultry fillets, with different production dates and a similar RAPD types, also suggests the presence of *Pseudomonas* in persistent biofilms in one or more steps in the processing line.

Additionally, it was confirmed that *Pseudomonas* spp. was the main spoilage bacteria, affecting the poultry quality of skinless marinated poultry breast fillets stored at 4 °C.

Spoilage bacteria, such as *Pseudomonas* spp., should be evaluated for incorporation in HACCP pre-requisites plans. Each poultry producer must recognize and maintain control over the microbial spoilage contamination of their product before this reach the

consumer, which is only achieved with strict prevention and control systems, as well as the application of traceability of microorganisms in the food during the production, processing, distribution, storage and retail.

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