MOLECULAR MONITORING OF MEAT SPOILING *PSEUDOMONAS* SPECIES AND ANALYSIS OF STAPHYLOCOCCAL ENTEROTOXIN EXPRESSION AND FORMATION

Theses

Dóra Márta

Corvinus University of Budapest
Faculty of Food Science
Department of Microbiology and Biotechnology

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PhD School/Program

Name: PhD School of Food Science

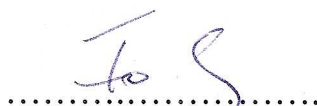
Field: Food Science

Head: **Prof. Péter Fodor**
University professor, DSc
Corvinus University of Budapest, Faculty of Food Science
Department of Applied Chemistry

Supervisors: **Prof. Anna Maráz**
University professor, CSs
Head of the Department of Microbiology and Biotechnology
Corvinus University of Budapest, Faculty of Food Science
Department of Microbiology and Biotechnology

Prof. Peter Rådström
University professor, PhD
Head of the Department of Applied Microbiology
Lund University, Faculty of Engineering (LTH), Sweden
Department of Applied Microbiology

The applicant met the requirement of the PhD regulations of the Corvinus University of Budapest and the thesis is accepted for the defence process.



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1. INTRODUCTION

The consumption of red meat represents a substantial part of human diet since the days of hunting and gathering. The domestication of animals (especially the sheep) which started at the dawn of the Neolitic revolution around 8500BC also contributed to this habit. The red meat contains different proteins, essential amino acids and trace elements that is necessary for the human health.

These characteristics and the high water activity of meat also ensure a perfect medium for the growth of microorganisms. The meat is contaminated after slaughtering with different psychrotrophic, psychrophilic and mesophilic microbes from the environment. On the surface of chilled, aerobically stored meat different *Pseudomonas* species play an important role and become dominant during storage. Their metabolic activity (especially proteolysis and lipolysis) contributes to the spoilage accompanied with off-odour and slime formation.

The characterization of *Pseudomonas* genus faces with difficulties based on their genetic heterogeneity. Reclassification of certain species originally belonging to the *Pseudomonas* genus is a continuous process based on the 16S rRNA studies. However, it is important to know the molecular diversity and characterization of *Pseudomonas* species to better understand the role of this type of bacteria in spoilage. In this work, different molecular methods were used to study the biodiversity of *Pseudomonas* species derived from pork meat which was stored at 4 and 8°C under aerobic conditions. These methods include RAPD, ARDRA, *rpoB*-RFLP, sequencing of 16S rDNA and *rpoB* genes and application of species-specific PCR. A genus-specific primer pair was also tested in parallel with the applicability of different media developed for detection of *Pseudomonas* species originated from various sources. To characterize the proteolytic and lipolytic activities of the isolates at different temperatures a semi-quantitative method was used.

Flavobacteria also play an important role in food spoilage by composing the initial part of the microbiota. The characterization of these microbes derived from meat is rare because the emending of the *Flavobacteriaceae* family started in the middle of '90s when the genus *Chryseobacterium* was also established and became the member of this family. There is only limited information regarding the *Chryseobacterium* isolates derived from spoilage. Characterization of the growth of a *Chryseobacterium* isolate in parallel with a type strain and cultivation in liquid cultures were performed at different temperatures during this work. Competition behaviour of the *Chryseobacterium* isolates was also tested with one *P. fragi* isolate combined in different ratios of inoculated cells.

Food safety is an essential issue for the food industry. Consumers demand continuously fresh food during the whole year containing less and less preservatives. The global distribution and consumption of fresh food emphasize the importance of food safety. Contamination of food with

pathogens presents greater economic and social problems than in the earlier years. The palette of food-borne infections changes continuously. While new pathogens appear, already existed pathogens may acquire new characteristics and occur in unexpected food matrices. The last outbreak that called the consumers' attention to the importance of food safety and prevention was detected in 2011 May in Germany and then at the end of June in France belonged to Shiga toxin 2a-producing *E. coli* serotype O104:H4.

Staphylococcus aureus is one of the most frequent pathogen that causes food-borne outbreaks. It is responsible for staphylococcal food poisoning (SFP) by producing heat-stable toxin. SFP was the fourth most common causative agent in food-borne illness within the EU in 2008. *Staphylococcus aureus* cells could be eliminated from the food but the toxin remains stable under rigorous heating. Contamination is mainly associated with improper and extensive manual handling of cooked or post-processed food rich in protein, that are combined with inadequate heating and/or improper storage temperature of the food. At present, 22 staphylococcal enterotoxins (SEs) or enterotoxin-like proteins were found. The two most often reported enterotoxins are, however, the SEA and SED which are encoded by genetically different genomic constituents. SEA (staphylococcal enterotoxin A) is encoded by the *sea* gene the expression of which is linked to the life cycle of SEA-encoded prophage. SED (staphylococcal enterotoxin D) is a plasmid-encoded protein that is connected to the *sed* gene and up-regulated by the accessory gene regulator (Agr) system via RNA III-mediated reduction of Rot (repressor of toxin) activity. The behaviour of pure bacterial cultures and the expression of their toxin genes in liquid culture media may be very different from those in food matrices where interaction of other bacteria through molecular signalling is current. To better understand the effects of these circumstances on SEA and SED formation, gene expression studies of *sea* and *sed* were performed on four processed pork products which possessed different intrinsic factors. As a control, the *Staphylococcus aureus* SA45 strain was cultivated under optimal growth conditions in pH controlled batch cultures. The relative expression of *sea* and *sed* as well as the extracellular SEA and SED production were determined by the application of quantitative reverse transcription polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA).

2. OBJECTIVES

The overall objective of this work was to study the dominant meat-associated bacteria (especially *Pseudomonas* species) involved in meat spoilage and the enterotoxin production of the important food poisoning *Staphylococcus aureus*. In the case of *Pseudomonas* species, the main objectives were (i) to work out a comprehensive methodology for molecular typing and identification and (ii)

to determine and characterise the diversity of the spoilage-causing microbiota on pork meat using conventional and molecular methods.

Regarding *Staphylococcus aureus*, the objectives were (i) to monitor the relative expression of enterotoxin genes (ii) to determine the production of enterotoxins and (iii) to compare the enterotoxin formation in food with batch cultures of a *Staphylococcus aureus* strain using molecular and immunological methods.

The following steps were determined for reaching objectives:

1. Evaluation of different *Pseudomonas* selective media for detection of pseudomonads

2. Molecular identification and assessment of genetic diversity of *Pseudomonas* spp. based on different PCR-methods

- a) evaluation of a *Pseudomonas* genus-specific primer pair published by Purohit et al. (2003)
- b) molecular typing and comparison of the isolates with RAPD analysis
- c) identification of the *Pseudomonas* isolates with species-specific PCR primers
- d) analysis of the 16S *rDNA* and *rpoB* genes by using PCR-RFLP and DNA sequencing

3. Determination of the proteolytic and lipolytic activities of the bacterial isolates to assess their spoiling potential

4. Study of the spoiling potential and competitiveness of a *Chryseobacterium antarcticum* isolate

5. Study of the growth behaviour of *Staphylococcus aureus* SA45 strain

- a) on different meat products
- b) in pH controlled batch fermentations
- c) as well as studying the expression of the enterotoxin encoding genes *sea* and *sed* in *S. aureus* by using qRT-PCR (referring to the circumstances in 5.a-b)
- d) and determine the extracellular SEA and SED production with ELISA (referring to the circumstances in 5.a-b)

3. MATERIALS AND METHODS

The *Pseudomonas* strains analysed in this work were derived from the spoiling microbiota of pork chops during 6 or 8 days of chilled storage at 4°C and 8°C. These strains were isolated at the Department of Microbiology, Central Food Research Institute, Budapest. Several other reference and type strains from different culture collection were also applied in these studies.

For the evaluation of selective and elective media aiming to detect different *Pseudomonas* species Cetrimide, GSP, *Pseudomonas* Agar F and P were applied and fluorescence ability as well as

pigment production was also tested at 20 and 30°C. For preliminary screening of *Pseudomonas* isolates in further studies connected to food, a genus-specific primer pair published by Purohit et al. (2003) was tested using the isolates, type strains as well as non-*Pseudomonas* strains. For typing of the *Pseudomonas* isolates RAPD-PCR was applied using four random primers with different sequences. Identification of *Pseudomonas* isolates was performed by the following methods: ARDRA, *Pseudomonas fragi* and *P. lundensis* species-specific primer pairs (Ercolini et al., 2007) as well as sequencing of the 16S rDNA and *rpoB* genes and *rpoB*-RFLP. Four restriction enzymes were applied for the restriction of the amplicons in 16S rDNA-RFLP and *rpoB*-RFLP analysis. Based on the RAPD restriction patterns as well as the 16S rDNA-RFLP and *rpoB*-RFLP patterns different dendograms were constructed by the GelCompare II software and then evaluated. In case of 16S rDNA and *rpoB* sequence analysis the sequences were aligned to the public sequence databases of GenBank to identify the isolates.

For testing the proteolytic and lipolytic activities of the isolates a semi-quantitative method was used by measuring the halo or the clearing zones around the macrocolonies on SM (Skim Milk agar), SMC (Standard Methods Caseinate Agar), PAT-80 (Tween^{TM} 80 Hydrolysis Medium) and PCATB (Tributyrin PCA) plates. The temperatures of incubation were 15, 20, 25 and 30°C and the clearing zones were checked after 24, 72 and 168 hours of incubation.

Characterization of the *Chryseobacterium antarcticum* F1445/3 isolate was also performed after the 16S rDNA sequence alignment to the database on EzTaxon server 2.1. Cultivation at different temperatures (from 5°C to 30°C) as well as lipolytic and proteolytic activities were also analysed in case of that isolate together with *Chryseobacterium antarcticum* JCM 12381^{T}. The competitiveness between *P. fragi* and *C. antarcticum* isolates was also tested with different cell ratios in liquid cultures.

In case of *Staphylococcus aureus* enterotoxin studies, SA45 strain derived from a boiled ham in a food-poisoning outbreak was applied. Four different processed meat products (smoked, cooked and Serrano ham, black pepper salami) were used for testing the behaviour of *S. aureus* SA45 focusing on the growth, the *sea* and *sed* gene expression as well as SEA and SED production. Gene expression studies were performed by qRT-PCR applying hybridization probes. The amounts of the produced SEA and SED toxins were detected separately by sandwich ELISA in which polyclonal antibodies were used. Fermentations in pH controlled batch cultures at pH7 were also performed with SA45 strain to follow the same parameters.

4. RESULTS

4.1. Characterization of the *Pseudomonas* isolates

Morphological, physiological and biochemical properties of representative bacterial strains isolated from pork meat stored at 4 and 8°C were determined in which altogether 34 psychrotrophic/psychrophilic bacterial isolates were characterised. All isolates were Gram negative, catalase and oxidase positive, non-spore forming. To determine the selectivity of different *Pseudomonas* media, GSP, Cetrimide, *Pseudomomas* Agar P and F were tested for growth and fluorescence of the isolates at 20 and 30°C for 72 h. As control PCA plates were also used. All the isolates had good growth on the different media at 20 and 30°C except F1445/3 that showed weak growth at 30°C. Non *Pseudomonas* species had weak growth on all media at both temperatures. Although growth of the *Pseudomonas* isolates was quite uniform there was difference in the fluorescence ability and pigment production at 20 and 30°C. Five isolates (F1443/4c, F1443/5, F1443/6, F1443/7, F1443/11) had greenish-yellow colour on Cetrimide and *Pseudomonas* Agar F. These isolates showed fluorescence more intensively under UV light at 366 nm on these media as well as on GSP agar than other isolates. Majority of the isolates did not show fluorescence at 20 and 30°C on *Pseudomonas* Agar P and PCAT and almost one third of them did not show fluorescence on GSP, Cetrimide and *Pseudomonas* Agar F. Four isolates (F1443/12b, F1443/13a and b, F1445/4) had dark brown pigment production on *Pseudomonas* Agar F while the same colour changing was observed on BHI agar after 3 days of incubation at 20 or 30°C.

All the selective and elective media were suitable for growth of *Pseudomonas* strains isolated from meat and distinction from other non-*Pseudomonas* species at 30°C as well as detection of their pigment production and fluorescence ability. According to the data, the majority of the isolates produced pyoverdine which is a potent siderophore for the fluorescent pseudomonads. It was found that pyoverine was less stable at 30°C than at 20°C.

4.2. Molecular characterization of the *Pseudomonas* isolates

For screening the food spoilage-causing *Pseudomonas* strains, a genus-specific primer pair (Psf-Psr) was tested which was published by Purohit et al. (2003). Altogether 14 *Pseudomonas* type and reference strains gave positive reactions with the Psf-Psr primer pair. Two *Pseudomonas* reference strains were negative in the test and six non-*Pseudomonas* species were positive in the PCR reaction. The sensitivity was calculated as 87.5% while the specificity was only 66.6%. The analysis of the specificity was also calculated after the exact identification of the putative *Pseudomonas* isolates had been done, which showed that the sensitivity decreased to 82,5%. Because of the low specificity of the PCR reaction the further use of this primer pair was abolished.

Pseudomonas isolates derived from meat spoilage were fingerprinted by RAPD-PCR analysis to characterize their diversity. The dendogram showed very divergent RAPD patterns. All the isolates had distinct position in the dendogram and showed only 60-70% similarity regardless being isolated from the same or different samples. *Pseudomonas* type and reference strains showed very low degree of similarity and they generally formed unique clusters below 50% similarity.

According to the cluster analysis in case of 16S rDNA-RFLP four different groups could be distinguished from each other at the level of 60% similarity. All the type and reference strains that belonged to *P. fragi*, *P. lundensis* and *P. fluorescens* separated into the first two clusters;.only *P. putida* ATCC 12633^T belonged to cluster 4 but showed less than 70% similarity.

For identification of *P. fragi* and *P. lundensis* species among the *Pseudomonas* isolates a multiplex PCR assay was used which was developed for the *car*A gene by Ercolini et al. (2003). The multiplex PCR had been optimized but the species-specific primer pairs were used in separate PCR reactions. From the 34 isolates altogether 25 were identified as *P. fragi*. None of the isolates generated amplicon with the *P. lundensis*-specific primer pair. For identification of nine *Pseudomonas* isolates which were negative in the species-specific PCR reaction, direct sequencing of the *rpoB* and 16S rDNA genes were used. Eight other isolates were also tested that originally gave positive results with the *P. fragi* specific primer pair but generated extra amplicons during that reaction. Based on the *rpoB* and 16S rDNA sequence analysis these eight isolates were identified as *P. fragi*. From the nine isolates which were negative in the species-specific PCR reaction eight were identified as *P. fluorescens.* One isolate namely the F1445/3 was previously found to be different from the *Pseudomonas* isolates (e.g. production of orange pigment, slow growth) not only in phenotypic characteristics but also in the analysed 16S rDNA sequence. Based on the database of the EzTaxon server 2.1. the F1445/3 isolate was identified as *Chryseobacterium antarcticum.*

In parallel the *rpoB*-RFLP was also tested as a cost-effective solution for molecular identification. In this analysis five clusters were formed where the similarity was higher than 60% but some solo strains have not been clustered. All the *P. fluorescens* and *P. fragi* type and reference strains were clustered into the same group similarly as it was found previously in the 16S rDNA-RFLP analysis.

4.3. Lipolytic and proteolytic activities of the *Pseudomonas i*solates

Proteolytic activites of *P. fluorescens* isolates on SM and SMC plates at 12, 20, 25, 30°C were more intense that of the *P. fragi* isolates in almost all cases. The proteolytic activity on SM plates was more intense than on the SMC plates. It was found that *P. fluorescens* isolates also possessed higher proteolytic and esterase activities than *P. fragi* isolates. However, the lipase activity of *P. fragi* isolates at 15 and 20°C was higher.

4.4. *Chryseobacterium antarcticum* and its potential role in meet spoilage

The optimal temperature of growth of the *Chryseobacterium antarticum* F1445/3 isolate that was derived from the initial population from meat stored at 8°C was between 15 and 25°C and possessed higher proteolytic activity at 15, 20, 25 and 30°C than the type strain *Chryseobacterium antarcticum* JCM 12381^T. This difference could be due to its origin from meat while the type strain was derived from the Antarctic habitat. Both the F1445/3 isolate and the type strain had lipase activity. The competition between *P. fragi* F1445/1b and *Chryseobacterium antarticum* F1445/3 strains inoculated in different cell ratio into liquid culture medium showed that if the cell ratio was equal or *P. fragi* had higher cell concentration the *Chryseobacterium antarticum* F1445/3 had been overgrown. However, when the *Chryseobacterium antarcticum* F1445/3 had higher cell concentration it was able to compete with the *P. fragi* F1445/1b strain.

4.5. Expression of the *S. aureus sea* and *sed* genes and enterotoxin formation

Four processed pork products were studied that possessed different intrinsic factors to see how *S. aureus* is able to grow and produce heat stable enterotoxin A and D which are encoded by the *sea* and *sed* genes, respectively, carried on different genetic elements. Gene expression and the production of SEA and SED were also followed in liquid culture. The Agr-regulated *sed* expression showed a similar expression behaviour as the phage-regulated *sea* expression in the pork products. The *sea* expression peaked in the transition from the exponential to the stationary growth phase in both meat products and broth cultures indicating that its expression is linked to the bacterial growth. However, the time scale differed significantly between the two matrices. In broth medium the *sea* expression peaked after 3–4 h of growth, while in the boiled and smoked ham products the expression peak was found after 1–2 days of incubation. The amount of SEA and SED toxins was much higher in the boiled and smoked ham slices already after one day of incubation. Smoked ham was found to contain much lower amounts of SED per CFU of *S. aureus* than boiled ham. In Serrano ham, growth of *S. aureus* was restrained and SED was not detected until five days of incubation. However, the amount of SEA and SED toxins that was detected on these products could also be potentially enough to cause SFP in sensitive individuals. The difference between the produced SEA and SED amounts could be attributed to the fact that the *sea* and *sed* gene expressions belong to different regulations in *S. aureus*. The growth of *Staphylococcus aureus* was supported on the three ham products while no growth was detected on black pepper salami as a possible consequence of lactic acid bacteria, the low pH and the spices.

5. NEW SCIENTIFIC RESULTS

1. Evaluation of selective and elective media for *Pseudomonas* isolates:

It has been proved that the combinations of *Pseudomonas* Agar F, *Pseudomonas* Agar P, GSP agar and Cetrimide agar were applicable for the detection and characterization of *Pseudomonas* species derived from meat spoilage when the incubation temperature was 30°C. *Chryseobacterium antarcticum,* however, could also grow on these media at 20°C. Fluorescence of the isolates at 30°C was less intensive than at 20°C that could be connected to the faster growth at 30°C and instability of pyoverdine in the stationary growth phase.

2. Molecular identification and typing *Pseudomonas* isolates:

The *Pseudomonas* genus-specific primer pair published by Purohit et al. (2003) had low specificity (66.6%) and sensitivity (82.5%) as calculated by the application of *Pseudomonas* isolates, type and reference strains, therefore it could be concluded that these primers are not suitable for preliminary screening of *Pseudomonas* species belonging to this genus.

It has been concluded that the RAPD analysis was not applicable for the determination of high similarity clusters of *Pseudomonas* isolates as the consequence of high level of their molecular diversity. However it could be applied for typing of *Pseudomonas* isolates. The 16S rDNA-RFLP method was not suitable either for identification or typing of *Pseudomonas* species. The *rpoB*-RFLP was not useful for identification purposes either.

From the end of storage of pork meat at 4°C *P. fluorescens* and *P. fragi* species were identified by sequencing of 16S rDNA and *rpoB* gene and using species-specific primer pair while the initial microbiota of pork meat stored at 8°C contained not only these species but the *Chryseobacterium antarcticum* (F1445/3) as well. At the end of storage at 8°C only *P. fragi* became dominant.

3. Proteolytic and lipolytic activites of the *Pseudomonas* isolates:

The *Pseudomonas fluorescens* isolates showed quite intense proteolytic activity on SMC plates in 15, 20, 25 and 30°C except F1443/2a and F1443/7 which showed activity only at 15 and 20°C. However the proteolytic activity of all *P. fluorescens* isolates on SM plates was higher than on SMC agar. The esterase activity of these *P. fluoresens* isolates was also intense and higher than that of the *P. fragi* isolates in all incubation temperatures. However, the lipase activity of *P. fluoresens* isolates was lower compared to *P. fragi* isolates at 15 and 20°C. The majority of *P. fragi* isolates had both proteolytic and lipolytic activities.

4. Spoilage potential of the *Chryseobacterium antarcticum* F1445/3 strain

It was the first case that *Chryseobacterium antarcticum* was isolated from surface of chilled pork meat. The optimal growth range of *Chryseobacterium antarcticum* F1445/3 was between 15 and 25°C. This isolate had proteolytic activity at 15, 20, 25 and even at 30°C. It also produced lipase but esterase production was not detected. Measurement of the proteolytic and lipolytic activities of this strain showed that this bacterium could also contribute to the initial spoilage process.

The competition between *Chryseobacterium antarticum* F1445/3 and *P. fragi* F1445/1b strains showed that when *Chryseobacterium antarcticum* was in higher cell ratio than the *P. fragi* F1445/1b it was able to grow and compete even after the *P. fragi* reached its maximal growth rate and started to decline. When *Chryseobacterium antarcticum* F1445/3 was in equal cell ratio with the *P. fragi* F1445/1b or *P. fragi* F1445/1b had higher cell ratio this competition between the two strains could not be demonstrated.

5. Growth of *Staphylococcus aureus* and its enterotoxin expression and formation:

It was the first time that the *sea* and *sed* enterotoxin gene expression of *S. aureus* was detected in combination with enterotoxin production in food, especially on cooked ham, smoked ham, Serrano ham and black pepper salami. Extended *sea* and *sed* expression were observed in ham products as well as during fermentations, the pattern of these gene expressions and enterotoxin formations, however, were different. The *sea* expression was continuous under pH controlled circumstances, while the *sed* expression had second peak during the expression after 4 days. The amount of SEA toxin was higher than the SED during fermentation and both of them proved to be stable in the liquid culture medium. The ratio and the amount of SEA and SED also altered in different ham products but could be enough to potentially cause SFP. In smoked ham the amount of toxins remained stable while in cooked ham it decreased after 5 days. In black pepper salami there was no growth and enterotoxin production at all.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

Based on the cultivation results on selective and elective media (Cetrimide, GSP, *Pseudomonas* agar F and P) all of them were suitable for selection and pre-characterization of the *Pseudomonas* isolates from meat but cultivation at 30°C is more advisable than at 20°C. To receive broader view from pigment production of the isolates derived from meat *Pseudomonas* Agar F and P is advised. Further studies on pigment production and analysis of its function in the life cycle of the spoilage causing pseudomonads could also contribute to the deeper characterization of the isolates. The results of investigations at the beginning of this century already indicated that (Meyer, 2000). The fluorescent pigment pyoverdine might be considered as potent and easy-to handle taxonomic

marker for the fluorescent species of the genus *Pseudomonas* (Meyer, 2000). It was also found in another investigation that different pigments have a function as signalling molecules controlling gene expression inside the bacterial cells (Lamont and Martin, 2003) as well as that in case of *P. aeruginosa* the pyocyanin production has potential virulence function (Liu and Nizet, 2009).

The RAPD analysis could be applied for pre-selection purposes of the isolates from large number of candidates. It was demonstrated that sequencing is a suitable tool for identification purposes. It provides more precise identification because it analyses the series of nucleotides in the DNA while the PCR-RFLP method focuses only few restriction sites in the DNA. Nevertheless, for establishing an in-house bacterial databank the PCR-RFLP analysis provided a more cost effective solution until now. As a promising method the MLSA (multi-locus sequencing analysis) technique seems more reliable for detection of *Pseudomonas* species in the future based on the combination and application of *rpoB*, *gyrB* or *rpoD* genes at the same time. The application of Rep-PCR (repetitive extragenic palindromic-PCR) is also an alternative method that focuses on repetitive chromosomal elements, which are randomly distributed in bacterial genomes and are the target of the PCR amplification. Designing further species-specific primer pairs e.g for *P. fluorescens* could be a future perspective based on genes that belongs to the metabolic pathway e.g. enzyme production or pigment production. However this perspective brings more difficulties in case of *P. fluorescens* because it has 5 biovars as it was found in variation of ribotyping patterns in *P. fluorescens*.

According to the data regarding the lipolytic and proteolytic activities and physiological properties of *Chryseobacterium antarcticum* this bacterium could be the member of the spoilage causing biota however it has only role at the beginning. Molecular characterization of species belonging to the young genus of *Chryseobacterium* could be a novel field in the identification and characterization of them. Some information is available from *gyrB* gene that encodes the subunit B protein of DNA gyrase in molecular database but only for a few *Chryseobacterium* species.

The extended *sea* and *sed* expression observed in ham products has provided new information about SEA and SED production in food products where *S. aureus* is immobilized in multi-cellular communities. Furthermore, the *sed* gene expression pattern in ham products indicates a complex behavior of the regulatory network controlling *sed* expression. These results may be used to enhance the assessment of SFP (staphylococcal food poisoning). However, more data is required on staphylococcal enterotoxin regulation and signal transduction to ensure the production of microbiologically safe food with high quality. Further investigations should therefore be carried out to study the effects of other intrinsic and extrinsic factors that influence the enterotoxin expression and then toxin production in *S. aureus* and growth in various food matrices. Microarray studies could be a help to understand the mechanism of the global gene regulation between these toxins and

how the quorum sensing influences the toxin production on these products when other microbial community also contribute to the deterioration of the food.

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7. PUBLICATION LIST

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In journals with impact factors

1. Belák, Á., Kovács, M., Hermann, Zs., Holczman Á.N., **Márta, D.**, CeniČ StojakoviČ, S., Bajcsi, N., Maráz, A. (2011) Molecular analysis of poultry meat spoiling microbiota and heterogeneity of their proteolytic and lipolytic enzyme activities. *Acta Alimentaria* **40** (Supplement): 3-22. (Impact factor: 0.379)

2. **Márta, D.[1]**, Wallin-Carlquist, N.[1], Schelin, S., Borch, E., Rådström, P. (2011) Extended staphylococcal enterotoxin D expression in ham products. *Food Microbiology* **28**: 617-620. **[1] These authors contributed equally to the paper.** (Impact factor: 3.320)

3. Wallin-Carlquist, N. [1], **Márta, D. [1]**, Borch, E., Rådström, P. (2010) Prolonged expression and production of *Staphylococcus aureus* enterotoxin A in processed pork meat. *International Journal of Food Microbiology* **141**: S69–S74. **[1] These authors contributed equally to the paper.** (Impact factor: 3.143)

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1. **Marta, D.** (2007) Immunanalytical methods for the detection of gliadin as wheat allergen protein, Journal of Food Investigations Food Quality-Food Safety Volume LIII. pp. 166-173.

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2. **Márta, D.**, Takács, T., Gelencsér, E. (2007) Enzyme-linked immunanalytical methods for the detection of cereal allergens in food, "Lippay János-Ormos Imre-Vas Károly" Scientific Conference, Faculty of Food Science, 7-8 November 2007, Budapest, Hungary, Book of Abstracts pp. 72-73.

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