VARIATION AND UNIFORMITY IN COOKED MEAT SPOILING BACTERIA.

Renate Zumbrink[1], Nicole de Rooij[1], Olav Sliekers[1], Peter Sijtsema[1] and Harmen Kroon[1]

[1] Corbion Purac, Arkelsedijk 46, 4206 AC Gorinchem, P.O. Box 21, 4200 AA Gorinchem, Netherlands

Abstract – The objective of this study was to investigate the variation in cooked meat spoilage between regions in the world and the variation in growth and lactic acid sensitivity between individual spoilage strains and species. More than 200 dominant isolates (60 from the US) were obtained from different regions in the world (US, Latin America, Asia and Europe), their 16 S r DNA sequenced and their growth rates and lactic acid sensitivity determined. The most frequently isolated species per region show some variability, but a breakdown to the top 3 per region show obvious similarities. The biological variation, in terms of growth rate however, between meat spoilage strains is considerable.

Key Words – Lactic acid bacteria, Cooked meat, spoilage

I. INTRODUCTION

Although cooked meat products like frankfurters and ham are subjected to a heating step of around 72 °C, it is not the spore forming bacteria that frequently spoil these meat products. It is well established that Lactic Acid Bacteria (LAB) are important spoilers of cooked meat and organoleptic defects and pH drop correlate strongly with the numbers of lactic acid bacteria [1,2]. Beside color, taste or odour defects, growth of LAB can result in gas formation and slime production [3]. A level of 10^7 LAB per gram in cooked meat can be regarded as spoiled (Kreyenschmidt). LAB like *Lactobacillus sakei* and *Leuconostoc mesentroides*, but also *Weisella* and *Carnobacterium* species have been found in spoiled cooked meat [4,5]. However, studies up to now don't give a clear picture of the most dominant species and could preferably use a reassessment using DNA based identification. Also, the studies so far do not answer the question whether there is variability between different regions in the world. Moreover, species may differ in terms of growth rate, and strains of the same species as well. Knowledge about the variability in spoilage flora is important for optimal design of antimicrobial solutions to extend shelf life of cooked meat. The objective of this study was to investigate the variation in cooked meat spoilage between regions in the world and the variation in growth and lactic acid sensitivity between individual spoilage strains and species.

II. MATERIALS AND METHODS

More than 200 dominant isolates (60 from the US) were obtained from spoiled cooked meat products from different regions in the world (US, Latin America, Asia and Europe). Their 16 S r DNA was sequenced, and the identity was established by comparing to the SILVA database with a homology cut off of at least 99%. A the top 5 list per region was established, by counting the frequency of isolation of a particular species from cooked meat products from a specific region. A considerable part of the strains (128 strains, and approximately 25 strains per species) was grown in MRS broth at 4°C and growth was measured using the optical density (turbidity) in broth. The logistic growth equation was fitted to each growth curve and a growth rate (μ) was established. Different species were also grown in cooked meat (2.3% salt, 1.5% maltodextrine, 0.42% STP, 3% modified corn starch, 0.7% carrageenan, 84.75% Turkey breast) at 4 C in absence and presence of 3% Purasal S. Purasal S is a Corbion Purac product containing lactate that has been shown to delay spoilage of cooked meat. To count the amount of bacteria present at a time point, meat samples were diluted 3 times with peptone saline water and homogenized with a stomacher for 60 seconds. The homogenized samples were diluted further and plated on MRS agar plates. The amount of colonies per gram was assessed after 2 days of growth at 30 °C.

III. RESULTS AND DISCUSSION

A clear top 5 of vacuum packaged cooked meat could be established (Table 1). Almost a quarter of all isolates consisted of *Lactobacillus sakei*. Other important spoilers were *Leuconostoc mesentroides, Leuconostoc carnosum, Lactobacillus plantarum* and *Lactobacillus curvatus*. Together, they have a 72% share of the isolates. Other species did not exceed 5% and most of the other species were only isolated a few times, indicating that the top 5 cooked meat spoilers were indeed the dominating flora.

Table 1: Top 5 spoilage bacteria found in cooked meat products. The total of isolated bacteria was 212 strains.

	Absolute count	Share
Lactobacillus sakei	48	23%
Leuconostoc mesenteroides	35	17%
Leuconostoc carnosum	24	11%
Lactobacillus plantarum	23	11%
Lactobacillus curvatus	22	10%
Total share		**72%**

The most frequently isolated species per region show some variability with respect to the most dominant species, but a breakdown to the top 3 per region show obvious similarities (Figure 1).

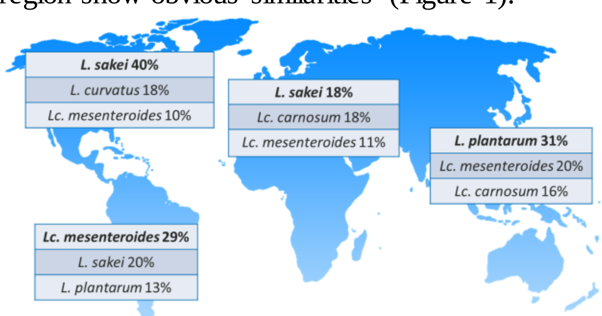

Figure 1. A breakdown of the top 3 cooked meat spoilers per region

In the US, the most frequently isolated species was *Lactobacillus sakei* (40%). In Europe both *Lactobacillus sakei* as well as *Leuconostoc carnosum* was found equally often (18% each). In Latin America, *Leuconostoc mesentroides* (29%) was the most common and in Asia, *Lactobacillus plantarum* (21%).

More striking was the variability in broth growth rates between the species and especially between different strains of the same species (table 2). *L.plantarum* grew by far the slowest in broth, whereas slow and fast growers could be found within the *L.sakei, Lc. mesentroides* and *Lc. carnosum* strains. Although the fastest growing strains could be found within the *L.sakei* species, the strains were very variable in terms of growth rate. Between the slowest strain and the fastest growing strain there was a factor of 5.

The fastest strain of each species could in theory outgrow the slowest strain of another species. An exception to this is *L. plantarum*. All 25 strains grew slower than the slowest strains of other species.

Table 2: mean, maximum and minimum growth rate per day at 4°C in MRS broth

	L.sakei	*Lc.mesentroides*	*Lc.carnosum*	*L.plantarum*	*L.curvatus*
strains tested	29	26	24	25	24
average µ per day	0.57	0.27	0.30	0.03	0.45
min	0.21	0.14	0.18	0.00	0.31
max	1.04	0.41	0.59	0.07	0.51

When grown in cooked Turkey roll, the growth rate of all species was diminished with almost the same factor by Purasal S (Figure 2). L. plantarum was unable to show substantial growth during the period tested. The current hypothesis is that this species shows better growth at higher temperatures (eg. 7°C). This hypothesis could also explain the prevalence of this species in Asia and South America (figure 1), where the distribution channel may be less reliable compared to the US and Europe.

It is also tempting to hypothesise that it is a matter of chance what species from the top 5 becomes dominant during shelf life. It may depend on the individual growth rate of all the competing species that attach to the cooked meat during processing and packaging and due to the shown variability, the growth rates of the individual species are sometimes higher and sometimes are lower than others. Luckily, lactic acid will slow down growth, independent of who is winning the competition.

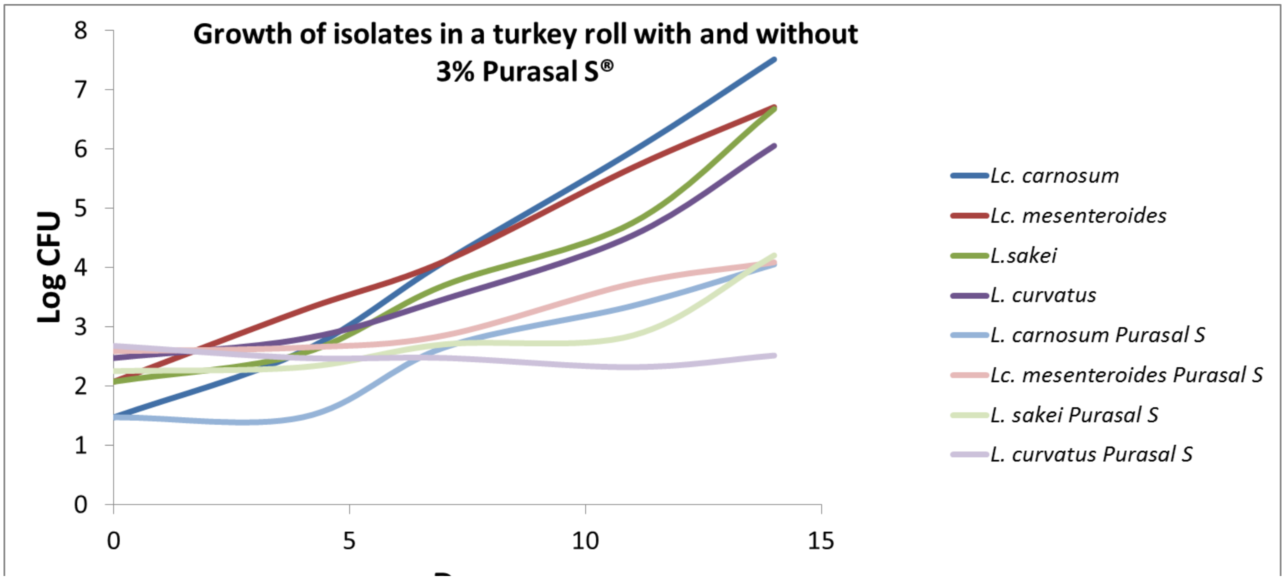

Figure 2 growth of isolates in a turkey roll with and without 3% Purasal

IV. CONCLUSION

The biological variation between meat spoilage strains is considerable. This in sharp contrast with the uniformity of the dominant flora that spoils cooked meat all over the world. All species were sensitive to lactic acid..

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