Food hygiene - the influence of microbial biofilms and problem identification

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Outline

- Introduction
- Problems caused by persistent microorganisms
- Why do they persist?
- Consequences
- Cleaning and disinfection

Importance of bacteria in foods

- Problems
  - Illness
  - Spoilage

- Advantages
  - Preservation
  - Flavour, texture, ...

- No importance

How is hygiene controlled in the food industry?

- GHP (good hygienic practice=SSOP=pre-requisites)
  - buildings, lay-out, training of personnel, pest control, cleaning and disinfection

- HACCP
  - hazards – what can happen (likely)
  - critical control points – how and where can the hazards be eliminated or reduced to an acceptable level
Problem 1: Spoilage of marinated herring

- Raw material (herring in salt/acid) “sterile”

- Acid flavour and gas/bombage
  - isolate lactic acid bacteria and yeast
  - LAB + yeast traced to surfaces and equipment in the processing environment

Problem 2: Spoilage of ground turkey

- Raw material contains many spoilage bacteria

- Rapid spoilage due to *Pseudomonas* spp.

- Isolate pseudomonads from raw material, processing equipment and final product
  - spoilage-sub-types traced to meat grinder
  - identical sub-type isolated after 6 weeks
  - => “resident” flora
Problem 3: 
*Listeria monocytogenes* on smoked fish

- Gram-positive, environmental bacteria
- Causes listeriosis (sepsis, meningitis, abortion)
- High mortality in risk groups
- Food-borne disease
- Psychrotrophic, halotolerant: grow in many foods
- Listeriosis may be caused by cheese, pate, frankfurters, smoked trout, smoked mussels

**Listeria - in the news**

- Maple Leaf Foods - *Listeria* linked to deaths
  - 57 reported cases, 22 deaths
- 220 products recalled; costs: 20 mill $ + 27 mill $
- One of 22 plants involved
- One-two of 11 production lines involved – found “lurking deep inside two meat-slicing machines”
- Re-opened after thorough cleaning and sanitizing (peroxy-acetic acid, QUATs, isopropyl alcohol, refrigeration gel, granular compound) and training of employees

**Listeria - in the DK news**

- Danish outbreak – spring/summer 2014
- 41 patients, 17 dead
- Source: “rullepølse” – up to 2 month incubation time
- ID’ed by genome analyses (SNP; single nucleotide polymorphism)

**Listeriose in DK – clinical picture**

Data from SSI
Problem 3: *Listeria monocytogenes* on smoked fish

• Preparation of cold-smoked fish
• processes do not eliminate *Listeria*
• parameters do not prevent growth
  • no CCP in the HACCP-plan
• ready-to-eat – no cooking by consumer

• Level should be as low as possible
• Source of product contamination?

Sources of *Lm* in smoked fish?

- Water
  • Low
  • 0-62% positive

- Raw fish
  • Typically 1%
  • 0 - 10% positive

- Final product
  • average on 5%
  • 0-84% positive;
  • large factory variation

Tracing *Lm* during fish processing

Differentiating between strains of the same bacterial species?

Subtyping (molecular, sero, phage, phenotype, genome)

Techniques used in epidemiology
Tracing *Lm* during fish processing

Randomly Amplified Polymorphic DNA (RAPD)

purify DNA, amplify with random primers, separate profile in agarose-gel

Strain number

1 2 3 4 5 6 7 8 9 10 ..........

Tracing *Lm* during fish processing

- Follow fish during processing
  - raw fish, fillets before salting, fillets after smoking, slicing, packaged product
- Sampling from surfaces during processing
- Sampling from processing environment
- Sampling after cleaning and disinfection

Fonnesbech Vogel et al. 2001. AEM

Number of isolates with RAPD type

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| Positive for *L. monocytogenes* | 17 | 13 | 05 | 08 | 39 | 19 | 09 | 03 | 15 |

| Total samples | 20 | 12 | 18 | 230 | 8 | 150 | 147 | 40 | 12 | 105 | 2 | 75 | 100 | 48 |

1. P = product, R = raw fish, R-A = raw fish area, S-A = smoking area; S-A = Slicing area

Wulff et al. 2006. AEM

Distribution of RAPD subtypes in fish processing environments

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X Total | 28 | 43 | 46 | 32 | 46 | 7 | 29 | 7 | 238
**Why does one Lm sub-type dominate?**

Persistent versus non-persistent

- Most common in the environment?
- Adhere better to surfaces?
- Higher growth rate?
- More tolerant to preservation?
- More tolerant to drying?
- More tolerant to cleaning and disinfection?

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Hansen et al. JFP 2006

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**Lm in the environment**

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**Lm adheres to inert surfaces**

- Scanning Electron Micrograph (SEM) of Lm on a plastic surface
- Atomic-Force-Microscopy (AFM) of Lm on surface

Jensen and Kastbjerg in collaboration with CU and MMU

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**L. monocytogenes adhesion and biofilms**

- Listeria monocytogenes biofilm formation in microtiter wells; measured as crystal violet staining

Jensen et al. JFP 2007
Growth rate, processing, drying, cleaning and disinfection

- \( Lm \) strains grow with similar growth rate
- \( Lm \) strains are inactivated similarly
- \( Lm \) survives drying for weeks/months in the presence of organic material and NaCl
- All \( Lm \) strains appear equally sensitive to disinfection

Process contamination - why

- Lack of cleaning and disinfection
- Wrong physical design
- Adhered bacteria
  - protected by slime, proteins
  - resistant to cleaning and disinfection?
- Special adhesion phenotype?

Proces contamination – why?
Lack of cleaning and disinfection

Cleaning and colonization of \( Listeria monocytogenes \)

Comparison of total aerobic count and % samples positive for \( Listeria monocytogenes \) from a food processing unit

Hejmarklaboratoriet and DTU Aqua 2008
Proces contamination – wrong design

Proces contamination – why?
Micro-design – surface topography

Listeria monocytogenes on a stainless steel surface
Shewanella putrefaciens on a stainless steel surface

Adhered bacteria may be resistant (?)

Reduction in *Listeria monocytogenes* by treatment with 0.1M NaOH / HCl

Survival of *L. monocytogenes* as planktonic (light) or adhered (dark) cells when exposed to Incimaxx

Kastbjerg and Gram 2008

Bakterial adhesion/removal depends on:

- The bacteria
  - species, strain, other bacteria, culture

- Surface
  - conditioning, roughness, hydrophobicity

- Flow
  - turbulent, stagnant

Presence of pseudomonads facilitate biofilm formation by *Listeria*

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<th>Surface</th>
<th>log cfu/cm² after days:</th>
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<td>rubber</td>
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Bourion and Cerf 1996. Sciences des aliments 16:151-156

Niche?

- A niche – also called "harbourage site"
  - a place in the processing area, where one/several microorganisms has become established and multiply
  - is a reservoir for the bacteria from where it spreads during processing
  - typically requires water (humidity) and dirt (nutrients)
Crack where cleaning is impossible

Cleaning and disinfection
- Purpose to remove/kill microorganisms
- Real life:
  - microbes detected after cleaning and disinfection
- Improvement:
  - new methods
  - how to compare?

“New” cleaning and disinfection
- Biochemically based
  - removal of microbial biofilms: cells, water, extracellular products: exo-polysaccharides, protein, DNA
- Physically based
  - removal by heat, ultrasound, oxidation, UV-light
    - sonosteam (steam and ultrasound)
    - ozon
    - UVC-light

“new” cleaning and disinfection

_Pseudomonas aeruginosa_ biofilm on stainless steel (A) before and (B) after treatment with Pectinex Ultra

Johansen et al. 1997. AEM
Quantifying bacteria on surfaces ??

- Fluorescens mikroscopy
  - DNA-staining, rRNA-staining
- Removal from surface – quantification by plating
  - ultrasound, glass beads a.o.
- ATP-measurements
- Conductometric methods
- Real-time PCR to quantify

Conclusions / perspectives

- Equipment must be designed to allow cleaning
- Surfaces minimizing adhesion should be developed
- Understanding adhesion/ resistance in food microorganisms is required
- Microbial ecology of food processing
  - hot spots?
  - methods for quantification
  - procedures against attached bacteria
  - tolerance / resistance to biocides
  - .............