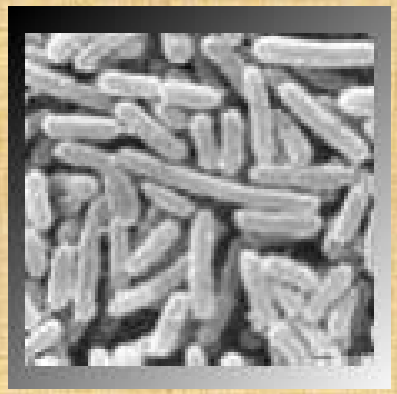
C. sakazakii: Advice, Policy and Research in Canada



Jeffrey M. Farber and Franco J. Pagotto
Bureau of Microbial Hazards
Food Directorate
Health Canada

Health Canada Santé Canada

Outline

- Introduction
- Canada's role in CCFH/Codex
- GMPs for Infant Formula
- Regulation of PIF and other human milk substitutes in Canada
- Microbiological Criteria for PIF in Canada
- Cases/Surveillance
- Guidelines for the Safe Preparation, Storage and Handling of PIF
- Research in Health Canada

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Powdered Infant Formula (PIF) in Canada

- Used to have 5 or 6 plants manufacturing PIF
- At present, none left and we import all PIF
- Nevertheless, PIF safety is an important issue for Canada

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Codex Committee on Food Hygiene

- In 2003, the Codex Committee on Food Hygiene (CCFH) requested a revision of the *Recommended International Code of Hygienic Practice for Foods for Infants and Children* (1979)
- The CCFH requested:
  - Canada to initiate work towards the revision of the Code with the help of a drafting group;
  - *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children*
- Two FAO/WHO Expert Group meetings were held in 2004 and 2006 on *E. sakazakii* and other microorganisms in PIF

CCFH - *Code of Hygienic Practice for Powdered Formulae for Infants and Young Children*

- ✓ Code was completed in 4 years
- ✓ Has helped contribute to an improvement in the hygienic conditions in plants manufacturing PIF
  - ✓ Microbiological criteria
  - ✓ "Safe preparation, storage and handling of PIF"
  - ✓ Used the RAs to help develop the Code
  - ✓ Web-based risk assessment tool

FAO/WHO Expert Group Meeting (2008) & CCFH (2008)

- Washington, 2008: Technical meeting with the objective of providing the scientific information to inform the decision-making process on the development of a microbiological criterion for *C. sakazakii* for follow up formulae (FUF) intended for infants 6–12 months of age

- Guatemala City, 2008, CCFH:
  - The meeting concluded that there was not a clearly defined scientific justification to support the establishment of a microbiological criterion for *C. sakazakii* in FUF as a RM option
  - Other RM options to consider include enhanced product labelling and education of caregivers and health professionals
  - Text to be added to Annex II to emphasize that FUF should be used for the target population for which is intended and to highlight the need for further education of caregivers and health professionals as to the appropriate uses of FUF

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Health Canada: **GMPs for Infant Formula**

Purpose

The purpose of this document is to establish and document the current GMP's for production & quality control of infant formula products manufactured or imported for sale in Canada

http://www.hc-sc.gc.ca/fn-an/legislation/codes/infant_formula_gmp-eng.php#0.3

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Health Canada: **GMPs for Infant Formula**
Scope

- The GMP's described in the document apply to the production of all domestic or imported Human Milk Substitutes (Infant Formulas) as described in Division 25 of the *Food & Drug Regulations*
- These GMPs also apply to new or changed infant formulas, and to third party facilities subcontracted to manufacture or package infant formula
- The GMPs encourage the application of HACCP and ISO 900 principles and programs in infant formula establishments

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Regulation of PIF and other human milk substitutes in Canada

- Division 25 of the *Food and Drug Regulations* requires that the manufacturer of a new infant formula or a formula which has undergone a major change, notify the Director of HPFB in writing, at least 90 days before sale or advertisement
- “Details” and “results” respecting manufacturing, quality control procedures, and determination of the expiration date, need to be provided
- Label should comply with the requirements set out under Section B.25.057 of the *Food and Drug Regulations*

Guidelines for pre-market notification of PIF and human milk substitutes

- **Criteria include:**
  - Nutritional, microbiological and chemical specifications for all ingredients
  - "Evidence" used to establish that formula is nutritionally adequate to promote acceptable growth and development in infants (clinical trials)
    Guidance document applicable: "*Clinical Testing of Infant Formulas with Respect to Nutritional Suitability for Term Infants*" (Committee on Nutrition of the American Academy of Pediatrics, 1988)
- PIF should be manufactured according to processes which are in line with Canadian GMPs for infant formulas

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Microbiological Criteria for PIF in Canada

Method	Guideline	Sampling Plan Parameters			
		n	c	m	M
MFHPB-18	ACC	5	2	10^3	10^4
MFHPB-19	*E. coli*	10	1	<1.8	10^1
MFHPB-20	Salmonella	20	0	0	0
MFHPB-21	*S. aureus*	10	1	10^1	10^2
MFLH-42	*B. cereus*	10	1	10^2	10^4
MFHPB-23	*C. perfringens*	10	1	10^2	10^3

Surveillance in Canada

- At present, there is no active or passive surveillance systems for *C. sakazakii*
- Number of reported cases of *C. sakazakii* in Canada is very small
- Three cases of illness due to *C. sakazakii* reported in 1991/1992
- One case of meningitis due to *C. sakazakii* reported in 2007; occurred in one of the twins

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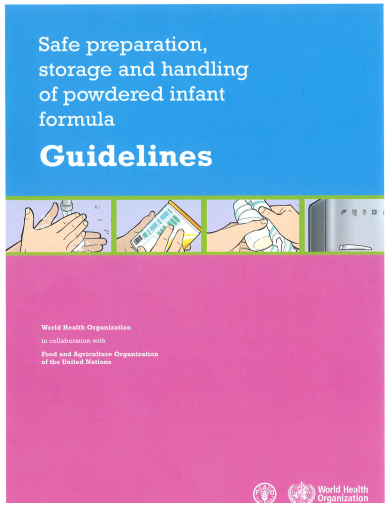
WHO/FAO Guidelines

Safe preparation, storage and handling of powdered infant formula

- Provide recommendations for the safe preparation, storage and handling of PIF in care settings and homes
- Covers general aspects of cleaning and sterilization of feeding and preparation equipment, and safe preparation of PIF
- Printed in four booklets targeting different groups (managers of organizations serving infants, home settings using bottles/cups, care settings)
- Sterile liquid infant formula is recommended for infants at highest risk of infection
- Preparation of PIF should be with water at a temperature no less than 70°C
- Minimizing the time from preparation to consumption and storage at temperatures no higher than 5°C for a maximum of 24h significantly reduce the risk



http://www.who.int/foodsafety/publications/micro/pif2007/en/index.html

Guidelines for the Safe Preparation, Storage and Handling of PIF

- The FAO/WHO guideline was developed to be a generic document that can provide guidance for countries and governments
- Health Canada has adapted and condensed the FAO/WHO guidelines to develop a guidance document on the preparation and handling of PIF in home and hospitals/care settings

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Guidelines for the Safe Preparation, Storage and Handling of PIF

- The PIF Guidance Document was initially reviewed by members of FPT Group on Nutrition, and health professionals in Ontario
- Consultation with health professionals across Canada in 2008
- The PIF Guidance Document was sent to over 10 companies involved in the manufacturing and sale of PIF in Canada
- Once finalized, the PIF Guidance Document will be published on Health Canada's website
- The Guidance Document can be used to educate parents, caregivers and staff in hospitals and day-care centres on the potential hazards associated with PIF products

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Some Industry Issues with the Guidelines

- Boiled water used for preparing PIF should be cooled down to a lower temperature than 70^0C (i.e., 37^0C) for several reasons:
  - Rapid degradation of heat-sensitive nutrients
  - Potential breakage of glass bottles
  - Water at 70^0C presents a safety hazard to the preparer and the baby
  - Households do not typically have thermometers

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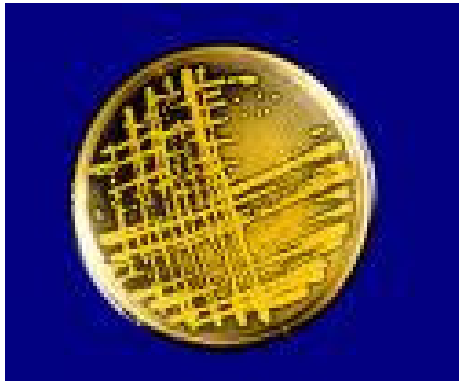
Research endeavours at Health Canada's BMH



Bureau of Microbial Hazards

BMH BDM

Bureau des dangers microbiens

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Research Themes

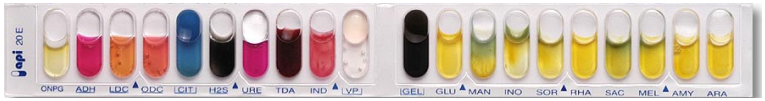
- **Genotypic characterization**
  - PFGE
  - Ribotyping
  - Bioinformatics (MLST, 16S rRNA)

- **Phenotypic assessment**
  - Isolation media (e.g., chromogenic agars)
  - Physiology (e.g., capsule production)

- **Pathogenicity**
  - In-vivo, using non-primate animal models
  - In-vitro, using blood-brain barrier cell lines
  - Production of enterotoxin(s)

Methods - phenotypic characterization

API 20E



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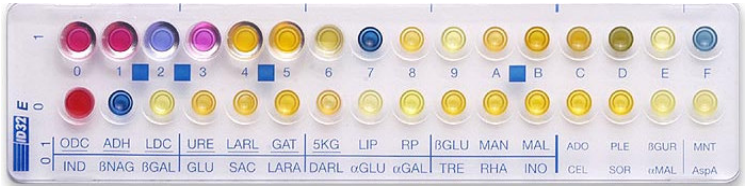
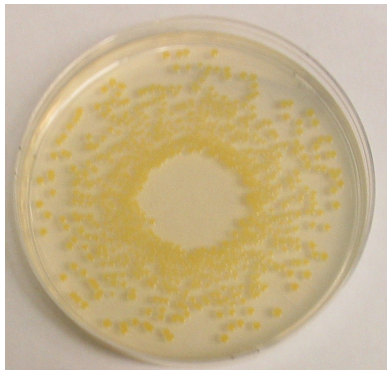
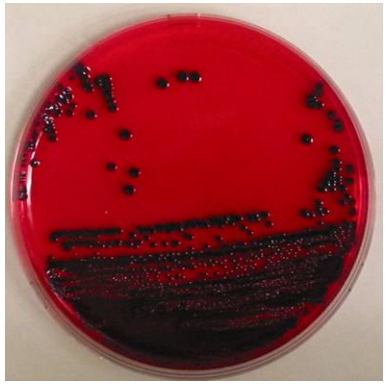
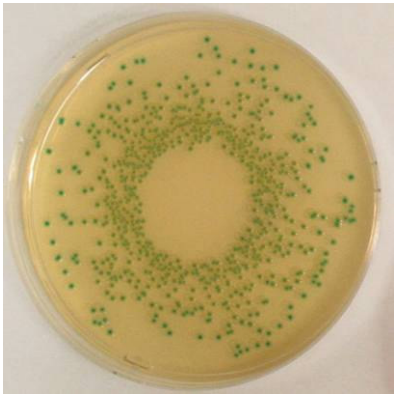




Yellow colonies on TSA

Blue-black colonies on ESPM





Blue-green colonies on DFI

Results – Phenotypic characterization

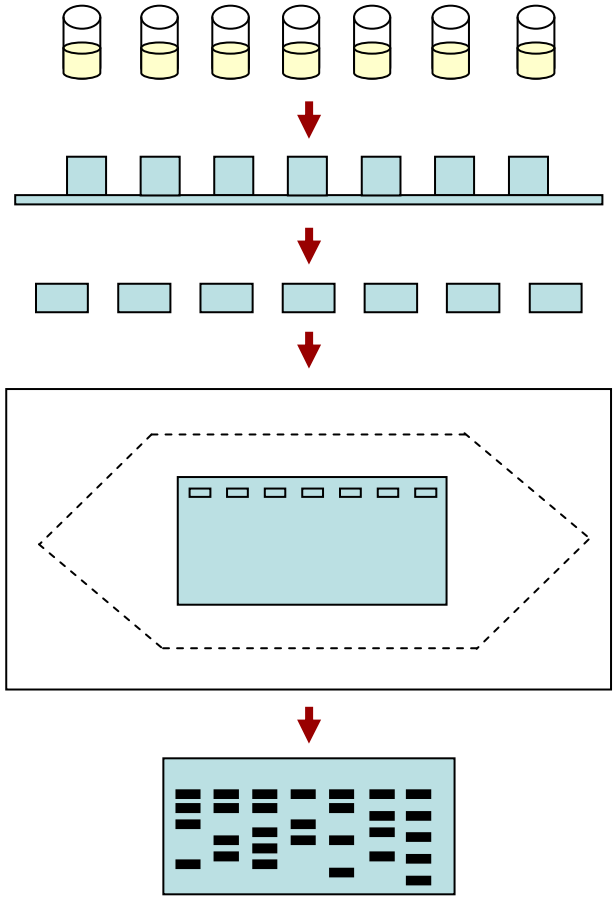
Using 247 strains from our database:

- ✓ 203 strains identified as *C. sakazakii*
- ✓ 30 strains identified as *C. malonaticus*
- ✓ 4 strains identified as *C. muytjensii*
- ✓ 1 strain identified as *C. turicensis*
- ✓ 1 strain identified as *C. dublinensis* subsp. *lactaridi*

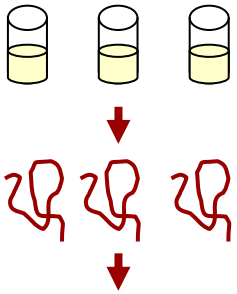
✓ Still working on 8 strains ☺

Methods - genotypic characterization

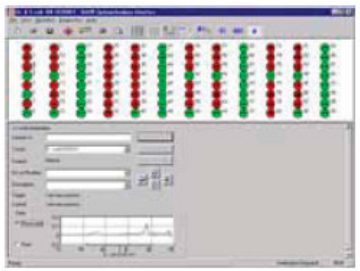
PFGE



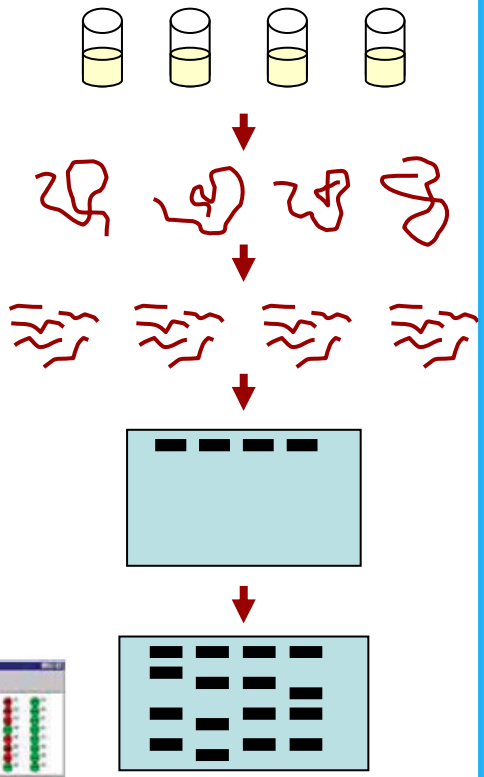
16S rDNA Sequencing



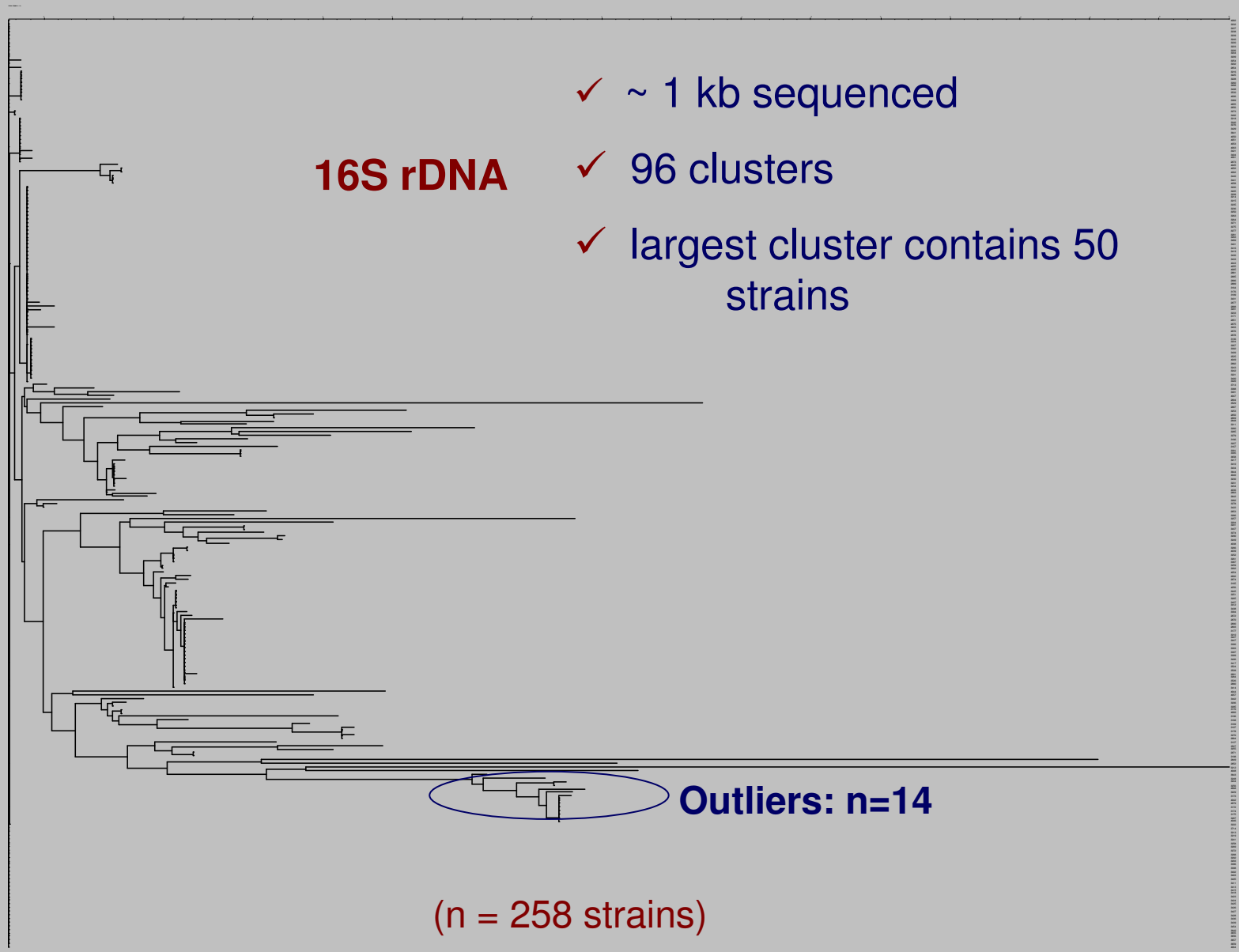
BAX™ PCR



Ribotyping



Genotypic characterization

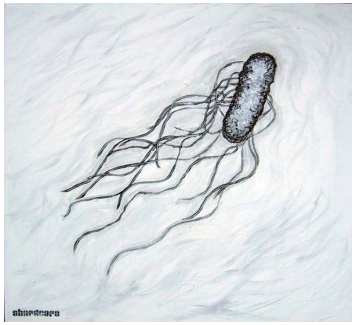


16S rDNA

- ✓ ~ 1 kb sequenced
- ✓ 96 clusters
- ✓ largest cluster contains 50 strains

Outliers: n=14

(n = 258 strains)



Other Research Activities

Non-Primate Animal Models to Assess C. *sakazakii* Virulence and Pathogenicity

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Young Animals

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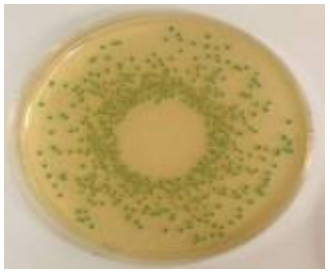
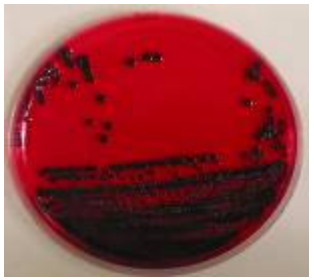
Bureau des dangers microbiens

Methods

- **Animal models (weight; age):**
  - Pigs (6.3 – 7.2 kg; 5 weeks)
  - Chicks (1 day)
  - Rabbits (2.7 – 3.0 kg; 2 months)
  - Guinea pigs (300 – 400 g; 3 – 4 months old)
  - Gerbils (40 – 50 g; 1 – 2 months)

- **Challenged with three core isolates at doses of 10^9 cells of C. *sakazakii* grown and suspended (1 ml) in PIF**





Young Animals – Results

- **No deaths or illness observed**
- ***C. sakazakii* was recovered from fecal samples of all animals tested (pigs not done)**
  - Up to day 14 for chicks, gerbils and guinea pigs
  - Up to day 7 for rabbits

Young Animals – Results

- **Chicks and young gerbils had organs positive for all three strains**

- **No other animals had positive organs**

Results (young gerbils)

C. sakazakii was recovered from gerbil organs

- Brains positive for all three strains, but no deaths
- Strain 3290 (CSF clinical): all organs except intestines positive on day 7 with high counts of *C. sakazakii*

Young gerbils: results

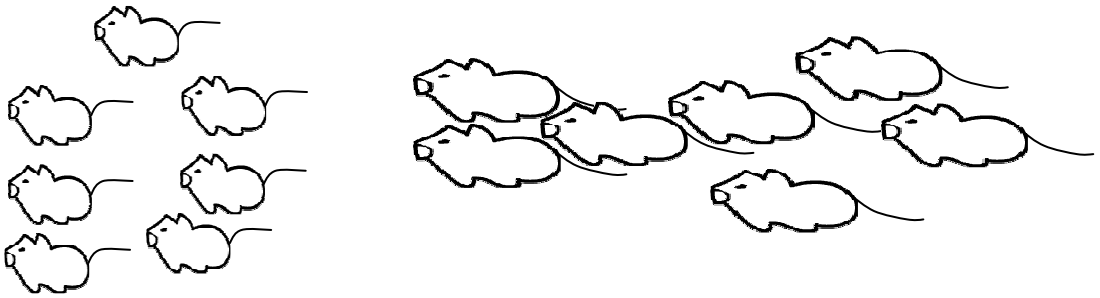
3290 – CSF Clinical		Brain	Heart	Spleen	Liver	Kidney	Intestine
Day 7 p.i.	Gerbil 01	++ >3000 cfu	++ 500 cfu	++ 330 cfu	++ 1600 cfu	++ 300 cfu	-
	Gerbil 02	++ >3000 cfu	++ 10 cfu	++ 90 cfu	++ 1100 cfu	++ 85 cfu	-
Day 14 p.i.	Gerbil 03	-	-	-	-	-	-
	Gerbil 04	-	-	-	-	-	-

(–) NG; (+) presence upon selective enrichment; (++) direct count

Young Animals – Conclusions

- **Strains differ in ability to infect**
- **Most animals clear infections by day 14**
- **Young gerbils most susceptible to *C. sakazakii***

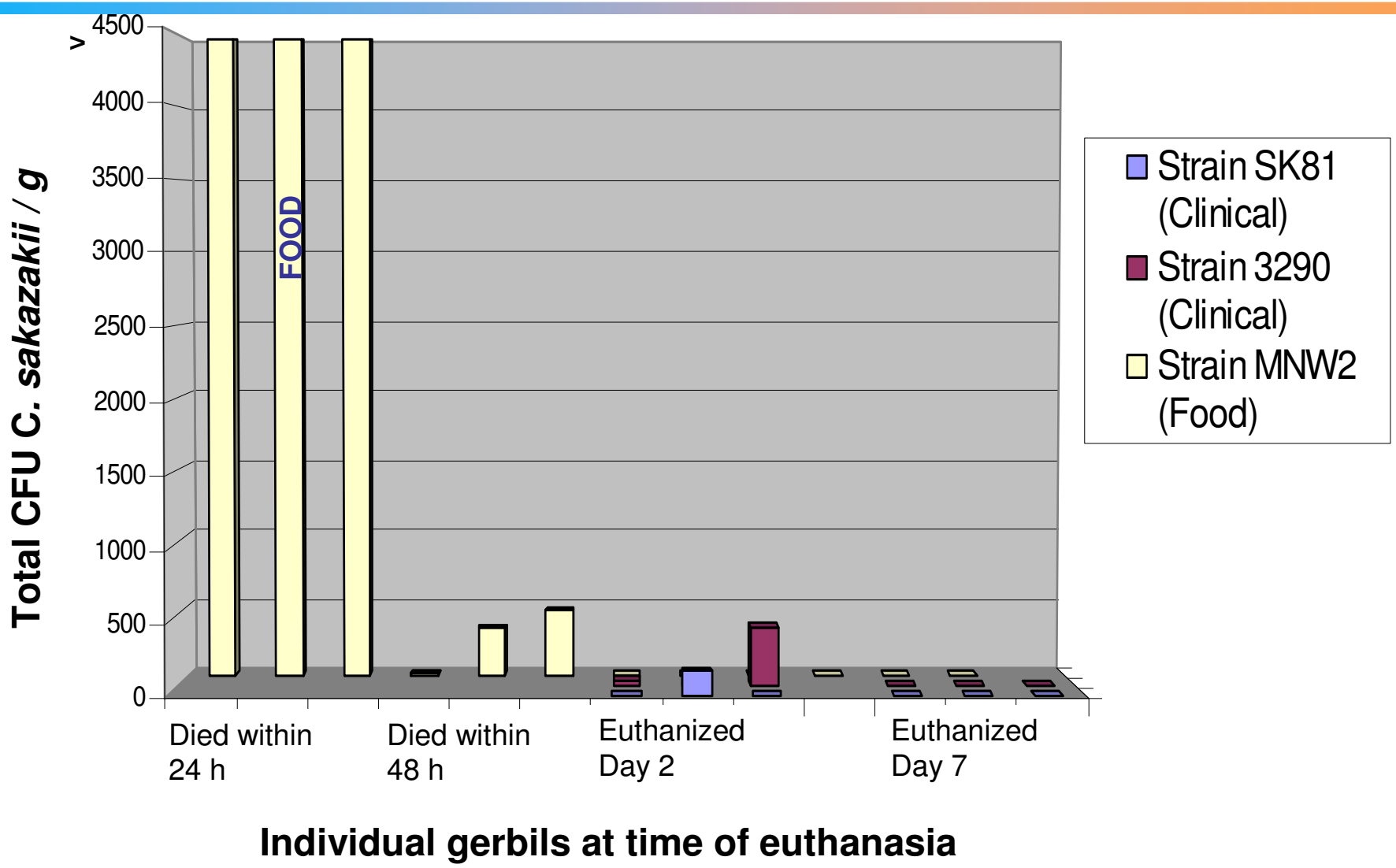
Neonatal Animals



Methods

- Neonatal gerbils
- Same C. *sakazakii* strains as for young animals
- Similar methodology used

Results for Brains – 3 Strains

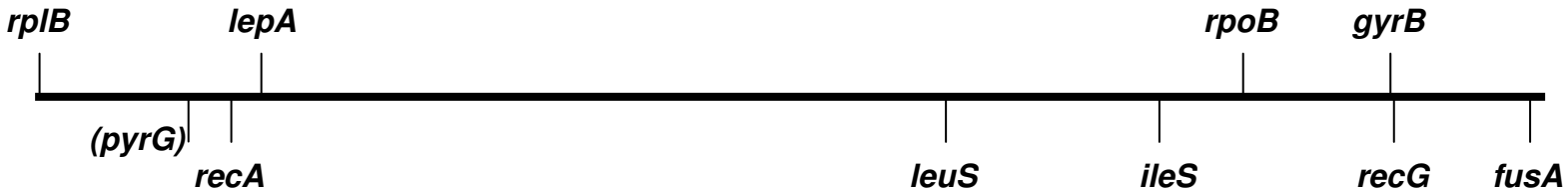


Summary – neonatal gerbils

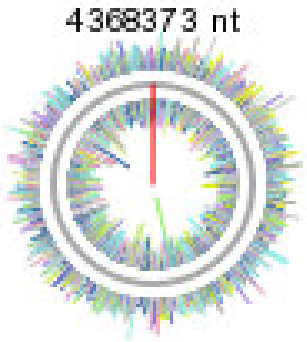
- **Food strain (MNW2): 6/12 died within 48 h of inoculation**
  - **3/9 oral and 3/3 i.p. inoculated**
- **No deaths with clinical strains**
- **Positive organs for all strains**
- **Intestines most highly infected organ for all strains**

Genotyping - MLST scheme

- Based on following 9 genes:
  - *fusA, gyrB, ileS, lepA, leuS, recA, recG, rplB, rpoB, (pyrG)*



Approximate positions based on *C. sakazakii* ATCC BAA-894



MLST Preliminary Data

Allele #

lepA - 11

fusA - 10

ileS - 12

gyrB - 13

rpoB - 10

rplB - 3

recA - 8

recG - 8

leuS - 10

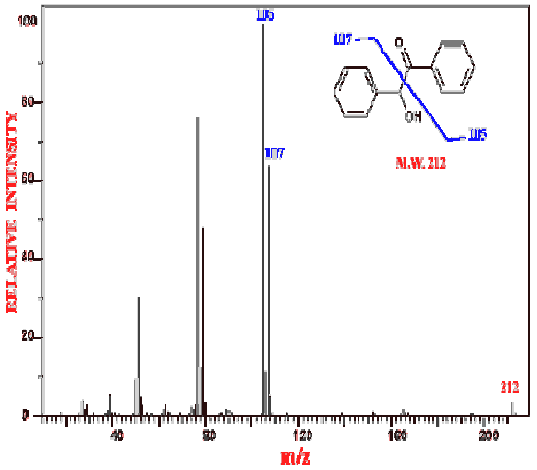
Strain	Ribogroup (Dupont)	Sequence type	16S rDNA
3420	<DUP-18790	6.2.2.10.5.2.7.5.2	A
3267	No match to database	10.8.11.1.7.3.3.2.8	A
3656	DUP-18755	4.1.6.9.8.1.3.4.1	B
3439	DUP-18620	7.5.8.2.2.1.4.6.5	B
3396	DUP-18799	5.3.5.8.8.1.3.4.1	C
3657	DUP-18755	5.1.4.12.8.1.1.4.1	C
3234	<DUP-14595	3.1.6.11.8.1.3.4.1	D
2871	DUP-18755	5.1.6.11.8.1.3.4.1	D
3434	<DUP-18790	5.1.6.11.8.1.5.4.1	D
3410	<DUP-18790	5.4.6.11.8.2.3.4.1	E
3403	Degradation	1.7.7.7.6.2.2.3.5	E
3428	DUP-18799	5.1.6.6.8.1.3.4.6	E
3436	No match to database	11.10.12.13.10.1.3.1.9	E

NOTE:

- *all have different PFGE profiles using XbaI*
- *all are C. sakazakii based on 16S rDNA*
- *strain 3267 (C. malonaticus) based on biochemical profiling*
- based on 20 entries of different source, origin and date of isolation used

Cell Wall Work

- LPS being targeted
- 2D 1H and 13C **NMR**
- **Mass spectroscopy**

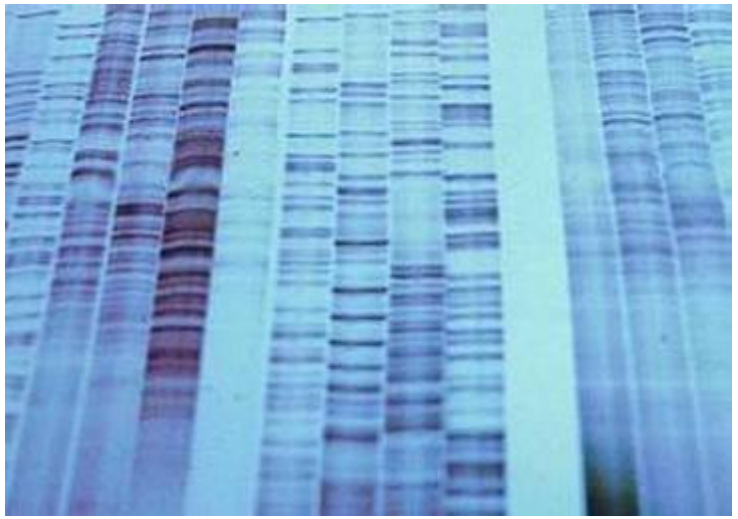


  - O-polysaccharide structure for *C. sakazakii** (Tennessee outbreak)
  - O-polysaccharide for *C. muytjensii**
  - Will be doing 3 other species

** Publications submitted*

Genome sequence project

- Collaboration with McGill Genome Centre
- Focus on 3 strains
  - *C. sakazakii*
  - *C. muytjensii*
  - *C. malonaticus*



Thank You


Similac
Organic
GOOD START 2
SUPREME
Pregestimil
Hypoallergenic Formula with MCT oil
Enfamil
LIPIL
Nutramigen
GOOD START
DHA & ARA