

Enterobacter sakazakii

General Microbiology

Contaminants and Natural Toxicants
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OVERVIEW

- **History**
- **Ecology**
- **Incidence on Canadian retail market**
- **Growth characteristics**
- **Phenotype characterization**
- **Genotyping**
- **Pathogenicity**

History

- Name *Enterobacter sakazakii* proposed in 1980
- DNA hybridization studies
- Biochemical reactions
- Yellow - pigmented colonies

Enterobacter sakazakii colony



Electron micrograph of
Entrobactor sakazakii



E. sakazakii and the environment

Surface water

Mud

Rotting wood

Bird dung

Rodents

Domestic animals

Cow's milk

Clinical Strains of *E. sakazakii*

- London 2
- Montreal 1
- Toronto 3
- LCDC 3

Dried-infant formula isolates

Company	Positive samples (%)
A	3/24 (12)
B	2/24 (8)
C	0/24 (0)
D	1/24 (4)
E	2/24 (8)
<hr/> total	8/120 (6.7)

Temperature Gradient Incubator

- 10 strains
- Temperature range 4-50°C
- 30 tubes in duplicate

Temperature Gradient Incubator

Strain Number	Minimum Growth Temp °C
1C [★]	7
2C	6
3C	6
4C	8
5C	5.5
6C	6.5
7F [★]	7.5
8F	5.5
9F	6
10F	7
11F	5.5



C = Clinical
F = Food

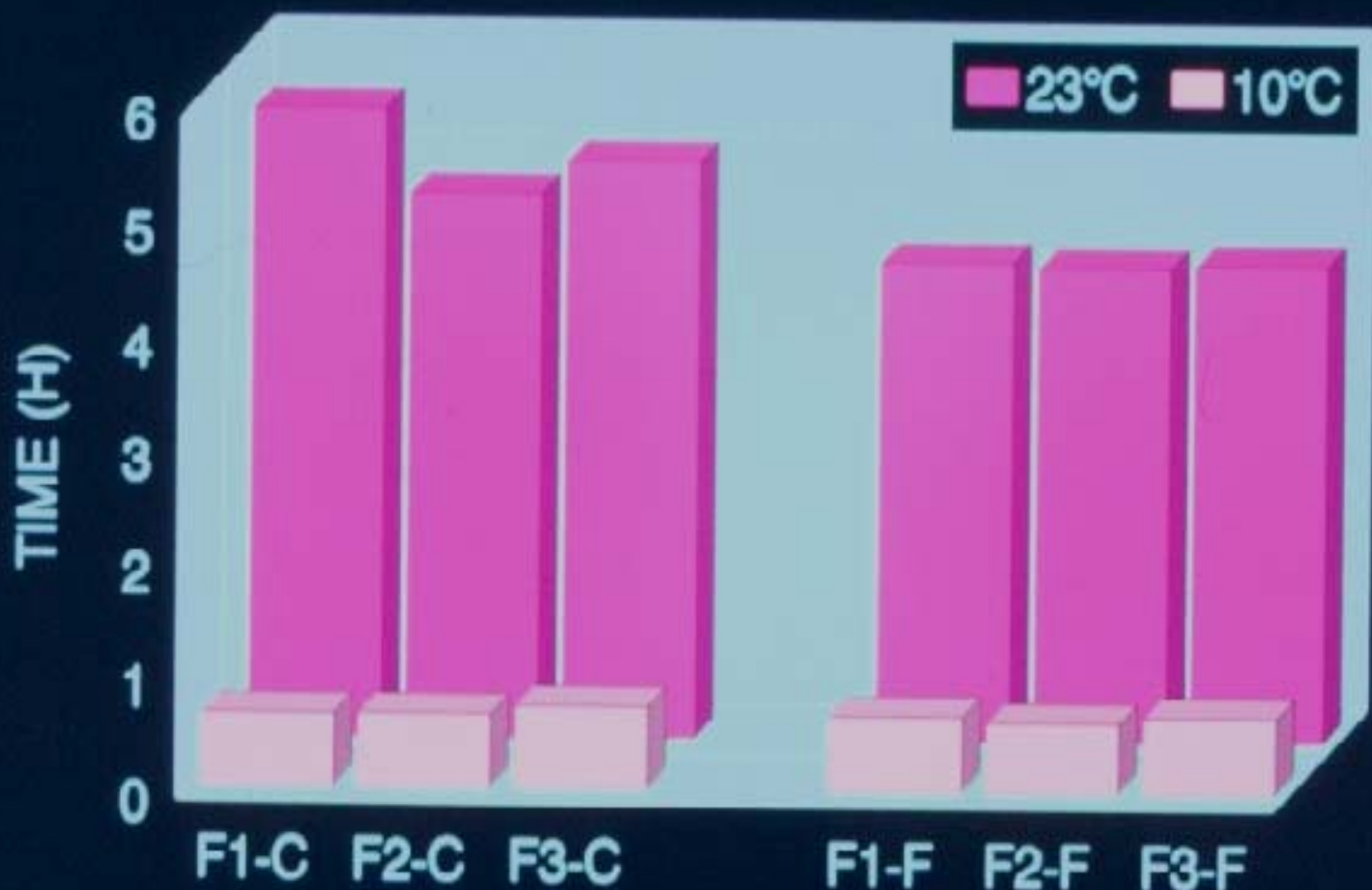
Experimental Protocol

Growth Study

- 3 Different formulae
- Cocktails
 - 5 clinical isolates
 - 5 food isolates
- 4, 10 and 23°C
- Direct plating
- Generation and lag time

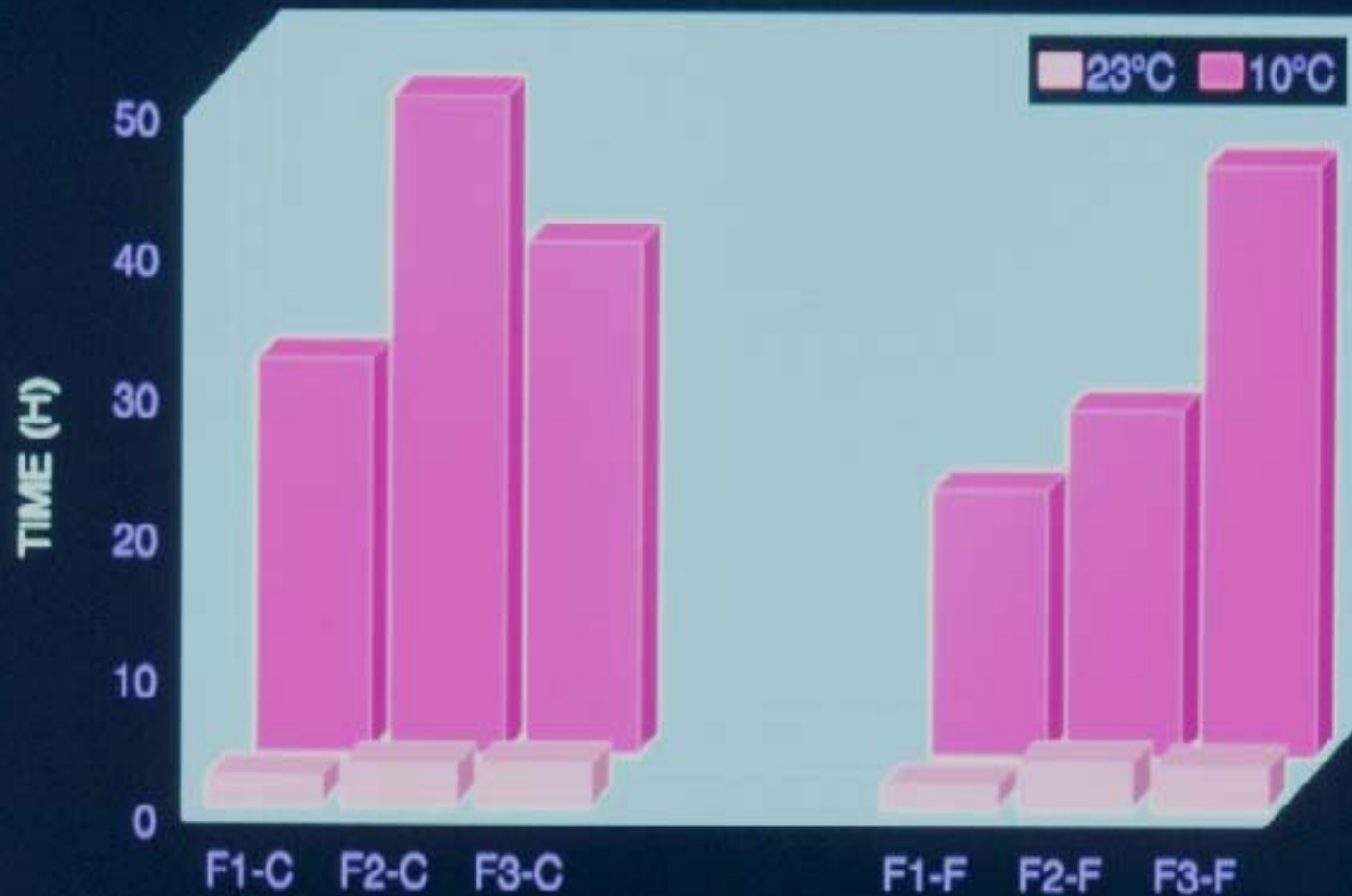
GROWTH STUDY - INFANT FORMULA

GENERATION TIME



GROWTH STUDY -INFANT FORMULA

LAG TIME



Phenotyping

- Biochemical 3 biotypes
- Antibidiograms 4 antibidiograms

Antibiotics used

- Ampicillin
- Cefotaxime
- Chloramphenicol
- Gentamicin
- Kanamycin
- Polymixin B
- Streptomycin
- Trimethoprim-Sulfamthoxazole
- Tetracycline
- Sulfisoxazole
- Cephalothin

Antibiotic Resistance Profile

Antibiotic	Number of strains (18)
• Ampicillin	1
• Tetracycline	2
• Chloramphenicol	3
• Cephalothin	17
• Sulfisoxazole	18

Genotyping

- Ribotype
- Pulsed Field Gel Electrophoresis (PFGE)
- Random Amplification of Polymorphic DNA (RAPD)

Genotypic profiles of *E. sakazakii*

Source	Strain	Ribotype	PFGE		RAPD	
			<i>Xba</i> 1	<i>Spe</i> 1	<i>Primer 1</i>	<i>Primer 2</i>
Clinical	Type strain	1	1	1	1	1
	LA	1	2	2	2	2
	LB	5	3	3	3	3
	MONT	6	4	4	4	4
	SK81	3	5	5	5	5
	SK90	3	6	6	6	6
	SK92	3	7	7	7	7
	LCDC1	7	8	8	8	8
	LCDC2	1	9	9	9	9
	LCDC3	1	10	10	10	10
Food	MNW1	8	11	11	11	11
	MNW2	4	12	12	12	12
	MNW3	2	13	13	13	13
	MNW4	2	14	13	13	14
	MNW5	2	15	14	14	15
	MNW6	10	16	15	15	16
	MNW7	9	17	16	16	17
	MNW8	2	18	17	17	18

Virulence Factors

- Suckling mouse assay
- Three cell culture lines
- Infectivity
 - intraperitoneal
 - oral challenge

Enterotoxin Screening

- 4 strains positive for enterotoxin production
- 3 clinical and 1 food

Morphological Responses of Cell Lines to Filtrates

- 1 clinical strain toxic to 3 cell lines
- more pronounced on Y-1 and Vero cells
- no effect of boiling on Y-1 and CHO cells
- cytopathic effect decreased on Vero cells
- 2 clinical strains, no effect on 3 cell lines

Infectivity studies

- i.p. injections lethal at 10^8 CFU per mouse
- within 3 days of dosing
- per oral route 2 strains caused death
- 1 clinical and 1 food

Summary

- Incidence in dried infant-formula on the Canadian market found to be 6.7%
- Minimum growth temperature 5.5-8.0°C
- Generation time at room temperature 40 min
- Four of eighteen strains produce enterotoxin
- Infectious dose
- Heterogeneity among strains

**What
do we
do now??**

