

# ***Cytotoxic Activity of Food Isolates of Cronobacter sakazakii and Cronobacter muytjensii from Indonesia***

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# Outline

- Introduction
- Methodology
- Result and Discussion
- Conclusion



# Introduction



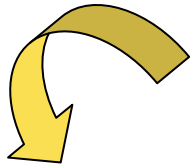
About *C. sakazakii* and *C. muytjensii*:

- Species in Genus *Cronobacter* spp.  
(*Enterobacter sakazakii*)
- Problem in several countries
- As contaminant in infant formulae, weaning food and dried food
- Caused meningitis and necrosis for “unhealthy infant”

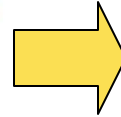


# Introduction

Our previous research:  
collected 33 isolates from foods

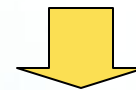


## Pathogenicity



Using one of indicator :  
Toxin production and  
its cytotoxic activity

- MTT Assay method using animal culture cell line :
- measured the absorbance of the living cell



Vero cells as a model cell line

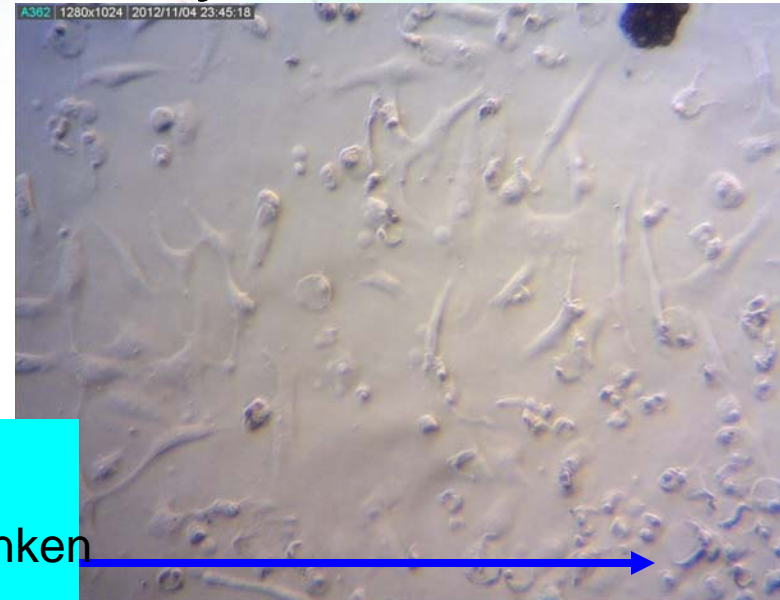
# Introduction

## Vero cell

- Cell line from monkey kidney



Polygonal  
shape



Rounded  
Shape and shrunken

Normal morphology  
(living cell)

alteration of  
morphology caused by toxin  
(died cell)

- Cytotoxic activity : calculated by comparing number of living cells and total of cell control (%)

## Objectives

*The objective of this study were:*

- *to assess the cytotoxic activity of twenty food isolates of Cronobacter spp. obtained from Indonesia*
- *to learn the cytotoxic effect to morphology of Vero cell*



# Methodology

## 1. Culture and Vero Cell preparation

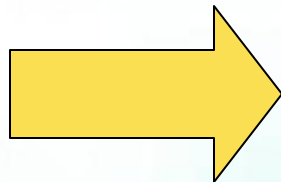
Sources	Name of Isolates	Number of Isolates	References
Starch/ flour	desc13, desc7, FWHb6, FWHc3	4	Dewanti-Hariyadi <i>et al.</i> 2010
Powdered Infant Formulae	desb10, YRt2a, YRw3	3	Meutia <i>et al.</i> 2008 Hamdani 2012
Weaning Food	YRc3a, YRsnkn, E1, E2, E4, E6, E7, E9, E11	8	Estuningsih <i>et al.</i> 2006
Spices	FWHd2u, FWHd11, FWHd16	4	
Sugar	FWHb15	1	
	<b>Total</b>	<b>20</b>	

**2. Crude toxin (supernatant free cell ) extraction, was sterilized by filtration**

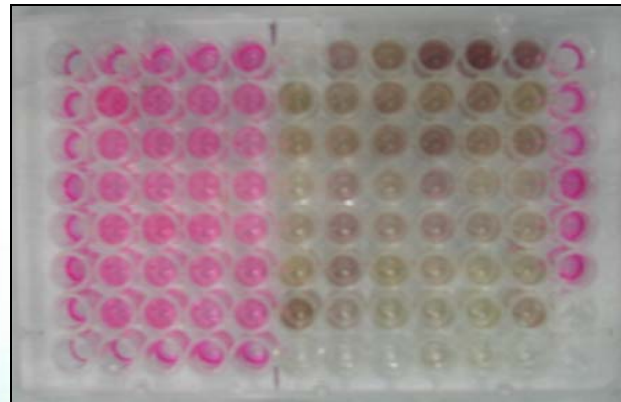
# Methodology

## 3. Cytotoxic evaluation by MTT Assay

- Carried out in tissue culture plate 96 wells
- Measured the Abs by ELISA reader



monolayer  
Vero cell

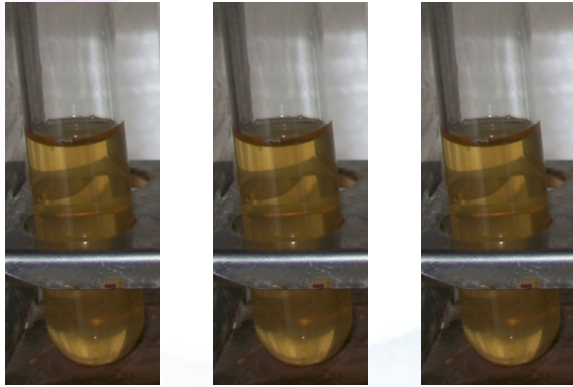


$$\% \text{ Cytotoxicity} = 1 - \frac{A_{595} \text{ treated cell}}{A_{595} \text{ cell control}} \times 100\%$$



# Methodology

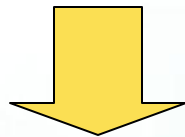
## 4. Cytotoxic effect vs age of cultures



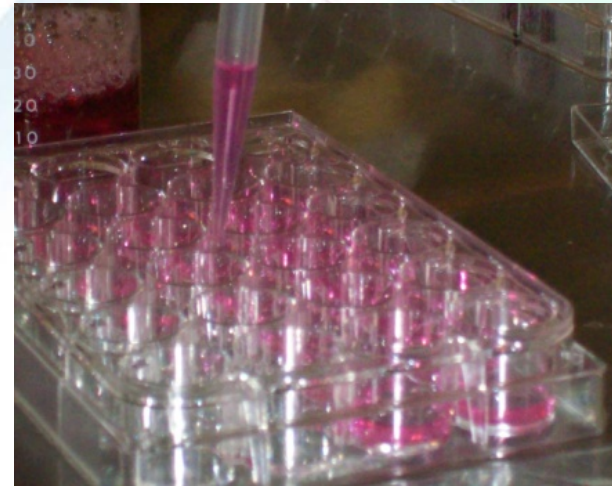
24 h

40 h

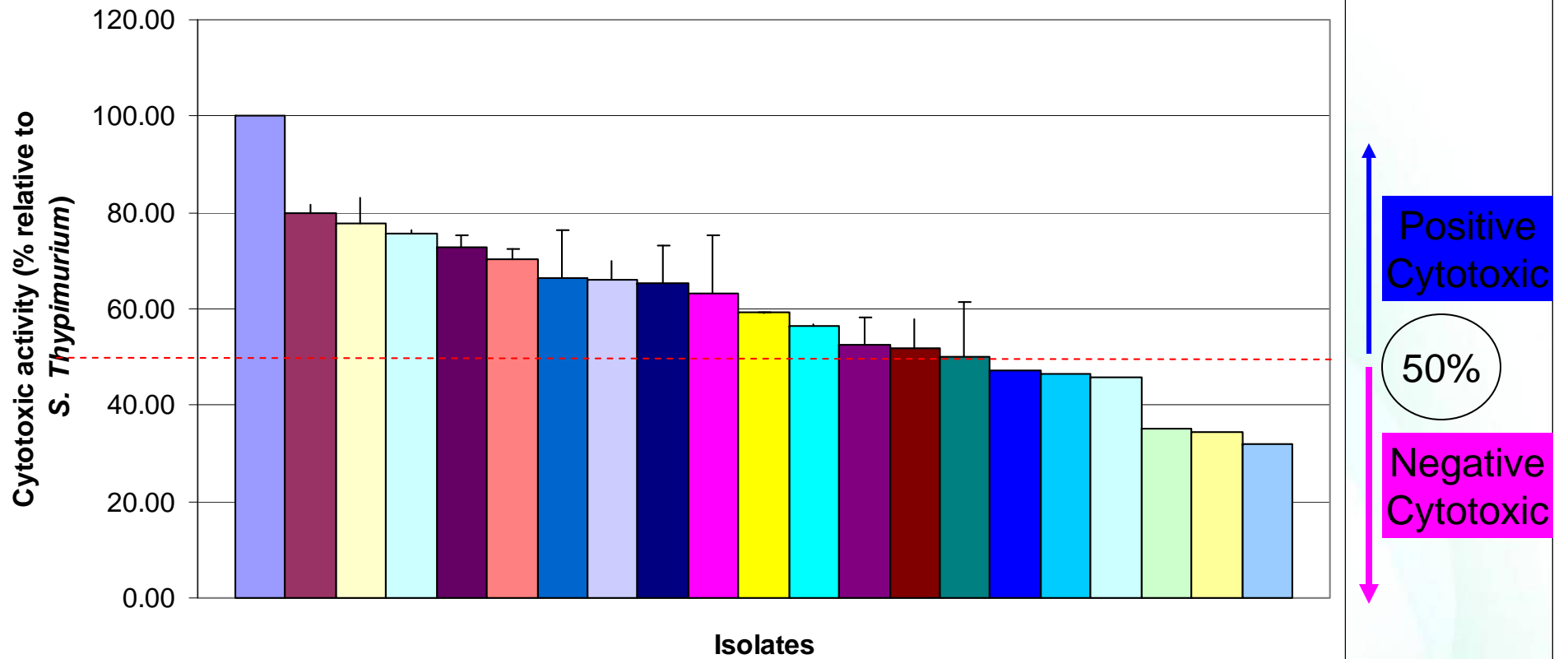
48 h



Staining the Vero cell by  
Haematoxylin-Eosin



# Result and Discussion



- Salmonella Thypimurium
- FWH b6
- E2
- FWH b15
- FWH c3
- FWH d16
- YRc3a
- E1
- FWH d11
- Des c7
- YRw3
- Des b10
- E4
- E11
- E6
- FWH d2u
- YRk2a
- E sakazakii ATCC
- E7
- E9
- YRt2a

## Result and Discussion

### Positive Isolates

1	<b>FWHb6</b>	<b>: 80%</b>
2	<b>E2</b>	<b>: 78%</b>
3	<b>FWHb15</b>	<b>: 76%</b>
4	<b>FWHc3</b>	<b>: 73%</b>
5	<b>FWHd16</b>	<b>: 70%</b>
6	<b>FWHd1</b>	
7	<b>YRc3a</b>	
8	<b>E1</b>	
9	<b>FWHd11</b>	
10	<b>Desc7</b>	
11	<b>YRw3</b>	
12	<b>Desb10</b>	
13	<b>E4</b>	

65% of 20 isolates have cytotoxic activity

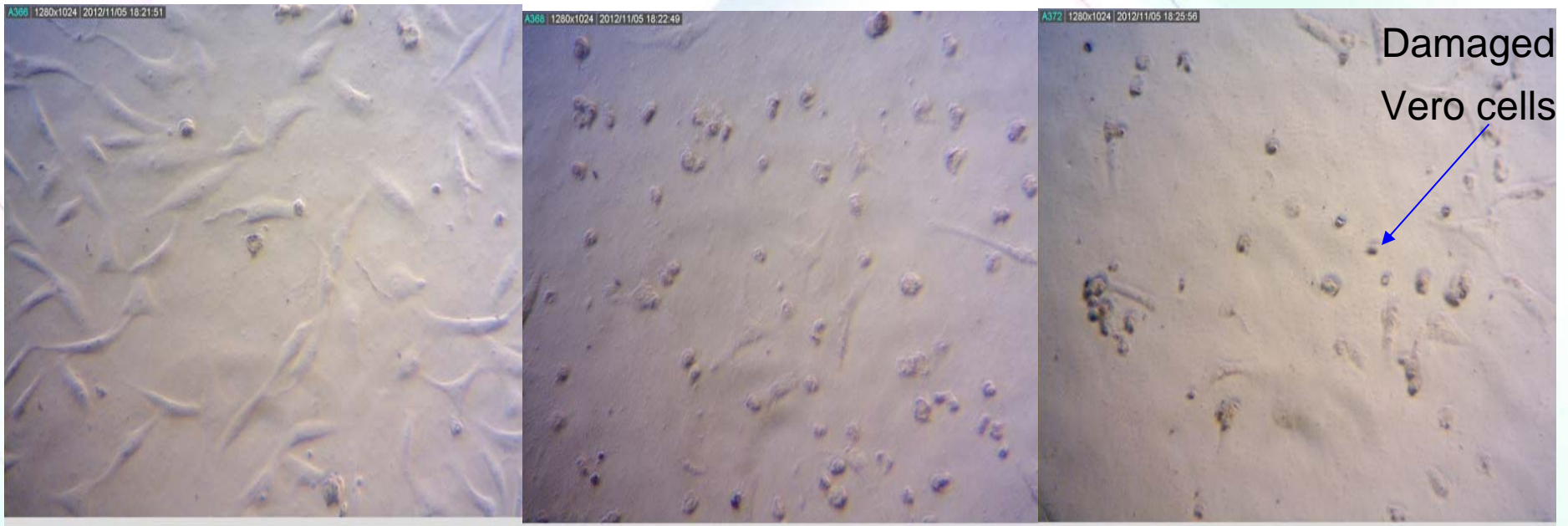
### Negative Isolates

14	<b>E11</b>
15	<b>E6</b>
16	<b>FWHd2u</b>
17	<b>YRk2a</b>
18	<b>E7</b>
19	<b>E9</b>
20	<b>YRt2a</b>



# Result and Discussion

## Effect of toxin of *C. Sakazakii* FWHd16



age of culture :

24 h

40 h

48 h

Increasing cytotoxic effect by increasing age of bacterial culture

# Result and Discussion

Effect of toxin of *C. Sakazakii* FWHc3

Damaged  
Vero cells



age of  
culture:

24 h

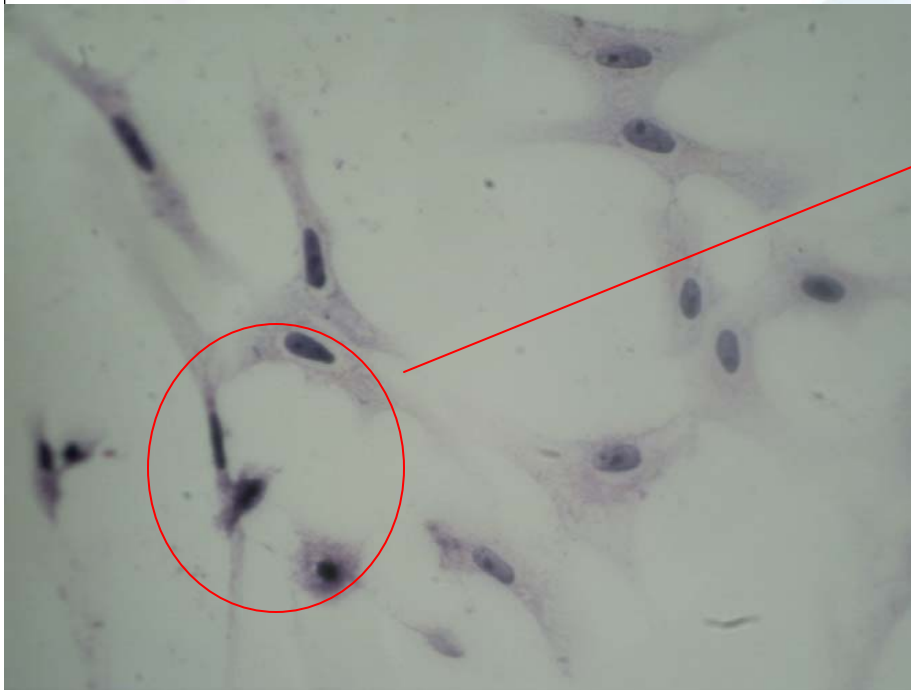
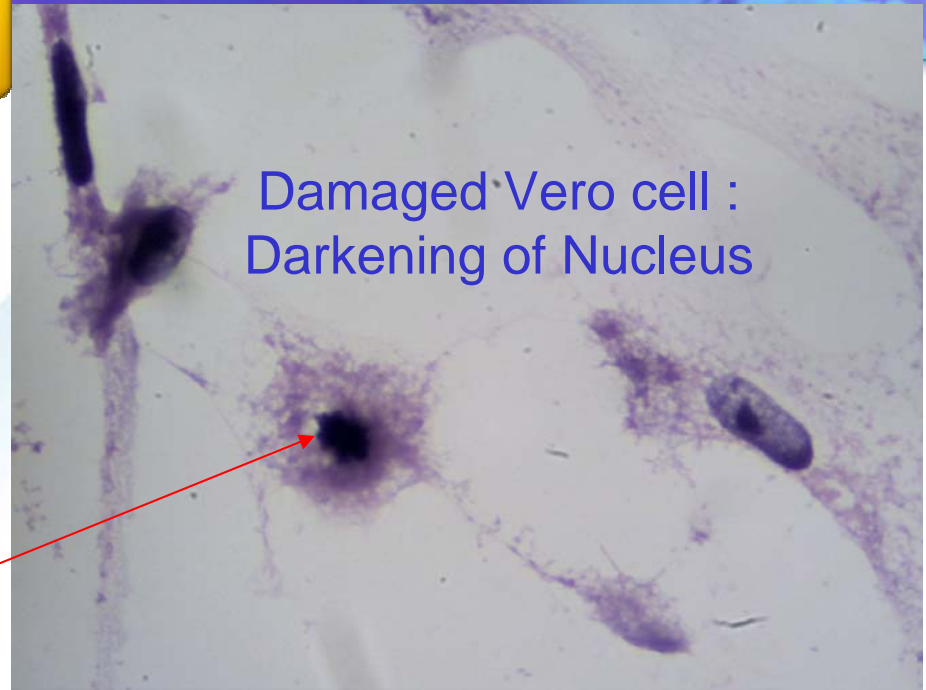
40 h

48 h

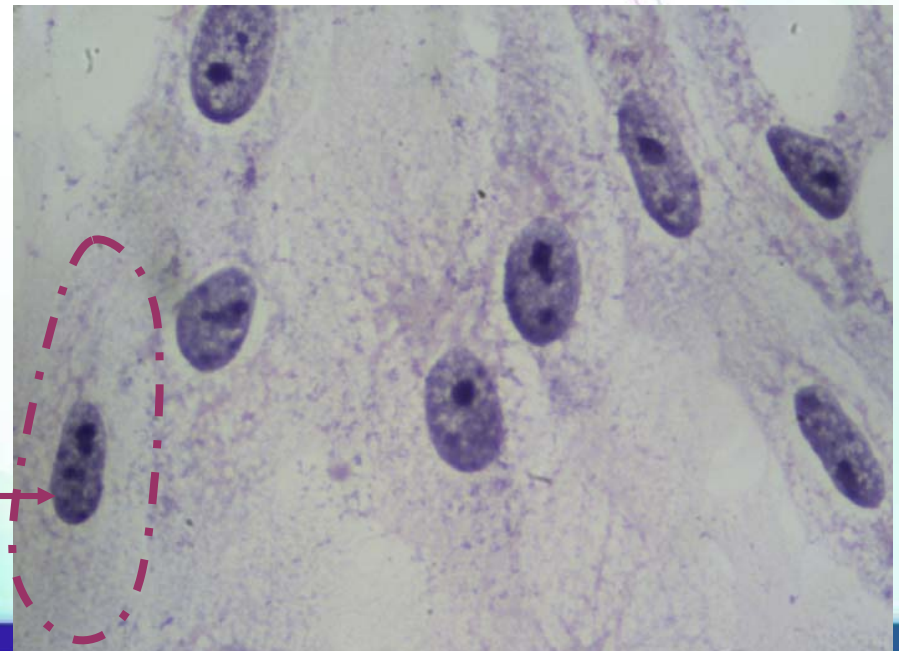
Increasing cytotoxic effect by increasing  
age of bacterial culture

# Result and Discussion

## EOSIN staining Result



Normal Vero cell :  
Complete of cytoplasm  
and clear nucleus





## Conclusions

- *13 out of 20 isolates positive for cytotoxic activity with varying between 50-80%*
- *The cytotoxic effect increase by increasing age of bacterial culture.*
- *Eosin staining of injured Vero cells showed the loss of cytoplasm and condensation of its nuclei*

# Funding research

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*THANK YOU*