Proceeding

International Seminar

CURRENT ISSUES AND CHALLENGES IN FOOD SAFETY:

science - based approach for food safety management

editor:

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SEAFAST CENTER

Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center
Bogor Agricultural University

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Bogor, December 2-3, 2009

Organized by:

Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center
Bogor Agricultural University

International Commision on Microbiological Specifications for Foods (ICMSF)

Norman Borlaug Institute for International Agriculture
Texas A & M University

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Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center,
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2010

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Publisher
Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center
Bogor Agricultural University
Bogor-Indonesia

National Library Republic of Indonesia
ISBN 978-602-96665-3-3

Acknowledgement
Thanks to Leo Wibisono Arifin, Yesica Dwi Ariesta, Amelinda Angela, Vita Ayu Puspita, Palestina Santana, Virna Berliani Putri, Zulaikhah and Nurwandi NC for preparing manuscript of this proceeding

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ISOLATION OF *ENTEROBACTER SAKAZAKII (CRONOBACTER SPP)* FROM POWDERED INFANT FORMULA AND OTHER DRY FOODS OBTAINED FROM BOGOR AREA, INDONESIA

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ABSTRACT

Recently there has been increasing concern related to the presence of *Enterobacter sakazakii* in powdered infant formula (PIF) which was linked to severe systemic infection in certain groups of neonates. *E.sakazakii* has been previously isolated from PIF in Indonesia; however more information regarding *E.sakazakii* is needed to assess the risk of this pathogen for infants in Indonesia.

The research was aimed to isolate *E.sakazakii* fromPIF, weaning foods and other dry foods obtained in Bogor, Indonesia. PIF and weaning food samples were obtained in 2006 and sampling were repeated in 2009. Several non PIF samples were collected in 2009. Isolation and detection were carried out according to FDA methods (2002) as modified by Iversen & Forsythe (2004).

Four *E. sakazakii* were isolated from 2 out of 16 PIF samples in 2006 but none was isolated from 3 PIF samples collected in 2009. Four isolates were obtained from 2 out of 9 weaning foods samples obtained in 2006 while two were isolated from two out of 15 samples taken in 2009. Two *E. sakazakii* were also isolated from two corn starch samples and one was isolated from chocolate powder sample. The result implied significant improvement of *E. sakazakii* management by PIF manufacturer in the past 2 years, while improved processing and or process control is probably needed for dry food production to minimize contamination by *E. sakazakii*.

BACKGROUND

Enterobacter sakazakii has been classified as severe pathogen to a restricted population. The bacterium has been reported to cause several cases of fatality and diseases in prematures baby, immuno-compromised newborn infants, and newborn infant up to few weeks of age. Meningitis and necrotizing enterocolitis (NEC) diseases caused by *E.sakazakii* in newborn infant has been linked to consumption of powdered infant formula (PIF) (van Acker *et al.* 2001).

Recent study on *E.sakazakii* in Indonesia has been done by Estuningsih *et al.* (2006) who reported the occurence of *E.sakazakii* from weaning food in Indonesia from 2 different manufacturers. The report has stirred fears in Indonesia, thus more research is should be condusted to understand the presence of this bacterium in PIF and other foods. This information is important to assess the risk of this pathogen and establish measures to control *E. sakazakii*. Therefore, data regarding the presence of *E.sakazakii* in PIF and weaning food in Indonesia is needed.

The aim of this research was to evaluate the presence of *E. sakazakii* in PIF, weaning food and other dry food obtained from Indonesia based on their biohemical properties

MATERIAL AND METHODS

Bacterial Isolation

A total 19 PIF samples and 9 weaning food were purchased from retail market in Bogor, Indonesia in 2006. Meanwhile, three PIF samples and 15 weaning food samples were obtained in 2009. Other food samples evaluated in 2009 include 5 cassava starch, 8 corn starch, 4 mung bean starch, 7 chocolate powder, 3 milk powder, 2 agar-agar powder and 4 instant cereal powder samples

All samples were obtained from products purchased in closed, intact packages, as offered in the shop. The media used for isolation of *E.sakazakii* were : *Buffered- Peptone Water* (BPW); *Enterobacter erichment* (EE) *broth*; *Violet Red Bile Glucose* (VRBG) Agar; *Druggan-Forsythe-Iversen* (DFI) Agar; *Trypticase (Tryptic) Soy Agar* (TSA); and also API 20E *Biochemical Strips.*

Isolation of *E.sakazakii* was carried out according to FDA recomemended methods (2002) modified by Iversen & Forsythe (2004). Each food samples were weighed aseptically (25 g) and diluted in nine parts of sterile BPW (1:10) then prewarmed to 45°C and gently shaken until the smples were uniformly suspended. The suspension were then incubated at 37°C for 24 hours. Ten mililiters of each suspensions were taken, placed in 90 ml EE *broth* and then incubated at 37°C for 24 h. After incubation, 1 loopful of the suspension were streaked onto VRBG agar and incubated at 37°C for another 24 h. The streaking was repeated on two VRBG agar plates. Presumptive colonies of *E.sakazakii* on VRBG agar were picked and re-streaked onto DFI agar plates and then incubated at 37°C for 24 h. Typical *E.sakazakii* colonies found on DFI agar were then streaked onto TSA agar plates and incubated at 37°C for 48 – 72 h. Each typical colony was confirmed by API 20E *biochemical identification system* and the results were analyzed using software api*web*™(Biomerieux).

RESULTS AND DISCUSSIONS

Bacterial Isolation from PIF and Weaning Foods

Two *E.sakazakii* isolates were obtained from two out of 16 PIF samples obtained in 2006, but none was isolated from three samples obtained in 2009. The results suggests that there was increased process or process control in the manufacturing of PIF which may have been caused by 2007 public outcry on the publication of *E. sakazakii* finding in the PIF.

Of the 9 weaning food samples obtained in 2006, two typical *E. sakazakii* were isolated. Two isolates were also isolated from 15 weaning food samples collected in 2009. The results also suggests decrease in the isolation frequency of *E. sakazakii* in weaning food product from 22% in 2008 and 14.3% in this research. This isolation frequency was also significantly lower than that of Estuningsih et al. (2006) who suggest an isolation rate of 34%.

Bacterial Isolation from Food Other than PIF or Weaning Foods

Fewer isolates were obtained from samples other than weaning food. Three isolates were obtained from 3 samples out of the 36 samples taken (8%). *E. sakazakii* was not found in cassava starch, mung bean starch, milk powder, agar-agar powder, instant cereal powder, non dairy creamer, instant coconut milk powder, instant spicy drink powder samples.

Two isolates were obtained from two out of 8 corn starch samples while one isolate was obtained from one out of 7 cholate powder samples. The results support Eddelson-Mammel and Buchanan (2004) who reported that *E. sakazakii* was able to survive for over 2 years in dry products. Table 1 shows the complete data.

Table 1. Isolation of *E. sakazakii* from dry foods obtained from Bogor area, Indonesia

Samples (year taken)	No of samples	No of positive sample	%
PIF (2006)	16	2	12.5
PIF (2009)	3	0	0
Weaning food (2006)	9	2	22
Weaning food (2009)	15	2	13
Cassava starch (2009)	5	0	0
Corn starch	8	2	25
Mungbean starch	4	0	0
Agar-agar powder	2	0	0
Milk Powder	3	0	0
Chocolate powder	7	1	14.3
Instant cereal powder	4	0	0
Non dairy creamer	1	0	0
Instant coconut milk powder	1	0	0
Instant spicy drink	1	0	0
Total	79	9	11.4

All isolates showed typical colonies on selective media (VRB agar) as well as chromogenic media (DFI agar). However, although showing typical *E. sakazakii* colonies in DFI, four isolates shows similarity with other *Enterobacteriaceae* when tested with API 20E. One PIF isolate showed 90.6% similarity with *Enterobacter amnigenus* while only had 2.6% similarity with *E.sakazakii.* Another isolate has 90.0% similarity with *E.amnigenus,* the third showed 18.5% similarity with *E.sakazakii* and has more similarity with *E.cloacae* (81,4%) while the fourth isolate is more related to Pantoea spp 3 (80,3%).

Acknowledgement

Thanks to Dr Sri Estuningsih for the confirmed cultures used as standards. The research was partially funded by SEAFAST Center, Bogor Agriculture University (2006) and Competitive Grant for Graduate Program of the Directorate General of Higher Education, Ministry of Education, Indonesia (2009)..

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