13TH ASEAN FOOD CONFERENCE 2013
MEETING FUTURE FOOD DEMANDS: SECURITY AND SUSTAINABILITY
9 - 11 SEPTEMBER 2013
MAX ATRIA, SINGAPORE EXPO

Molecular Confirmation and Detection of Genes Encoding for Enterotoxin Production in *Staphylococcus aureus* Food Isolates

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Food Microbiology (I)
Session Co-Chairs: Prof Luu Dzuan & Dr Mathew Lau

FMB-O 1.1: 14:00 – 14:15
Enumeration and Identification of Dominant Lactic Acid Bacteria in Indonesian "Tempoyak" During Low Temperature Fermentation
Mrs Tri Wardani Widowati, Sriwijaya University, Indonesia

FMB-O 1.2: 14:15 – 14:30
Cytotoxic Activity of Food Isolates of Cronobacter Sakazakii and Cronobacter Muytjensii from Indonesia
Mrs Siti Nurjanah, Bogor Agricultural University, Indonesia

FMB-O 1.3: 14:30 – 14:45
Fast Screening of Antibacterial Compounds from Kesum Leaves by Ultrasound Assisted Extraction and Bioautographic Method
Dr Harsi Dewantari Kusumaningrum, Bogor Agricultural University, Indonesia

FMB-O 1.4: 14:45 – 15:00
It's Now Time to Look at the Good Guys: Whole Genome Sequencing of Food Fermentation and Probiotic Bacteria
Dr Mark Turner, University of Queensland, Australia

FMB-O 1.5: 15:00 – 15:15
Molecular Confirmation and Detection of Genes Encoding for Enterotoxin Production in Stapylococcus Aureus Food Isolates
Dr Ratih Dewanti-Hariyadi, Bogor Agricultural University, Indonesia

Introduction

Staphylococcus aureus

- a non sporeformer, Gram positive bacterium; cocci, single or in group , grape-like in shape
- diameter 0.5-1.5 μm, aerobic or facultatively anaerobic, non motile,
- belongs to Staphylococcaceae family, coagulase, catalase positive and capable of mannitol fermentation
- commonly found in human skin, nose, throat
- have been isolated from various foods and clinical isolates
- local isolates were obtained from steamed coconut rice and shredded chicken (Dewanti-Hariyadi et al. 2011) ; biochemically confirmed but not known to form toxins

Introduction

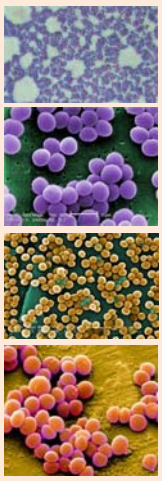
- *S. aureus* causes foodborne intoxication due to *S. aureus* enterotoxins (SE): SEA, SEB, SEC (SEC1,SEC2,SEC3), SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO
- SEs are heat stable, 26,900-29,600 Dalton, globular proteins rich in lysine, aspartate, glutamic acid and tyrosine
- Most frequently associated with foodborne intoxication : SEA. SEC, SED
- Prior report from Indonesia : of 20the SE produced from milk isolates, the most frequently isolated toxin is SEC (30%) (Salasi et al., 2009)

Introduction

Staphylococcus aureus Food Poisoning

Year	Location	Food	No. of cases	Reference
1989	Starkville, MS, US	Can mushroom	22	CDC, 1989
1990	Hospital, Puerto Rico	Ham	NA	Bergdoll, 1992
1996	Germany	Schwarzwalden Schinken		
1997	FL, US	Ham	31	
2000	Japan	Pasteurized milk	14,555	
2002	Australia	Rice,potato,beef	250	
2007	Padang, Indonesia	Sticky rice dish	36	Gentina et al., 2008

Objectives



- To identify the relatedness of *Staphylococcus aureus* previously isolated from coconut rice and shredded chicken using Polymerase Chain Reaction (PCR)
- To detect the presence of enterotoxin genes for SEA and SEC in the local isolates using PCR

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Methods

Microorganisms

- 14 local isolates *S. aureus* from shredded chicken (AS) and coconut rice (NU1, NU2, NU3, NU4, NU5, NU6, NU7, NU8, NU9, NU10, NU11, NU13, NU14 and a non-enterotoxigenic *S. aureus* ATCC 25923

Primers

63f/1387r encoding for universal 16S rRNA (Marchesi et al. 1998)
CAG GCC TAA CAC ATG CAA GTC
GGG CGG WGT GTA CAA GGC
SEA-1/SEA-2 encoding for SEA (Johnson et al. 1991)
TTG GAA ACG GTT AAA ACG AA
GAA CCT TCC CAT CAA AAA CA
SEC1-1/SEC1-2 encoding for SEC1 (Johnson et al. 1991)
GAC ATA AAA GCT AGG AAT TT
AAA TCG GAT TAA CAT TAT CC

Methods

- Isolation

Bacterial DNA was extracted with phenol chloroform isoamylalcohol, lysozyme and proteinase K (Doyle and Doyle 1990 with modifications). DNA was measured for its quality by spectrophotometry and verified on agarose gel after EtBr staining

- Amplification :

amplification of gene encoding for universal 16S rRNA was done using a primer pair 63f and1387r (Marchesi *et al,* 1998). The PCR protocol :

pre-PCR (95^{o}C, 3 min), denaturation (94^{o}C, 30 sec), annealing (55^{o}C, 30 sec), elongation (72^{o}C, 1 min) and post-PCR (72^{o}C, 5 min; 30. Visualization of the amplicon was observed by agarose (1.5%; w/v) gel electrophresis at 50 V, 45 min

- Sequencing :

DNA fragment obtained from PCR was sequenced with ABI PRISM™ 3100-Avant 4-*Capillary System* Genetic Analyzer (Applied Biosystem), by PT. Macrogen Inc., Seoul, Korea Selatan. The resulted sequence was processed with BioEdit and analyzed using BLAST from NCBI at www.ncbi.nlm.nih.gov.

Methods



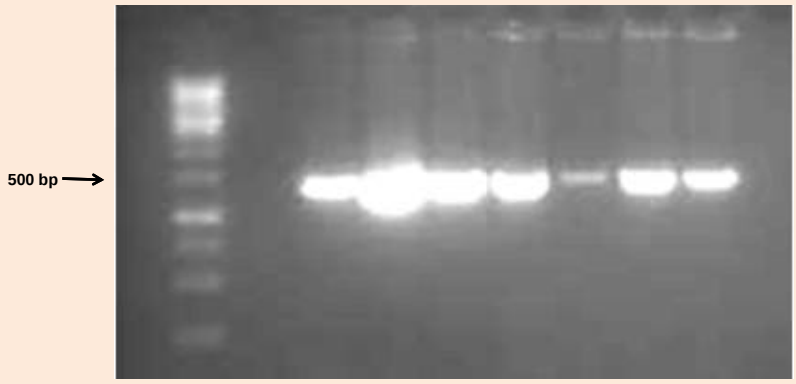

- Amplification of enterotoxin genes for SEA and SEC1 (Johnson et al., 1991).

PCR for *sea* (30 cycles)
- pre-PCR (95°C, 3 min)
- denaturation (94°C, 2 min)
- annealing (55°C, 90 sec)
- elongation (72°C, 1 menit)
- post-PCR (72°C, 5 min)

PCR for *sec* (30 cycles)
- pre-PCR (95°C, 3 min)
- denaturation (94°C, 30 sec)
- annealing (54°C, 30 sec)
- elongation (72°C, 1 min)
- post-PCR (72°C, 5 min)

Visualization of the amplicons was observed by agarose (1.5%; w/v) gel electrophresis at 70 V for 60 minutes

Results



Visualization of amplified DNA fragment encoding for universal 16S rRNA using 63f/1387r primer pairs from local isolates of *S. aureus* dan *S. aureus* ATCC 25923 on 1.5% agarose gel : negative control (1), ATCC 25923 (2), AS (3), NU1 (4), NU2 (5), NU3 (6), NU4 (7), NU5 (8). M is a 1 kb DNA ladder

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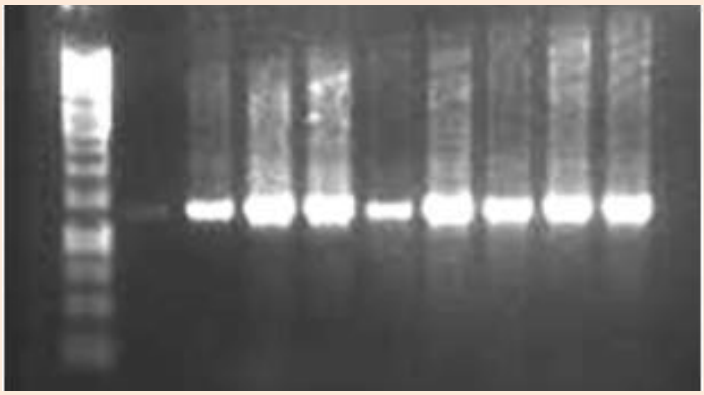
Results



Visualization of amplified DNA fragment encoding for universal 16S rRNA using 63f/1387r primer pairs from local isolates of *S. aureus* dan *S. aureus* ATCC 25923 on 1.5% agarose gel : ATCC 25923 (1), NU6 (2), NU7 (3), NU8 (4), NU9 (5), NU10 (6), NU11 (7), NU13 (8), NU14 (9). M is a 1 kb DNA ladder marker

Percent similarity of local isolates with several *S. aureus* from GenBank based on partial gene sequence of 16S rRNA

Isolate	Total score	Ref strain (source)	% similarity
AS	239	*S.aureus* subsp.aureus T0131 (clinical, China) *S.aureus* subsp.aureus str JKD6008 (clinical, Australia) *S.aureus* subsp.aureus TW20 (clinical, London) *S.aureus* subsp.aureus USA300 TCH1516 (clinical, US) *S.aureus* subsp.aureus str Newman DNA (clinical, Japan)	76%
NU1	542	*S.aureus* subsp.aureus T0131 (clinical, China) *S.aureus* subsp.aureus str JKD6008 (clinical, Australia) *S.aureus* subsp.aureus TW20 (clinical, London) *S.aureus* subsp.aureus USA300 TCH1516 (clinical, US) *S.aureus* subsp.aureus str Newman DNA (clinical, Japan) *S.aureus* subsp.aureus NTCC 8325 (clinical,US) *S.aureus* subsp.aureus USA300 FPR3757 (clinical, US) *S.aureus* subsp.aureus clone sabac-1 (not known, US)	85
NU4	566	*S.aureus* subsp.aureus ECT-R2 (human, Sweden) *S.aureus* subsp.aureus ED98 (animal, US) *S.aureus* subsp.aureus Mu3DNA (clinical, Japan) *S.aureus* subsp.aureus JH1 (not known, US) *S.aureus* subsp.aureus MU50 DNA (not known, Japan)	86

Percent similarity of local isolates with several *S. aureus* from GenBank based on partial gene sequence of 16S rRNA

Isolate	Total score	Ref strain (source)	% similarity
NU5	239	*S.aureus* subsp.aureus ECT-R2 (human, Sweden) *S.aureus* subsp.aureus ED98 (animal, US) *S.aureus* subsp.aureus Mu3DNA (clinical, Japan) *S.aureus* subsp.aureus JH1 (not known, US) *S.aureus* subsp.aureus MU50 DNA (not known, Japan)	92
NU9	865	*SS.aureus* subsp.aureus ECT-R2 (human, Sweden) *S.aureus* subsp.aureus ED98 (animal, US) *S.aureus* subsp.aureus Mu3DNA (clinical, Japan) *S.aureus* subsp.aureus JH1 (not known, US) *S.aureus* subsp.aureus MU50 DNA (not known, Japan) *S.aureus* subsp.aureus T0131 (clinical, China)	96
ATCC 25923	375	*S.aureus* subsp.aureus str JKD6008 (clinical, Australia) *S.aureus* subsp.aureus TW20 (clinical, London) *S.aureus* subsp.aureus USA300 TCH1516 (clinical, US) *S.aureus* subsp.aureus str Newman DNA (clinical, Japan) *S.aureus* subsp.aureus NTCC 8325 (clinical,US) *S.aureus* subsp.aureus USA300 FPR3757 (clinical, US)	81

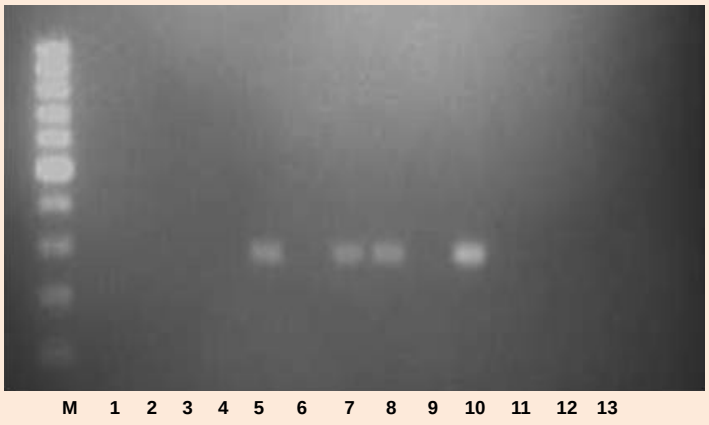
Percent similarity between local isolates of *S. aureus* based on partial gene sequence of 16S rRNA

	AS	**NU1**	**NU4**	**NU5**	**NU9**	**ATCC 25923**
AS	100	76	-	-	-	-
NU1		100	-	-	-	-
NU4			100	87	87	-
NU5				100	92	-
NU9					100	-
ATCC 25923						100

Visualization of amplified DNA fragment encoding for *Staphylococcus* enterotoxin A (sea) using SEA-1/SEA-2 primer pair in local isolates of *S. aureus* dan *S. aureus* ATCC 25923 on 1.5% agarose gel

Negative control (1), ATCC 25923 (2), AS (3), NU1 (4), NU3 (5), NU4 (6), NU5 (7), NU6 (8), NU7 (9), NU8 (10), NU9 (11), NU11 (12), NU13 (13). M is a 1 kb DNA ladder marker

Visualization of amplified DNA fragment encoding for *Staphylococcus* enterotoxin C1 (SEC1) using SEC1-1/SEC1-2 primer pair in local isolates of *S. aureus* dan *S. aureus* ATCC 25923 on 1.5% agarose gel



Negative control (1), ATCC 25923 (2), AS (3), NU1 (4), NU3 (5), NU4 (6), NU5 (7), NU6 (8), NU7 (9), NU8 (10), NU9 (11), NU11 (12), NU13 (13). M is a 1 kb DNA ladder marker

Conclusions .. (1)

- Five (AS, NU1, NU4, NU5, NU9) out of 12 of presumptive *S. aureus* isolated from RTE foods in Indonesia can be confirmed as S. aureus using primer for gene encoding for universal 16S rRNA
- The five local isolates showed sequence similarity between 76 - 96% with other (mainly) human/clinical isolates
- Similarity between the 5 local isolates is low (76-92%), needs to establish phylogenetic tree

Conclusions ..(2)

- Of the 5 local isolates, NU1 was found to possess gene encoding for SEA, while NU5 has both genes for SEA and SEC1. However several isolates not confirmed by universal 16S rRNA seems to possess SEA or SEC1 gene (NU3, NU6,NU8), thus use of primer for universal 16S rRNA may not be adequate/efficient for molecular confirmation of *S. aureus*

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Thank You

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