การประเมินความจำเพาะของไพรเมอร์ที่ใช้ตรวจ *Salmonella enterica* subspecies *enterica*

Evaluation of the Specificity of Published Primers for Detecting *Salmonella enterica* subspecies *enterica*

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บทคัดย่อ

การศึกษานี้เป็นการประเมินความจำเพาะของไพรเมอร์ที่ตีพิมพ์แล้วจำนวนห้าคู่ที่ใช้ตรวจและจำแนก *Salmonella enterica* subspecies *enterica* ด้วยวิธี Polymerase Chain Reaction (PCR) ผลการศึกษาพบว่าไพรเมอร์ ST11-ST15 มีความจำเพาะร้อยละ 100 กับ *Salmonella enterica* subspecies *enterica* จำนวน 59 สายพันธุ์ใน 22 ซีโรกรุ๊ป ขณะที่ไพรเมอร์ *sip*B-*sip*C, *iro*B, *stn* และ *hil*A มีความจำเพาะร้อยละ 93.2, 89.8, 88.1 และ 84.7 ตามลำดับ ผลที่ได้จากการศึกษานี้จะเป็นประโยชน์สำหรับการพัฒนาการตรวจสายพันธุ์ของ *Salmonella enterica* subspecies *enterica* ได้อย่างรวดเร็วด้วยวิธี PCR ต่อไป

ABSTRACT

The present study were evaluated the specification of five published primers for detecting/identifying *Salmonella enterica* subspecies *enterica* strains by polymerase chain reaction (PCR) assay. The results demonstrated that ST11-ST15 primers showed 100% specification of amplification to 59 *Salmonella enterica* subspecies *enterica* strains in 22 serogroups, whereas primers of *sip*B-*sip*C, *iro*B, *stn* and *hil*A were 93.2%, 89.8%, 88.1% and 84.7%, respectively. The results from this study will be useful for further development of rapid detection of strains in *Salmonella enterica* subspecies *enterica* by PCR.

คำสำคัญ: ซัลโมเนลลา, พีซีอาร์, ไพรเมอร์จำเพาะ

Keywords: *Salmonella enterica* subspecies *enterica*, PCR, specific primer

INTRODUCTION

Salmonella are Gram-negative rod, non-spore forming, facultative anaerobic bacteria, and motile by peritrichous flagella. These organisms can be classified into two species; *S. enterica* and *S. bongori*. In addition, *Salmonella* can also be classified more than 2,500 serovars using serological method based on the antigenic variability of lipopolysaccharides (O antigens), flagella proteins (H1 and H2 antigens), and capsular polysaccharides (Vi antigens). The conventional methods to detect *Salmonella* are laboring, time – consuming, expensive, and low in specificity and sensitivity (Pui, 2011), and must be performed key the reference laboratory only.

A polymerase chain reaction (PCR) technique has been accepted as a rapid-reliable tool, time saving and high sensitivity-specificity for detecting salmonellae isolates by designing the primers specific to the target genes. Nowadays, hence, various primers specific to different target genes have been established. However, some primers show the negative amplification with some salmonellae serovars (Ziemer and Steadham, 2003). Additionally, those published primers were applied for detecting various serogroups and serovars of *S. enterica* subsp. *enterica* from different sources of samples (Pui, 2011).

Therefore, the present study evaluated the specification of those published primers for detecting various serovars in different serogroups of *S. enterica* subsp. *enterica*. The result from this study will be useful for further rapid alternative salmonellae-detection of salmonellae strains by multiplex PCR.

MATERIALS AND METHODS

1. Bacterial strains

A total of 59 *Salmonella* strains in 22 serogroups belonged to *Salmonella* subspecies *enterica* and 12 non-*Salmonella* strains including *Vibrio vulnificus*, *V. cholerae*, *V. parahaemolyticus*, *Yersinia enterocolitica*, *Serratia marcessens*, *Bacillus cereus*, *Shigella* spp., *Escherichia coli*, *Klebsiella* spp., *Citrobacter diversus*, *Proteus mirabilis*, and *Enterobacter aerogenes* were used in this study. These strains were obtained from Nation Institute of Health (NIH), Department of Medical Science, Ministry of Public Health, Nonthaburi and Department of Microbiology, Faculty of Public Health, Mahidol University. In addition, *Salmonella* Typhimurium ATCC13311 was used as a reference strain.

2. Genomic DNA preparation

The *Salmonella* strains were inoculated into Luria-Batani (LB) broth. One milliliter of cell suspension was washed and then heated at 95°C for 10 min. The tubes were then placed on ice immediately for 5 min. The gDNA was collected by centrifugation at 16,000 x g for 10 min.

3. Primers and PCR condition

Five published sequence primers of salmonellae are shown in Table 1. The PCR mixture was prepared as 25 µl containing 1 µl of DNA template, 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM of each primer, and 0.5 U of *Taq* polymerase. The condition of PCR were used initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 55°C for 45 s, DNA extension at 72°C for 45 s and final extension at 72°C for 5 min. The products were analyzed using 1.5% (w/v) agarose with 100 V for 45 min. The gel was stained with ethidium bromide for 5 min and destained with distilled water for 10 min. The amplicon sizes were visualized by Gel documentation.

Table 1 Sequence primers for *salmonellae* detection

	Primer sets	Primer sequence	Target gene	Size (bp)	Reference
Group 1	Junction of *sip*B and *sip*C	5´-acagcaaaatgcggatgctt-3´ 5´-gcgcgctcagtgtaggactc-3´	*sip*B - *sip*C	250	Carlson *et al*. (1999)
	ST11 ST15	5´-gccaaccattgctaaattggcgca-3´ 5´-ggtagaaattcccagcgggtactgg-3´	random fragment	429	Aabo *et al*. (1993)
	Fur-regulated gene	5´-tgcgtattctgtttgtcggtcc-3´ 5´-tacgttcccaccattcttccc-3´	*iro*B	606	Baumler *et al*. (1997)
Group 2	Enterotoxin gene	5´-ctttggtcgtaaaataaggcg-3´ 5´-tgcccaaagcagagagattc-3´	*stn*	260	Makino *et al*. (1999)
	SPI1 invasion gene	5´-ctgccgcagtgttaaggata-3´ 5´-ctgtcgccttaatcgcatgt-3´	*hil*A	497	Guo *et al*. (2000)

RESULTS AND DISCUSSION

The results of gDNA amplification from 59 strains of *Salmonella* strains and 12 non-*Salmonella* strains using 5 different primers are shown in Figure 1. The ST11-ST15 primers showed positive amplicons for all strains (100%) at the expected size of 429 bp. In contrast specificity of *sip*B-*sip*C, *iro*B, *stn*, and *hil*A were 93.2%, 89.8%, 88.1%, and 84.7%, respectively (Table 2). None of the primers amplified DNA from non-*Salmonella* strains.

Our result of ST11-ST15 amplification was agree to those of previous studies (Soumet *et al*., 1999, Pui *et al*., 2011). The results demonstrated that this primer set is specific for all 59 representative salmonellae strains documented in 22 serogroups belonging to subspecies *enterica*. In contrast, the other 4 published primers did not amplify totally different 14 salmonellae serovars as shown in Table 2. However, some serovars in certain serogroups are not totally included to be tested in the present study. Therefore, ST11-ST15 primers specific to *S. enterica* subsp. *enterica* are required to further detect other various serovars in the other subspecies in order to confirm specificity of these primers for specific detecting *S. enterica* subsp. *enterica* strains.

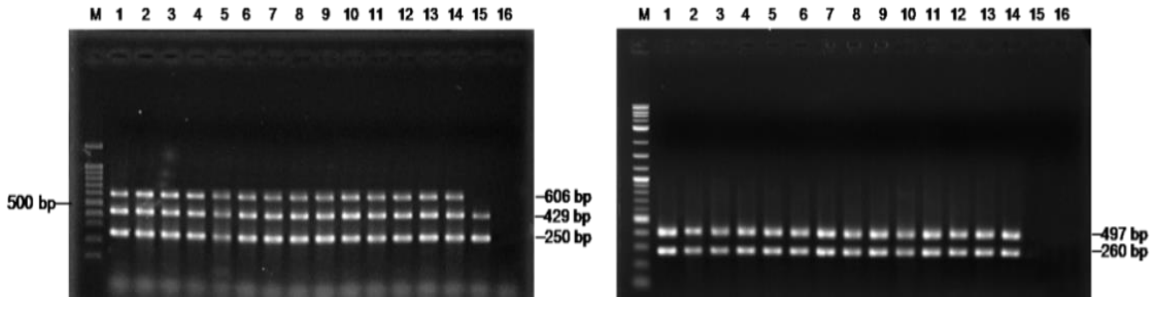

Figure 1 Multiplex PCR amplification using 5 different published primers. **A**: primer of Junction of *sip*B and *sip*C genes, ST11 ST15, Fur-regulated gene (*iro*B). **B**: primer of enterotoxin gene *(stn)* and SPI invasion gene (*hil*A). M: DNA ladder, Lane 1: *S.* Typhimurium ATCC 13311, Lane 2: *S.* Derby, Lane 3: *S.* Heidelberg, Lane 4: *S.* Paratyphi B variety Java, Lane 5: *S.* Stanly, Lane 6: *S.* Oritamerium, Lane 7: *S.* Bareilly, Lane 8: *S.* Choleraesuis, Lane 9: *S.* Infantis, Lane 10: *S.* Montevideo, Lane 11: *S.* Ohio, Lane 12: *S.* Rissen, Lane 13: *S.* Albany, Lane 14: *S.* Thompson, Lane 15: *S.* Singapore, and Lane 16: Negative control.

Table 2 PCR amplification of gene fragments by five selected primer sets in *Salmonella* strains.

Serogroup	Serovar	Primers 1	2	3	4	5	Serogroup	Serovar	Primers 1	2	3	4	5
B	*S.* Typhimurium ATCC 13311	■	■	■	■	■	E	*S.* Lexington	■	■	■	■	■
	S. Agona	□	■	■	■	■		*S.* Weltevereden	■	■	■	■	■
	S. Derby	■	■	■	■	■		*S.* Krefeld	■	■	■	■	■
	S. Heidelberg	■	■	■	■	■		*S.* Senftenberg	■	■	■	■	■
	S. Paratyphi B variety Java	■	■	■	■	■		*S.* Rubislaw	■	■	■	■	■
	S. Saintpaul	■	■	■	■	■	G	*S.* Poona	■	■	■	■	■
	S. Schwarzengrund	□	■	■	■	■		*S.* Putten	■	□	■	■	■
	S. Stanley	■	■	■	■	■		*S.* Idikan	■	□	■	□	■
	*S.*Indiana	■	■	■	■	■		*S.* Kedougou	■	■	■	■	■
C	*S.* Oritamerin	■	■	■	■	■	I	*S.* Hvittinggfoss	■	■	■	■	■
	S. Bareilly	■	■	■	■	■		*S.* Yoruba	■	■	■	■	■
	S. Choleraesuis	■	■	■	■	■	H	*S.* Carro	■	■	■	□	■
	S. Infantis	■	■	■	■	■	K	*S.* Cerro	■	□	■	□	■
	S. Montevideo	■	■	■	■	■	L	*S.* Minnesto	■	□	■	□	■
	S. Ohio	■	■	■	■	■	M	*S.* Chicago	■	■	■	■	■
	S. Rissen	■	■	■	■	■	N	*S.* Ramatgan	■	■	■	□	■
	S. Singapore	■	□	■	□	□	O	*S.* Addelaide	■	■	■	■	■
	S. Thompson	■	■	■	■	■		*S.* Alachua	■	■	■	■	■
	S. Albany	■	■	■	■	■	P	*S.* Bangkok	■	■	■	■	■
	S. Brunei	■	■	■	■	■	Q	*S.* Wandsworth	■	■	■	■	■
	S. Emek	■	■	■	■	■	R	*S.* Johannesburg	■	■	■	■	■
	S. Hadar	■	■	■	■	■	S	*S.* Waycross	■	■	■	■	■
	S. Kentucky	■	■	■	■	□	W	*S.* Jodhar	■	■	■	■	■
	S. Newport	■	■	■	■	■		*S.* Apapa	■	□	■	□	■
D	*S.* Enteritidis	■	■	■	■	■	Y	*S.* Bergen	■	■	■	■	■
	S. Panama	■	■	■	■	□		*S.* Dakata	■	■	■	■	■
	S. Fresno	■	■	■	■	■	O:51	*S.* Treforest	■	■	■	■	■
E	*S.* Amsterdam	■	■	■	■	■	O:53	*S.* Hamber	□	■	■	■	■
	S. Anatum	■	■	■	■	■	O:56	*S.* Artis	□	■	■	■	■
	S. Give	■	■	■	■	■	O:60	*S.* Arizonar	■	■	■	■	■

1 = Junction of *sip*B and *sip*C, 2 = Fur-regulated gene, 3 = ST11, ST15, 4 = Enterotoxin gene, 5 = SPI1 invasion gene

■ Positive □ Negative

CONCLUSION

The five sets of PCR primers were evaluated, only ST11-ST15 primers shows as reliable primers with high specification. Therefore, the ST11-ST15 alternatively primers can be applied for detecting *S. enterica* subsp. *enterica* strains in this study

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