

## Occurrence of Virulence Determinants in Fecal *Enterococcus faecalis* Isolated from Pigs and Chickens in Korea

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**Forty-one *Enterococcus faecalis* (*E. faecalis*) isolates from feces of pigs and chickens in Korea were screened for the presence of virulence factors. Gelatinase activity (85.4%, 35/41) was the more commonly observed phenotype of virulence in *E. faecalis*, compared with hemolytic activity (12.2%, 5/41). Thirty-one of 35 (88.6%) gelatinase-positive *E. faecalis* isolates harbored the *gelE* and *fsrABC* genes. A gene encoding for the enterococcal surface protein (Esp) was detected in 24.4% (10/41) of the isolates. All beta-hemolysin-producing isolates harbored the *esp* gene.**

**Keywords:** *Enterococcus faecalis*, virulence factors, livestock animals

Enterococci, as members of the commensal flora in human and animal, are also found in some foods and food products such as milk and cheese, meat, and vegetable. They have also emerged as the major causes of nosocomial infections, including urinary tract, endocarditis, bloodstream, and wound infections [18, 22]. In addition, the importance of these microorganism has been focused on their ability to acquire virulence factors or antimicrobial-resistant genes [7]. Different virulence determinants have been introduced in enterococci including gelatinase, extracellular surface protein, cytolysins, hyaluronidase, and adhesins binding to host cell and biofilm formation.

Gelatinase is an extracellular zinc-endopeptidase, and the contribution of this virulence factor has been well studied in other reports. The encoding gene is *gelE*, which is commonly accompanied with the *fsr* gene in *E. faecalis* [9, 23] and able to hydrolyze gelatin, casein, hemoglobin, and other bioactive peptides [27]. Hemolysin (called

cytolysin) displays bacteriocidal and hemolytic activities to a number of Gram-positive bacteria [25], and mediates enterococcal virulence through its toxic ability or by destroying the ecology. The encoding gene was identified as *cylA*, *cylB*, and *cylM* [10]. The enterococcal surface protein (Esp) contributes to colonization and persistence of enterococci during infections such as of the urinary tract [26] and biofilm formation [28].

Virulence factors were frequently found in *E. faecalis*, being *E. faecium* generally free of them [8, 16]. *E. faecalis* is responsible for up to 80% of enterococcal-associated nosocomial infections [9]. Although virulence factors of *E. faecalis* are harmless in normal status, enterococcal clinical isolates have pathogenic potential to unhealthy patients with impaired immune systems. Virulence determinants of *E. faecalis* are closely related to severity of disease [20], and the effects of virulence factors have also been demonstrated in animal models and cells [14, 15]. However, studies of virulence in enterococci of food and animal origin are scarce. Therefore, the aim of the present study was to assess the occurrence of putative virulence factors in *E. faecalis* strains isolated from chickens and pigs in Korea.

*E. faecalis* isolates used in this study were all recovered from fecal samples of chickens and pigs collected from Korean slaughterhouses in our previous studies [11, 12]. Briefly, fecal samples were inoculated into bile-esculin azide broth (BD BBL, Spark, MD, USA) at 35°C for 24 h. Isolates were then streaked on mEnterococcus agar (BD BBL) and incubated at 35°C for 24 h. Identification of enterococci was performed using a VITEK system (bioMérieux, Hazelwood, MO, USA). Confirmation of *E. faecalis* was done by polymerase chain reaction (PCR) as described previously [6].

The 41 isolates of *E. faecalis* (chicken: 22 isolates; pig: 19 isolates) were screened for virulence factors. Expression

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**Table 1.** PCR primers used for detection of virulence determinants.

Primer	Amplicon (bp)	Sequence of the primer (5'–3')	Reference
<i>gelE</i>	419	ACCCCGTATCAGTGGTTT ACGCATTGCTTTTCCATC	[7]
<i>esp</i>	933	TTGCTAATGCTAGTCCACGACC GCGFCAACACTTGCATTGCCGAA	
<i>fsrA</i>	740	ATGAGTGAACAAATGGCTATTTA CTAAGTAAGAAATAGTGCCTTGA	
<i>fsrB</i>	566	GGGAGCTCTGGACAAAGTATTATCTAACCG TTGGTACCCACACCATCACTGACTTTTGC	[20]
<i>fsrC</i>	1,343	ATGATTTTGTCTGTTATTAGCTACT CATCGTTAACAACCTTTTTTACTG	
<i>cylM</i>	742	CTGATCGAAAGAAGATAGTAT TGAGTTGGTCTGATTACATTT	
<i>cylB</i>	843	ATTCTACCTATGTTCTGTGA AATAAACTCTTCTTTTCCAAC	[7]
<i>cylA</i>	517	TGGATGATAGTGATAGGAAGT TCTACAGTAAATCTTTCGTCA G	

of gelatinase activity and hemolysin activity was also investigated in parallel with the detection of virulence determinants including *gelE*, *fsr* locus, *esp*, *cylA*, *cylB*, and *cylM* in those isolates. Gelatinase activity was determined on tryptic soy agar plates containing 1.5% skim milk (Difco, MD, USA) after overnight incubation at 37°C and then cooling to room temperature for 2 h. The appearance of a transparent halo around the colonies was considered to be a positive indication of gelatinase production [9]. The production of hemolysin was determined on Columbia Blood Agar (Oxoid, UK) containing 5% defibrinated horse blood (Oxoid, UK). Plates were incubated at 37°C for 48 h. Clear zones around colonies were interpreted as  $\beta$ -hemolytic activity. PCR was performed to detect the virulence genes involved in gelatinase (*gelE*), *fsr* locus, enterococcal surface protein (*esp*), and the expression of cytolysin (*cylA*, *cylB*, and *cylM*). Primer sets and PCR conditions are described in Table 1. DNA of *E. faecalis* ATCC51299 was used as a positive control in PCR reactions.

The distribution of virulence determinants among 41 *E. faecalis* isolates from chickens and pigs as well as the gelatinase and  $\beta$ -hemolysis activities are shown in Table 2. Gelatinase activity (85.4%) was the more commonly observed phenotype of virulence in *E. faecalis* compared with hemolytic activity (12.2%). Most of the chicken isolates (20/22, 90.9%) presented gelatinase activity, which is slightly higher than data reported for isolates of poultry origin (88%) [22]. Compared with the chicken isolates, *E. faecalis* isolates of swine origin showed lower rate of gelatinase activity (68.4%, 15/19). Overall, the prevalence of gelatinase activity observed in this study is higher than those reported in food (56%) and medical (55–33% and 45%) *E. faecalis* isolates [7, 17]. Compared with gelatinase activity,  $\beta$ -hemolysis was observed rarely in this study: In total, 5 of 41 (12.2%) *E. faecalis* isolates showed  $\beta$ -hemolysis, and most of them (4/5, 80%) were originated from pigs. Only one (4.6%) of 22 *E. faecalis* isolates from chicken showed  $\beta$ -hemolysis, which is almost similar to

**Table 2.** Distribution of virulence determinants in *E. faecalis* isolated from feces of chickens and pigs in Korea.

Species	Origin (No. of isolates)	Phenotype					Genotype			
		Hemolytic activity			Gelatinase activity	<i>esp</i>	<i>gelE</i> - <i>fsr</i> ABC	<i>cylM</i>	<i>cylB</i>	<i>cylA</i>
		$\alpha$	$\beta$	$\gamma$						
<i>E. faecalis</i>	Chicken (22)	13.6% (3)	4.6% (1)	81.8% (18)	90.9% (20)	9.1% (2)	90.9% (20)	18.2% (4)	9.1% (2)	18.2% (4)
	Pig (19)	10.5% (2)	21.1% (4)	68.4% (13)	79.0% (15)	42.1% (8)	68.4% (13)	36.8% (7)	26.3% (5)	31.6% (6)
Total	(41)	12.2% (5)	12.2% (5)	75.6% (31)	85.4% (35)	24.4% (10)	80.5% (33)	26.8% (11)	17.1% (7)	24.4% (10)

**Table 3.** Correlation between gelatinase activity and *gelE* and *fsrABC* genes in *E. faecalis* from chickens and pigs in Korea.

Species (No. of isolates)	Gelatinase activity	No. of isolates	Gene detected by PCR	
			<i>gelE</i> <sup>+</sup> - <i>fsrABC</i> <sup>+</sup>	<i>gelE</i> <sup>-</sup> - <i>fsrABC</i> <sup>-</sup>
<i>E. faecalis</i> (41)	Positive	35 (85.4%)	31 (88.6%)	4 (11.4%)
	Negative	6 (14.6)	2 (33.3%)	4 (66.7%)

**Table 4.** Relationship between *esp* gene and other determinants in *E. faecalis* isolates.

Isolates	Origin	Genotype		Phenotype	
		<i>esp</i>	<i>gelE</i>	Hemolytic activity	Gelatinase activity
NVRQS1200311	Chicken	+	-	β	-
NVRQS2200102		+	-	β	-
NVRQS2200141		+	-	β	-
NVRQS2200151		+	-	β	-
NVRQS 2200311		+	-	β	-
NVRQS 1200104	Pig	+	+	α	+
NVRQS 2200522		+	-	γ	+
NVRQS 2200531		+	+	γ	+
NVRQS 2200571		+	-	α	+
NVRQS 2200601		+	+	α	+

data reported for *E. faecalis* recovered from poultry (2.3%, 1/43) [21]. The PCR amplification revealed that 24.4% (10/41) of isolates represented gene encoding for the enterococcal surface protein (Esp), which was identified at high frequency, particularly in *E. faecalis* isolated from pigs (42%, 8/19). Our result is lower than those reported by other authors [5, 7, 29]. The presence of the *esp* gene in *E. faecalis* also suggests a role in colonization, persistence within the urinary tract [27], and formation of biofilm [28]. It could be detected from human isolates with bacteremia and endocarditis [1, 2]. As described in Table 4, all of the five *E. faecalis* isolates producing beta-hemolytic activity harbored the *esp* gene. Among the *esp*-positive *E. faecalis* isolates, strains with beta-hemolysis activity did not show any gelatinase activity.

The prevalence of β-hemolysis (12.2%) observed in this study is much lower than those of other reports (range: 17–60% in clinical and food isolates) [4, 13, 24]. All hemolysin-positive *E. faecalis* contained only the *esp* gene, with the loss of gelatinase activity. Five *E. faecalis* isolates that showed β-hemolysis harbored all *cyl* genes, which is in agreement with other reports [3, 21]. The other 36 isolates lacked at least one of the whole *cyl* set and did not show hemolytic activity. Previously, it was reported that low levels or down-regulation of gene expression could be a reason of lacked phenotype [3, 24].

Correlation between gelatinase activity and genotypes of *gelE* and *fsr* locus (*fsrA*, *fsrB*, and *fsrC*) in the isolates is presented in Table 3. It was reported that all the *E. faecalis* isolates that exhibited gelatinase activity harbored the *gelE*

and *fsr* genes [21], which is in disagreement with our results. In this study, only 31 of 35 (88.6%) gelatinase-positive *E. faecalis* isolates harbored the *gelE* and *fsrABC* genes, and 2 of 6 gelatinase-negative isolates contained those genes. Although we cannot explain the reason for this result, discrepancy between the genotype and phenotype in some of the *E. faecalis* isolates was also reported by other authors.

In this study, we described the occurrence of virulence factors in *E. faecalis* isolates from fecal samples of pigs and chickens in Korea. The data of this study could help to figure out the prevalence of virulence factors in enterococcal isolates from livestock animals in Korea, and indicate that *Enterococcus* species should be considered as a possible reservoir for virulence determinants from food products of animal sources. Further studies are needed to investigate the pathogenic role of the virulence factors in animals.

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