Enterococcus spp. 를 이용한 미생물 오염 추적 기술

Possibilities in using Enterococcus spp. in Microbial Source Tracking

Tatsuya Unno and Hor-Gil Hur*

1. Department of Environmental Science and Engineering, Gwangju Institute of Science and Technology, 1 Oryong dong, Buk-gu, Gwangju 500-712.[2]International Environmental Research Center, Gwangju Institute of Science and Technology, 1 Oryong dong, Buk-gu, Gwangju 500-712.

*Corresponding author: hghur@gist.ac.kr

Enterococcus is a fecal indicator bacterium and often used to indicate fecal contamination in the environment. Carbohydrates fermentation patterns of *Enterococcus* isolates were investigated as a way to differentiate the source of fecal contamination. Total 1826 *Enterococcus* isolates were obtained from cows, pigs, chickens, ducks, and humans in two geographically different locations. Distributions of carbohydrate fermentation patterns showed discrepancies among sources. This study suggest that the possibility of the use of *Enterococcus* in microbial source tracking.

*Enterococcus*는 fecal indicator bacterium의 하나로서 환경내에서 fecal contamination을 탐지 하는 지표로서 사용되며, 현재 각 *Enterococcus spp.* 의 서로 다른 당 분해능을 이용한 분별법이 개발되었으며, 이를 위해 서로 다른지역의 소, 돼지, 닭, 오리 그리고 사람으로 부터 총 1826의 *Enterococcus* 샘플을 획득 하였음. 이 연구는 *Enterococcus*를 통해 microbial source tracking 의 가능성을 제시하고 있음.

INTRODUCTION

Fecal contamination is the one of the most frequently occurred pollution found in lakes and rivers. There are cases that recreational use of water was banned after excessive numbers of fecal indicator bacteria were detected. There are three cases causing fecal contamination in rivers and lakes. First, urban storm water combined sewer overflows (CSOs) is a typical example of anthropogenic fecal contamination, and second, in many of the cases in general, wild animals such as migrating birds are the contributors to fecal contamination in lakes and water reservoirs. Thirdly, livestock feces contained in runoff from farms flow into rivers or lakes.

Enterococcus is a common inhabitant of the intestinal tract of warm-blooded animals, and its presence in water samples is an indication of fecal pollution and the possible presence of enteric pathogens and the *Enterococcus* test is recommended as a measure of ambient recreational fresh water quality (EPA method 1600). It is also stated that the significance of finding *Enterococcus* in recreational fresh water samples is the direct relationship between the density of *Enterococcus* and the risk of gastrointestinal illness associated with swimming in the water.

There have been several methods reported for tracking sources of fecal contamination using microbes and those are generally called microbial source tracking (MST). Many MST methods have been developed with *E. coli* strains, while the other fecal indicator bacterium, *Enterococcus*, has not been well studied as a way of MST. In 1999 Manero et. al reported that *Enterococcus* spp. can be well differentiated into species by several biochemical keys.

In this study, biochemical species differentiation of Enteorcoccus isolates obtained from ducks,cows, humans, pigs and chickens are investigated as a way to differentiate sources.

METHODS

Fecal Sample Collection

Swab samples from chickens, beef cows, dairy cows, and pigs were collected at farms in DamYang, South Korea in April 2006. Another sample collection was made for ducks, chickens, dairy cows, beef cows, and pigs at farms in Naju, South Korea in June 2007. Also swab samples from healthy human feces were collected at hospital in South Korea in June 2007. The swab samples were kept onice and processed within 12～24 hours.

Enterococcus isolation

The swab samples were directly streaked onto mEnterococcus agar plates. Plates were incubated at 42℃ for 48 hrs. Only pink～red colonies were transferred to new agar plates for colony purification. Five to eight colonies from each swab sample were transferred to 96-well microplate containing Brain Heart Infusion Broth (BHIB). Enriched cells were stamped on Bile Esculine agar plates. Only black colonies were considered to be *Enterococcus* isoaltes.

Biochemical tests

Carbohydrate fermentation tests were performed with the basal medium phenol red broth. L-Arabinose, ribose, sorbitol, mannitol, and methyl-alpha-D-Glucopyranoside were added at 1% concentration to phenol red broth for the corresponding tests. Decarboxylation of the arginine was checked by using Moller decarboxylase base medium.Isolates were also incubated in 6.5% NaCl BHIB in order to test resistance to salts. Colony pigmentation was also checked on Brain Heart Infusion Agar after 48 hrs of incubation at 37℃.

Analysis

Biochemical results were shown in+for positive, - for negative, and W for weak reaction. 9 different biochemical tests results for one isolate were defined as one type. Types representing less than 5% were not taken into analysis.

RESULTS

Types in biochemical test results

Most of the isolates were grouped into 13 types with biochemical tests. (Table 1)

Table 1. types of biochemical tests results

	Ara	YP	Rib	GPS	Sor	Suc	Arg	Man	NaCl
A	−	−	+	−	−	−	+	−	−
B	−	−	+	−	−	−	+	−	+
C	−	−	+	−	−	+	+	−	−
D	−	−	+	−	−	−	+	+	+
E	−	−	+	−	−	+	+	−	+
F	+	−	+	−	−	+	+	−	−
G	−	−	+	−	−	+	+	+	+
H	−	−	+	−	+	−	+	+	+
I	+	−	+	−	−	−	+	+	+
J	+	−	+	−	−	+	+	−	+
K	−	−	+	−	+	+	+	+	+
L	−	−	+	+	+	−	+	+	+
M	+	−	+	−	−	+	+	+	+
N	+	+	+	−	−	+	+	+	+
O	−	−	+	−	+	w	+	+	+

Depending on sources, Enterococcus isolates showed different distributions in biochemical results. High percentage can be seen in type E in livestock isolates whereas type M was the majority of Human isolates. Poultry isolates were distributed in many different types. Based on Manero's report, type E is durans or hirae, and type M is raffinosus or faecium. Another commonly isolated species faecalis can be best matched with type K.

Table 2. Distribution in biochemical test results types

		percentages in biochemical test results type of														
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
Livestock	Beefcow					35			6				5		9	6
	Dairycow					90										
	Pig					75						18				
Poultry	Chicken	6		14		26	8		14		11	7				
	Duck		6		15	5		6	13	18				13		
	Human					9					7			54		6

SUMMARY

Enterococcus isolates were typed with biochemical tests. Although there were trends in species distributions, it does not seem strong enough to differentiate sources. However, this study suggests the possibility of the use of *Enterococcus* isolates in microbial source tracking. Further typing with molecular technologies would add more precise discrimination in species level and possibly source differentiation as reported by Pavel et. al.

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