Campylobacter mucosalisとCampylobacter hyointestinalisの選択培地

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New Selective Media for the Isolation of *Campylobacter mucosalis* and *Campylobacter hyointestinalis*

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Campylobacter mucosalis and *Campylobacter hyointestinalis* are definitive etiological agents of proliferative enteritis (PE) in swine [1, 2, 5, 11]. Each of these bacteria shows distinctive biochemical characteristics compared with those of other *Campylobacter* species [3, 6]. But their growth on solid media is poor and form small colonies 1.0 to 2.0 mm in diameter. This often causes some difficulties for the isolation of them from clinical specimens by overgrowth of coexisting bacteria. Selective medium for *C. mucosalis* was first reported by Lawson and Rowland [5], which contains novobiocin and brilliant green as selective reagents, and improved by McCartney *et al.* [7] by the addition of trimethoprim or rifampicin. As for *C. hyointestinalis* selective media constituted by the addition of trimethoprim and sulfamethoxazole were described by Gebhart *et al.* [2]. Lambert *et al.* [4] employed selective medium contains trimethoprim, polymyxin, actidione and nalidixic acid as selective antibiotics and reagents for the detection of hydrogen sulfide. But the selectivity of these media are based on inhibitory effects of added antibiotics or dye against coexisting bacteria and it requires much experience to discriminate the small colonies in question from those of other bacteria. And in our preliminary study, it proved that fresh isolates of *C. mucosalis* or *C. hyointestinalis* were sometimes inhibited their growth by some kind of antibiotics or dye added at the concentration recommended. The abundant production of hydrogen sulfide by *C. mucosalis* and *C. hyointestinalis* was applied for the preparation of new selective media. In this study, the improved selective media supplemented by reagents for the detection of hydrogen sulfide production in addition to reasonably small amounts of antibiotics and dye as selecive agents, at the concentration exert no effect for the growth of *Campylobacter* species in question, are described.

Two kinds of selective media are developed. The constituents of these media are listed in Table 1. For incubation, and anaerobic jar containing plates was evacuated to a negative pressure of 650 mmHg and refilled with mixed gas (80%N_2-10%H_2-10%CO_2) or equipped with Gas Pak (BBL) without catalyst. Then the jar was maintained at 37°C for three days. Sodium thiosulfate and ferric ammonium citrate were added to detect hydrogen sulfide production. Medium A is prepared by slight modification of selective medium reported by Lawson and Rowland [5] which is frequently employed for the cultivation of *C. mucosalis* and *C. hyointestinalis* [8, 9, 10, 11]. In our preliminary works, it was revealed that the selective activity of brilliant green was enhanced on serum agar than blood

Table 1. Constitution of selective media

Medium A	
Basal medium	: Blood agar base No. 2 (Oxoid) supplemented with 5% sheep blood and 0.5% yeast extract (Difco)
Additives	: 5 μg/m*l* of novobiocin, 1/120,000 of brilliant green 0.05% of sodium thiosulfate, 0.025% of ferric ammonium citrate
Medium B	
Basal medium	: Blood agar base No. 2 (Oxoid) supplemented with 5% of horse serum and 0.5% yeast extract (Difco)
Additives	: 5 μg/m*l* of novobiocin, 5 μg/m*l* of nalidixic acid, 1/120,000 of brilliant green, 0.05% of sodium thiosulfate, 0.025% of ferric ammonium citrate

agar and *C. hyointestinalis* was less sensitive to brilliant green than *C. mucosalis*. Medium B was prepared for the strictly selective isolation of *C. hyointestinalis*. In field cases of PE which was characterized by acute death of animal, *C. hyointestinalis* was frequently isolated [8, 9]. And at the same time, occurence of diarrhea which was suspected the involvement of *C. hyointestinalis* is increasing in recent years. Medium B was intended for the isolation of *C. hyointestinalis* from such cases as described above. Both *C. mucosalis* and *C. hyointestinalis* grew well on medium A and showed colonies with blackened center by the production of hydrogen sulfide. *C. coli* also grew well on medium A forming opaque colonies. This organism failed to produce hydrogen sulfide on this medium. Colonies of *C. hyointestinalis* on medium B are characterized by blackened center accompanied opaque peripheries and the growth of coexisting bacteria was inhibited remarkably.

The efficacy of these two new media was compared with Lawson's selective medium and non-selective blood agar for the recovery of reference strains of *C. mucosalis*, NCTC 11000 (serovar A), NCTC 11419 (serovar B) and NCTC 11420 (serovar C) and *C. hyointestinalis* ATCC 35217 from artificially contaminated fecal specimens. (Table 2). Three fecal specimens which had been checked for the absence of *Campylobacter* species were collected from healthy pigs and corresponding reference strains were inoculated into each fecal specimen at the level of finally 10^3, 10^5 and 10^7 CFU/g. These reference strains were consistently recovered on each medium employed if they exist at the number of more than 10^5 CFU/g in feces. Recovery rate of *C. mucosalis* from feces harboring 10^3 CFU/g of this organism decreased remarkably by overgrowth of coexisting bacteria. *C. mucosalis* was barely cultured on Lawson's medium and medium A from one or two specimens. *C. hyointestinalis* was recovered well on medium B even if it presents at the low number as 10^3 CFU/g by strong inhibition of coexisting bacteria. The number of coexisting bacteria other than *Campylobacter* species in question was about 10^7 CFU/g for each specimen.

Based on these findings, the efficacy of these two media was also compared for the isolation of *Campylobacter* species concerned from the lesions of PE, mainly observed at caudal part of ileum. (Table 3). Viable bacterial count of *Campylobacter* species was ranged 10^5 to 10^8 CFU per gram of affected mucosa. The recovery of *C. mucosalis* was considerably high on medium A. As for *C. hyointestinalis* significant difference was not observed. The rate of coexisting bacteria decreased on medium A and medium B compared with Lawson's medium.

Table 2. Recovery of *C. mucosalis* and *C. hyointestinalis* from artificially contaminated fecal specimens

Bacteria	NO. of organisms (CUF/g)[a)]	Blood agar	Lawson's	Medium A	Medium B
C. mucosalis	10^3	0/3[b)]	0/3	1/3	0/3
NCTC 11000	10^5	2/3	3/3	3/3	0/3
	10^7	3/3	3/3	3/3	0/3
C. mucosalis	10^3	0/3	0/3	2/3	0/3
NCTC 11419	10^5	2/3	3/3	3/3	0/3
	10^7	3/3	3/3	3/3	0/3
C. mucosalis	10^3	0/3	1/3	1/3	0/3
NCTC 11420	10^5	3/3	3/3	3/3	0/3
	10^7	3/3	3/3	3/3	0/3
C. hyointestinalis	10^3	0/3	0/3	1/3	3/3
ATCC 35217	10^5	3/3	3/3	3/3	3/3
	10^7	3/3	3/3	3/3	3/3

a) No. of corresponding bacteria in feces artificially contaminated.
b) No. of specimens positive for recovery of corresponding bacteria from three specimens.

Table 3. Comparison of isolation rate of *C. mucosalis* and *C. hyointestinalis* from clinical specimens on each medium

Specimens	Bacteria	n[a)]	Blood agar	Lawson's	Medium A	Medium B
PE lesions	*C. mucosalis*	9	100[b)]	71.6±22.2[c)]	104.7±45.3	0
	C. hyointestinalis	7	100	111.1±28.2	95.8±18.5	102.2±22.1
	Coexistant[d)]	16	100	26.5±23.2	16.4±10.0	8.6±11.9
Diarrhea	*C. hyointestinalis*	3	100	126.3±23.6	143.9±24.4	129.3±27.5
	Coexistant	3	100	19.3±17.1	1.1± 1.0	0.1± 0.1

a) No. of specimens.
b) No. of colonies of corresponding *Campylobacter* recognized on blood agar was converted to 100 as an index.
c) Mean ± standard deviation.
d) Coexisting bacteria other than *C. mucosalis* and *C. hyointestinalis*.

Detection of suspicious colonies was much easier on these new media. Isolation of *C. hyointestinalis* from an occurrence of diarrhea is also listed in Table 3. Freshly collected three fecal specimens were subjected for bacteriological study. No other significant bacteria was isolated in this case. *C. hyointestinalis* was isolated 10^6 to 10^7 CFU per gram of feces. Significant difference for the recovery was not clear in three selective media. But the coexsisting bacteria decreased remarkably on medium A and medium B. On medium B, coexisting bacteria decreased to about 1/200 compared with Lawson's medium. After medication by antibiotics when diarrhea ceased, rectal swabs from eight clinically healthy pigs were subjected for the recovery of *C. hyointestinalis*. Bacterial count of *C. hyointestinalis* ranged 10^3 to 10^4 CFU per gram of feces. The rate of isolation of *C. hyointestinalis* on Lawson's medium, medium A and medium B is 2/8, 7/8 and 8/8, respectively. Adoption of medium B was proved to be considerably effective for the detection of animal in carrier state harboring relatively low number of organism. The reason for remarkable decrease of coexisting bacteria in fecal specimen on these new media compared with that in mucosal specimen may lies in the difference of bacterial population between ileum, where lesions of PE present, and feces. The selective medium contains detection reagents for hydrogen sulfide was also reported by Lambert *et al.* [4]. They employed sodium metabisulfite and ferrous sulfate as detection reagents, but there was no description for the detection of hydrogen sulfide on that medium. The combined use of medium A and medium B make it easier to isolate *C. mucosalis* and *C. hyointestinalis* by characteristic appearance of colonies due to the detection of hydrogen sulfide production and by growth inhibition of coexisting bacteria.

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要　約

Campylobacter mucosalis と *Campylobacter hyointestinalis* の選択培地（短報）：大宅辰夫・久保正法・渡瀬弘（農林水産省家畜衛生試験場九州支場）——豚の増殖性腸炎（PE）の原因菌とされる *Campylobacter mucosalis* 及び *Campylobacter hyointestinalis* の選択分離培地として，両菌による硫化水素産生を指標とした新しい選択培地2種（培地A及び培地B）を作製した．培地Aには *C. mucosalis* と *C. hyointestinalis* が，培地Bには *C. hyointestinalis* のみが，いずれも中心部が黒色のコロニーを形成し発育した．これらA，B両培地の併用はPE病変部のみならず，混在菌の多い糞便材料からの *C. mucosalis* 及び *C. hyointestinalis* の選択分離に特に有効であった．