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Preparation and Characterization of Monoclonal Antibodies Suitable for Detection of Foodborne Pathogens by Biosensor

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Monoclonal antibodies (MAbs) for detection of *Escherichia coli* O157:H7 (O157:H7), *Salmonella* Enteritidis (SE) and *Listeria monocytogenes* (LM) using surface plasmon resonance (SPR) biosensor were prepared and characterized. Indirect enzyme–linked immunosorbent assay (ELISA) and SPR biosensor were used for screening of the hybridoma cells secreting MAbs specific to the pathogens. Based on the reactivity of MAbs against the target pathogens by SPR biosensor, MAbs were selected. For O157:H7, the clones 3–11B–3F–8 and 3–11B–3F–11, which culture supernatants reacted strongly with boiled O157:H7 and sonicated O157:H7 cells were obtained and their culture supernatants were used for purification of anti–O157:H7 MAb. For SE, the clone 1–11G–8, which generated high response to sonicated SE cells and the lowest response to the sonicated mixture cells was obtained and the culture supernatant was used for purification of anti–SE MAb for detection of sonicated SE cells. The clone of 3–5H–3F was found with high reactivity against boiled SE and very low against the other boiled samples and was selected for purification of anti–SE MAb for detection of boiled SE cells. For LM, the clone of 2F6–7 that reacted strongest with boiled LM 4b was obtained and the culture supernatant was used for purification of anti–LM 4b MAb. The clone of 13H9–2 was found to generate almost greatest response against sonicated LM 1/2a and low response to sonicated *L. innocua*. Moreover, this MAb reacted strongly with boiled LM 4b without cross–reactivity against the other bacteria. This clone was selected for purification of anti–LM MAb for the detection of sonicated LM 1/2a or LM 4b cells and boiled LM 4b cells. Although MAbs for LM showed cross–reactivity against *L. innocua*, the MAbs obtained after screening by the combined method showed a capacity to detect target pathogens by using SPR biosensor as well as ELISA. These MAbs are useful for detection of pathogens by biosensor and are expected to contribute to the development of rapid detection of the pathogens.

Key words: SPR biosensor, ELISA, foodborne pathogen, hybridoma, monoclonal antibody

INTRODUCTION

Surface plasmon resonance (SPR) biosensors, which allow for real–time and label–free detection, have been applied to the detection of foodborne pathogens by many researchers (Karoonuthaisiri *et al.*, 2014; Meeusen *et al.*, 2005; Subramanian *et al.*, 2006; Tawil *et al.*, 2012; Wei *et al.*, 2007). Previous studies showed that the lower detection limit for pathogen using a SPR biosensor was not only dependent on the sensitivity of the instrument, but also on the specificity and sensitivity of the antibody applied in the detection (Subramanian *et al.*, 2006; Taylor *et al.*, 2005). Polyclonal antibodies have been used for several decades as detection devices for determining foodborne pathogens (Hochel *et al.*, 2007; Velusamy *et al.*, 2010). Although polyclonal antibodies can be raised quickly and cost effectively, they have some disadvantages such as higher potential for cross reactivity due to the recognition of multiple epitopes and variability among different batches produced in different animals at different times (https://www.pacificimmunology.com/resources/antibody–introduction/polyclonal–vs–monoclonal–antibodies, Jan. 9, 2018). In contrast, monoclonal antibodies (MAbs) are more useful for specific detection of foodborne pathogens and other molecules than polyclonal antibodies (Velusamy *et al.*, 2010), as they are homogeneous, no variability among different batches, and recognize only one epitope on an antigen (http://www.abcam.com/protocols/a–comparison–between–polyclonal–and–monoclonal, Jan. 9, 2018).

In this study, hybridoma technology was applied to prepare novel MAbs with high sensitivity and specificity that could be used for detection of foodborne pathogens by using SPR biosensor, as well as other immunological detection methods, such as enzyme–linked immunosorbent assay (ELISA). As cross–reactivity is the most important factor in determining the value of an antibody (Tanaka *et al.*, 2003), the specificities of MAbs were tested with various target pathogen–related representative bacterial strains during the screening of MAbs in

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order to guarantee high specificities of the MAbs against target pathogens.

MATERIALS AND METHODS

Bacteria and cultivation

Listeria monocytogenes 1/2b LIS 16 No. 22 (LM 1/2b), *L. monocytogenes* 4b LIS 9 No. 20 (LM 4b) and the following *Escherichia coli* strains were obtained from the Fukuoka City Institute for Hygiene and the Environment, Fukuoka, Japan: *E. coli* O157:H7 No. 28 (O157:H7), *E. coli* O157:H7 No. 30 (O157:H7 No. 30), *E. coli* O157 No. 139 (O157 No. 139), *E. coli* O157 No. 144 (O157 No. 144), *E. coli* O157 No. 166 (O157 No. 166), *E. coli* O157:H7 No. 196 (O157:H7 No. 196), *E. coli* O128:H2 No. 20 (O128:H2), *E. coli* O26 No. 100 (O26), *E. coli* O91 No. 104 (O91), *E. coli* O111 No. 107 (O111), O serogroup untypable *E. coli* No. 110 (OUT No. 110), O serogroup untypable *E. coli* No. 116 (OUT No. 116). *L. monocytogenes* 1/2a SNU A21 No. 136 (LM 1/2a) was obtained from Seoul National University, Seoul, Korea. *Salmonella* Enteritidis IFO 3313 (SE), *S.* Typhimurium IFO 12529 (ST), *E. coli* IFO 3301 (EC) and *Enterobacter aerogenes* IFO 13534 (EA) were purchased from the Institute for Fermentation, Osaka, Japan. *Klebsiella pneumoniae* JCM 1662 (KP) and *Bacillus cereus* JCM 2152 (BC) were purchased from the Japan Collection of Microorganisms, RIKEN Bioresource Center, Tsukuba, Japan. *L. innocua* (field isolate), *L. ivanovii* (ATCC 19119), *L. seelingeri* (ATCC 35967), *L. welshimeri* (ATCC 35897) and *L. grayi* (ATCC 25401) were obtained from Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan.

Each of the bacterial strains of O157:H7 No. 30, O157 No. 139, O157 No. 144, O157 No. 166, O157:H7 No. 196, O128:H2, O26, O91, O111, OUT No. 110 and OUT No. 116 was cultured in 5 ml of Brain Heart Infusion broth (BHI; Oxoid Ltd., Basingstoke, Hampshire, England) at 37°C for 18 h with shaking at 130 rpm. Each of the other bacterial strains was cultured in 5 ml of Tryptic Soy Broth (TSB; Becton, Dickinson and Company, Sparks, MD, USA) at 37°C for 18 h with shaking at 130 rpm. Cultures were used for preparation of MAbs and samples for detection.

Preparation of bacterial cells for production of MAbs

To harvest each of the bacterial cells, each culture of O157:H7 No. 30, O157 No. 139, O157 No. 144, O157 No. 166, O157:H7 No. 196, SE, and LM 1/2a was centrifuged at 5,800 × g for 5 min. The cells were resuspended in equal amount of Phosphate Buffered Saline (PBS; 137 mM NaCl, 8.10 mM Na_2HPO_4, 2.68 mM KCl, 1.47 mM KH_2PO_4, pH 7.4) to wash cells. The cells were washed twice with PBS as described above. The cells washed triplicate with PBS were used for preparation of MAbs.

Preparation of MAbs

Anti–O157:H7 MAbs

The cells from 1 ml of the respective cultures of O157:H7 No. 30, O157 No. 139, O157 No. 144, O157 No. 166, and O157:H7 No. 196 were resuspended in 1 ml of PBS. The bacterial suspensions were boiled for 10 min and then mixed together. The mixture was used as an immunogen. Six–week old female BALB/c mice were immunized with the immunogen by intradermal injection for five times every two weeks. First immunization was performed with 50 µg Freund's complete adjuvant, other immunizations with 50 μg Freund's incomplete adjuvant. On the third day after the final immunization, spleen cells were isolated and fused with P3X63Ag8U1 (P3U1) myeloma cells. Hybridomas were cultured at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in RPMI 1640 medium supplemented with 15% heat–inactivated fetal bovine serum (FBS; Life Technologies Corporation, Carlsbad, CA, USA), 100 mg/l of kanamycin sulfate (Meiji Seika, Tokyo, Japan), and HAT supplement (Thermo Fisher Scientific Inc., Waltham, MA, USA). Culture supernatants of hybridomas were collected and used for evaluation for antibody production by ELISA and SPR biosensor.

Anti–SE MAbs

The cells from 10 ml of the SE culture were resuspended in 10 ml of PBS. The bacterial suspension was boiled for 10 min, and then diluted in PBS to attain cell concentration of approximately 10^7 CFU/ml. The heat–treated bacterial suspension was used as an immunogen. Six–week old female BALB/c mice were immunized via the peritoneal cavity with the heat–inactivated SE cells for five times every two weeks. On the third day after the final immunization, spleen cells were isolated and fused with P3U1 myeloma cells. Hybridization and culture of hybridomas were performed as the same with O157:H7. Culture supernatants were collected and used for evaluation for antibody production by ELISA and SPR biosensor.

Anti–LM MAbs

The cells harvested from 8 ml of the LM 1/2a culture were resuspended in 2 ml of PBS. The bacterial suspension was boiled for 10 min and used as an immunogen. Six–week old female BALB/c mice were immunized via the peritoneal cavity with heat–inactivated LM 1/2a cells in Freund's complete adjuvant twice, with an interval of one week. Hybridization and culture of hybridomas were performed as the same with O157:H7. Culture supernatants were collected and used as samples for screening of antibodies by ELISA and SPR biosensor.

Preparation of samples for screening of antibodies by SPR biosensor

After the cultivation, cells were harvested from 1 ml of each culture of O157:H7, EC, EA, KP, SE, ST, LM 1/2a, LM 1/2b, LM 4b, *L. innocua*, *L. ivanovii*, BC, *L. seelingeri*, *L. welshimeri*, *L. grayi* by centrifugation at 5,800 × g for 5 min. Cells were resuspended in 1 ml of HBS–EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% (v/v) surfactant P20), centrifuged at 5,800 × g for 5 min, and the cells were resuspended in 1 ml of HBS–EP buffer. The bacterial suspensions were used for following sample preparation.

Samples for screening of anti–O157:H7 MAbs

Each bacterial suspension of O157:H7 and EC was 100–fold diluted with HBS–EP buffer and 10 ml of the 100–fold dilutions with bacterial cells at approximately 10^7 CFU/ml were boiled for 10 min or disrupted thoroughly by sonication using a TOMY Ultrasonic Disruptor UD–201 (TOMY SEIKO CO., LTD, Tokyo, Japan) at 50 W. Each bacterial suspension of EA, KP, SE, LM 1/2a and BC were 5–fold diluted with HBS–EP buffer. One ml of each 5–fold dilution was mixed with 5 ml of HBS–EP buffer. The mixture containing bacterial cells at approximately 10^8 CFU/ml was boiled or sonicated as described above.

Samples for screening of anti–SE MAbs

Each bacterial suspension of SE and ST was 100–fold diluted with HBS–EP buffer and 10 ml of the 100–fold dilutions with bacterial cells at approximately 10^7 CFU/ml were boiled for 10 min or sonicated as above. One ml of each bacterial suspension of EC, EA, KP, and BC was mixed with 6 ml of HBS–EP buffer. The mixture with each bacterium at approximately 10^8 CFU/ml, designated mixture–1, was boiled for 10 min or sonicated. Each bacterial suspension of O157:H7, LM 1/2a, EC, EA, KP, and BC was 5–fold diluted with HBS–EP buffer. One ml of each 5–fold dilution was mixed with 4 ml of HBS–EP buffer. The mixture containing bacterial cells at approximately 10^8 CFU/ml, designated mixture–2, was boiled for 10 min or sonicated.

Samples for screening of anti–LM MAbs

Each bacterial suspension of LM 1/2a, LM 1/2b, LM 4b, *L. innocua*, *L. seelingeri*, *L. welshimeri*, *L. grayi* and *L. ivanovii* was 10–fold diluted with HBS–EP buffer and the 10–fold dilutions with bacterial cells at approximately 10^8 CFU/ml were boiled for 10 min or sonicated. Each bacterial suspension of O157:H7, EC, EA, KP, SE, and BC was 5–fold diluted with HBS–EP buffer. One ml of each 5–fold dilution was mixed with 4 ml of HBS–EP buffer. The mixture containing bacterial cells at approximately 10^8 CFU/ml was boiled for 10 min or sonicated.

All the samples prepared for screening of antibodies by SPR biosensor were stored at –20˚C until use.

Screening of MAbs using indirect ELISA

Activity of MAbs was examined using an indirect ELISA method. The wells of a Nunc Maxisorp™ plate were coated with 40 μl aliquots of respective target antigen (the immunogen used in immunization for raising anti–O157:H7 MAbs and anti–SE MAbs, 100–fold dilution of the immunogen in PBS for raising anti–LM MAbs) or where a mixed bacterial suspension of non–O157 pathogenic *E. coli* strains was used, *i.e.*, O128:H2, O26, O91, O111, OUT No. 110 and OUT No. 116 in PBS. The plates were incubated overnight at 4°C and then blocked with a 1% (w/v) solution of block ace (Dainippon Sumitomo Pharma Co., Ltd., Tokyo, Japan) in PBS. A 50 μl sample of culture supernatant of hybridoma was added to the wells of the plate. Plates were incubated at 37°C for 1.5 h on a rocking platform. The wells were washed three times with PBS, and 50 μl of secondary antibody [horseradish peroxidase–labeled goat anti–mouse immunoglobulin (IgG, IgM, and IgA) antibody for anti–O157:H7 MAbs and anti–SE MAbs screening; alkaline phosphatase–labeled goat anti–mouse immunoglobulin (IgG, IgM, and IgA) antibody for anti–LM MAbs screening, Merck, Darmstadt, Germany] diluted in PBS was added. After a 1.5 h–incubation at 37°C, the wells were washed five times with PBS, and 50 μl of substrate was added to each well. After incubation in the dark at 37°C for 1 h, absorbance was determined at the most appropriate wavelength, 490 nm for screening anti–O157:H7 MAbs & anti–SE MAbs and 510 nm for anti–LM MAbs, using a microplate reader (Model 680; Bio–Rad, Tokyo, Japan).

SPR instrumentation

Analysis was carried out on a Biacore X instrument (GE Healthcare Bio–Sciences AB, Uppsala, Sweden) using sensor chip CM3 (GE Healthcare Bio–Sciences AB). Biacore X was equipped with two flow channels: flow channel 1 for reference and flow channel 2 for detection.

Immobilization of anti–mouse IgG antibody on sensor chip

Anti–mouse IgG antibody (GE Healthcare Bio–Sciences AB), which binds to all IgG subclasses, IgA, and IgM antibodies from mouse, was used as a capture antibody for the MAbs. The amine–coupling method was used for antibody immobilization (Löfås and Johnsson, 1990). Anti–mouse IgG antibody was immobilized on flow channel 2 by injecting the antibody at 50 μg/ml in 10 mM acetate buffer (pH 5.0) at a flow rate of 15 μl/min for 7 min. Flow channel 1 was left blank to serve as a reference channel. After antibody immobilization, the surfaces of both flow channels were blocked by injecting 1.0 M ethanolamine–HCl (pH 8.5) for 7 min to minimize non–specific adsorption (Rich and Myszka, 2001).

Screening of MAbs using SPR biosensor

The sensor chip CM3 with anti–mouse IgG antibody was docked into the Biacore X instrument. HBS–EP buffer was used as a running buffer and run at a flow rate of 15 μl/min and 25˚C. Culture supernatant of hybridoma was diluted appropriately with HBS–EP buffer and then injected for 6 min at a flow rate of 15 μl/min. MAb in the culture supernatant was captured by anti–mouse IgG antibody immobilized on sensor chip CM3 as the culture supernatant passed over the sensor chip surface. After immobilization of MAb, HBS–EP buffer was injected for 5 min to establish a baseline. Then the corresponding bacterial samples were injected one by one for 5 min for antigen–antibody binding. Signal change for each sample was obtained by subtracting the signal recorded at 30 s before the start of the sample injection from the signal recorded at 60 s after the end of the sample injection. After measurement of all the samples, regeneration solution (10 mM glycine–HCl, pH 1.7) was injected for 3 min to dissociate antigen and MAb from anti–mouse IgG antibody. After regeneration, sensor chip surfaces were equilibrated with running buffer (HBS–EP buffer). This sensor chip immobilized with anti–mouse IgG antibody was then used to evaluate another culture supernatant of hybridoma.

RESULTS

Screening and characterization of anti–O157:H7 MAbs

A total of 621 hybridoma cell lines were obtained after the cell fusion. The cell lines producing MAbs specific to O157:H7 were screened by ELISA. The reaction between the culture supernatants of the hybridomas and target antigen (O157 antigen), i.e., the mixture of bacterial suspension of boiled O157:H7 No. 30, O157 No. 139, O157 No. 144, O157 No. 166, and O157:H7 No. 196 in PBS that was used as the immunogen, was tested. After this preliminary screening, 43 cell lines were found to secrete MAbs specific to O157:H7 (Table 1). Then these selected cell lines were screened by using SPR biosensor. Cells of EC, O157:H7, and the mixture including EA, KP, SE, LM 1/2a and BC, were boiled or sonicated before applying to SPR biosensor. As shown in Table 2, the culture supernatants form 4 cell lines, 1–1A, 3–11B, 6–4G, and 6–7H, reacted strongly with boiled O157:H7, without cross–reactivity against boiled EC or boiled mixture sample. Culture supernatants from other cell lines showed relative high response to boiled O157:H7 (2–10D and 5–12A) or sonicated O157:H7 (4–8A, 5–12F, 6–3H, and 7–4A) although they showed cross–reactivity against EC or the mixture samples. These 10 cell lines were selected for subsequent cloning. Twenty–nine individual clones were obtained after cloning and re–screened by ELISA. The reactivity of the culture supernatants of the hybridomas with target antigen (O157 antigen) as well as non–target antigen (non–O157 antigen, *i.e.*, the mixture of bacterial suspension of boiled O128:H2, O26, O91, O111, OUT No. 110 and OUT No. 116 in PBS) was investigated. Reactivity of the culture supernatants from 7 clones, 3–11B–3F–2, 3–11B–3F–3, 3–11B–3F–7, 3–11B–3F–8, 3–11B–3F–9, 3–11B–3F–11, and 6–7H–1, was high enough against O157 antigen, but very low against the

Table 1. Reactivity of culture supernatants of hybridoma cell lines raised against O157 determined by ELISA

Cell line	Reactivity (A_{490})	Cell line	Reactivity (A_{490})
1–1A	2.956	5–2D	0.261
2–3B	0.214	5–8D	2.714
2–6A	0.331	5–10D	0.286
2–10A	2.978	5–12A	2.845
2–10D	1.833	5–12F	0.337
3–1D	0.225	6–1A	0.326
3–3B	0.220	6–1B	0.276
3–3D	2.869	6–1H	2.376
3–3F	1.077	6–3H	2.514
3–11B	2.949	6–4G	2.891
3–12A	2.611	6–5D	2.910
4–5D	0.503	6–7H	2.948
4–6A	2.923	6–10G	0.434
4–7D	0.256	6–12B	0.394
4–8A	0.497	7–1D	0.274
4–9D	2.998	7–1G	0.255
4–11D	2.406	7–2A	2.860
4–11F	0.382	7–3B	0.273
4–12A	1.119	7–4A	2.891
4–12B	1.325	7–5A	0.935
4–12D	0.375	NC*	0.069
4–12H	0.967	PC**	2.947
5–1D	0.271		

*NC: the blank control with the coating buffer only.
**PC: the positive control with 1000–fold diluted antiserum of immunized mouse.

Table 2. Reactivity of culture supernatants of hybridoma cell lines raised against O157 against various bacteria by SPR biosensor

Cell line	Signal change (RU)							
	Immobilization of antibody	HBS –EP	Boiled samples			Sonicated samples		
			EC	O157:H7	Mixture*	EC	O157:H7	Mixture*
1–1A	631.6	–11.0	–6.7	9.5	–6.5	–3.3	12.4	22.0
2–3B	511.1	–8.6	–5.5	–2.6	0.4	–2.0	–1.8	12.9
2–6A	439.6	–7.0	–5.1	–2.1	–2.3	–1.3	0.0	12.8
2–10A	267.1	–3.4	–3.2	–1.7	1.7	–1.6	–0.5	12.6
2–10D	180.3	–5.0	16.5	11.8	18.2	20.4	1.3	61.1
3–1D	620.0	–6.0	–3.8	–1.6	–0.5	–0.2	1.8	13.4
3–3B	182.4	–2.3	–2.5	–1.0	–0.2	1.7	1.3	12.1
3–3D	371.8	–7.0	–4.6	–1.7	–1.1	–2.0	–0.9	9.6
3–3F	244.2	–6.7	–6.6	–2.1	–2.9	–3.9	0.2	15.8
3–11B	444.1	–2.3	–2.3	39.0	–0.9	0.9	102.5	13.5
3–12A	462.8	–2.1	–2.3	–0.1	1.3	0.8	3.5	16.5
4–5D	151.7	–7.4	–6.2	–3.3	–3.3	–1.4	–0.9	10.6
4–6A	721.4	–10.8	–2.3	–2.2	9.0	8.3	7.0	70.0

Table 2. (Continued).

Cell line	Signal change (RU)							
	Immobilization of antibody	HBS –EP	Boiled samples			Sonicated samples		
			EC	O157:H7	Mixture*	EC	O157:H7	Mixture*
4–7D	406.1	–5.7	–5.4	–3.4	–1.7	–0.1	1.2	22.4
4–8A	511.0	–3.1	–3.5	–2.0	–1.1	15.0	26.3	21.0
4–9D	631.4	–5.7	11.1	0.7	35.5	45.7	4.3	29.9
4–11D	413.3	–13.1	–3.5	0.4	–1.4	5.1	7.5	38.1
4–11F	155.2	–4.1	–2.1	–0.6	1.1	2.3	4.3	34.7
4–12A	198.7	–5.2	–4.8	–0.3	–0.2	2.5	4.9	33.3
4–12B	495.7	–9.8	–7.4	–2.6	–3.0	0.6	3.4	29.4
4–12D	294.4	–5.7	–5.2	0.6	–1.0	2.9	3.1	36.4
4–12H	321.7	–7.8	–7.0	–1.0	–2.9	1.6	1.7	33.5
5–1D	177.2	–5.6	–5.4	–0.8	–2.5	1.9	2.8	31.5
5–2D	160.0	–5.1	–3.3	–1.5	–2.5	0.3	2.0	30.0
5–8D	791.1	–13.1	–9.4	–6.3	–6.3	2.1	5.0	37.0
5–10D	145.3	–3.6	–2.9	–1.4	0.1	1.6	4.3	36.8
5–12A	701.2	–8.0	8.3	7.8	10.2	22.2	3.6	66.0
5–12F	335.7	–10.8	–8.3	–4.2	–4.9	14.2	22.2	60.8
6–1A	224.9	–6.9	–6.6	–3.5	0.5	0.5	7.6	49.4
6–1B	194.8	–9.8	–7.9	–3.6	–2.9	1.7	5.4	44.4
6–1H	299.8	–7.2	–5.6	–2.7	–1.9	0.8	4.9	38.1
6–3H	303.0	–9.2	2.0	–0.9	8.6	16.8	10.6	83.4
6–4G	99.1	–3.1	–3.7	6.5	–0.3	4.6	20.4	53.7
6–5D	276.7	–4.4	–4.7	–2.7	–0.6	3.5	7.6	57.2
6–7H	194.0	–3.3	–3.3	20.0	–1.1	3.0	53.0	43.2
6–10G	138.4	–3.7	–1.7	0.6	3.0	4.1	6.5	56.8
6–12B	94.1	–3.4	–3.6	–2.1	–1.5	3.6	5.6	55.4
7–1D	399.3	–5.7	–4.6	–2.2	–1.7	2.1	4.6	48.3
7–1G	187.0	–7.0	–3.9	–2.4	–0.5	2.5	6.3	46.9
7–2A	294.3	–11.1	–7.1	–2.9	–3.8	3.2	7.2	65.4
7–3B	96.1	–3.9	–3.7	–1.1	0.0	3.0	6.8	51.0
7–4A	353.3	–7.2	–5.3	–0.6	2.7	5.0	10.3	54.2
7–5A	202.6	–6.9	–4.0	–1.6	–0.9	3.2	7.8	40.4

*Mixture: the mixture of bacterial suspension of EA, KP, SE, LM 1/2a and BC in HBS–EP.
Cell concentrations of O157:H7 and EC were approximately 10^7 CFU/ml, mixture was approximately 10^8 CFU/ml.

non–O157 antigen (Table 3). These culture supernatants were re–screened by using SPR biosensor. The culture supernatants from clones 3–11B–3F–8 and 3–11B–3F–11 strongly reacted with boiled O157:H7 and sonicated O157:H7 samples, without cross–reaction against the other samples (Table 4). These 2 clones were selected for purification of anti–O157:H7 MAb.

Screening and characterization of anti–SE MAbs

A total of 432 hybridoma cell lines were obtained after the cell fusion. The reaction between the culture supernatants of the hybridomas and the target antigen of boiled SE was tested. After the preliminary screening, 28 cell lines were found to secrete MAbs specific to boiled SE (Table 5). Then these selected cell lines were screened by using SPR biosensor. Cells of SE, ST, and mixture–1 including EC, EA, KP, and BC, were boiled or sonicated before applying to SPR biosensor. As shown in Table 6, culture supernatants from 10 cell lines (1–4A, 1–4B, 1–11G, 2–7H, 2–8H, 3–5H, 3–7A, 3–8F, 3–10F, and 5–6C) strongly reacted with boiled SE or sonicated SE with low reactivity to the other samples (Table 6). They were selected for subsequent cloning. Twenty–four individual clones were obtained after the cloning and re–screened by using SPR biosensor. Table 7 shows the reactivity of the culture supernatants from the selected clones against SE and the other bacterial samples. Several culture supernatants from the selected clones reacted strongly with boiled SE. In particular, clone of 3–5H–3F was found to produce MAb with high reactivity

Table 3. Reactivity of the selected culture supernatants of hybridomas raised against O157 against O157 and non–O157 by ELISA

Clone	Reactivity (A_{490})		Clone	Reactivity (A_{490})	
	O157#	Non–O157##		O157#	Non–O157##
3–11B–3F–2	2.889	0.114	5–12A–4	2.929	2.932
3–11B–3F–3	2.981	0.100	5–12A–5	2.999	3.026
3–11B–3F–7	2.865	0.111	5–12F–1	0.892	1.009
3–11B–3F–8	2.992	0.108	5–12F–4	0.865	0.969
3–11B–3F–9	2.881	0.111	5–12F–5	0.812	0.913
3–11B–3F–11	2.927	0.132	5–12F–9	0.735	0.826
4–8A–1	0.899	1.105	5–12F–11	0.683	0.755
4–8A–2	0.696	1.000	5–12F–12	0.761	0.810
4–8A–3	0.999	1.201	5–12F–14	0.977	1.107
4–8A–4	0.988	1.182	6–3H–1	0.778	1.236
4–8A–5	1.016	1.151	6–3H–2	0.749	1.190
4–8A–6	1.021	1.207	6–3H–3	1.381	1.296
4–8A–7	0.967	1.094	6–7H–1	2.911	0.099
5–12A–1	2.911	2.930	NC*	0.111	0.100
5–12A–2	2.909	2.924	PC**	2.922	2.918
5–12A–3	2.899	2.945			

O157# : the mixture of bacterial suspension of boiled O157:H7 No. 30, O157 No. 139, O157 No. 144, O157 No. 166, and O157:H7 No. 196 in PBS that was used as immunogen in the immunization.
Non–O157## : the mixture of bacterial suspension of boiled O128:H2, O26, O91, O111, OUT No. 110 and OUT No. 116 in PBS.
*NC: the blank control with the coating buffer only.
**PC: the positive control with 1000–fold diluted antiserum of immunized mouse.

Table 4. Reactivity of the selected culture supernatants of hybridomas raised against O157 against various bacteria by SPR biosensor

Clone	Signal change (RU)							
	Immobilization of antibody	HBS –EP	Boiled samples			Sonicated samples		
			EC	O157:H7	Mixture*	EC	O157:H7	Mixture*
3–11B–3F–2	569.3	–9.4	–4.3	64.7	–5.8	–2.8	77.8	4.6
3–11B–3F–3	509.5	–13.1	–14.1	49.4	–6.2	–4.9	69.8	4.8
3–11B–3F–7	341.3	–1.6	–1.8	44.1	–2.0	–3.1	50.2	–0.9
3–11B–3F–8	770.9	–1.6	–1.3	83.7	–5.6	–2.5	111.0	–1.2
3–11B–3F–9	459.4	–23.1	–14.7	53.0	–8.9	–8.3	71.0	0.5
3–11B–3F–11	519.3	–4.5	–0.4	72.6	–6.1	–1.9	84.1	–1.8
6–7H–1	361.8	–5.1	–3.3	41.2	–2.6	–1.3	48.7	13.6

*Mixture: the mixture of bacterial suspension of EA, KP, SE, LM 1/2a and BC in HBS–EP.
Cell concentrations of O157:H7 and EC were approximately 10^7 CFU/ml, mixture was approximately 10^8 CFU/ml.

against boiled SE and very low against the other boiled samples. This clone was selected for detection of boiled SE and MAb was purified from the culture supernatant. All tested culture supernatants from the clones reacted positively with both sonicated SE and sonicated mixture–2 including O157:H7, LM 1/2a, EC, EA, KP, and BC. Since the reactivity of the culture supernatants from clone 1–11G–8 to the sonicated mixture–2 sample was the lowest among them, MAb to detect sonicated SE was purified from the culture supernatant of the clone.

Screening and characterization of anti–LM MAbs

A total of 120 hybridoma cell lines were obtained after the cell fusion. The cell lines producing MAbs specific to boiled LM 1/2a were screened. After this preliminary screening, 24 cell lines were found to secrete MAbs specific to boiled LM 1/2a (Table 8). These selected cell lines were screened by using SPR biosensor. Cells of LM 1/2a, LM 1/2b, LM 4b, *L. innocua*, and mixture including O157:H7, EC, EA, KP, SE, and BC, were boiled or sonicated before applying to SPR biosen-

Table 5. Reactivity of culture supernatants of hybridoma cell lines raised against SE determined by ELISA

Cell line	Reactivity (A_{490})	Cell line	Reactivity (A_{490})
1–4A	2.977	3–8F	3.000
1–4B	2.959	3–10F	2.983
1–5F	2.934	4–3G	2.925
1–7D	3.000	4–4B	3.000
1–8A	2.968	4–5A	2.969
1–8B	2.974	4–6B	3.000
1–11G	2.937	4–8A	2.907
2–7H	3.000	4–9D	3.000
2–8H	2.999	4–10H	2.975
3–2E	2.910	4–11C	2.972
3–2H	2.953	5–2D	2.963
3–5E	2.936	5–6C	2.956
3–5H	2.962	5–6D	3.000
3–7A	2.989	NC*	0.166
3–7B	3.000	PC**	3.000

*NC: the blank control with the coating buffer only.
**PC: the positive control with 1000–fold diluted antiserum of immunized mouse.

sor. As shown in Table 9, almost all culture supernatants from the selected cell lines showed no reactivity against boiled LM 1/2a, the target antigen, while the culture supernatants from 4 cell lines (1F10, 2A6, 2F6, and 13H9) showed high reactivity only against boiled LM 4b. Against sonicated LM 1/2a, the culture supernatants from 5 cell lines (1F2, 1F10, 2A7, 2F6, and 13H9), especially 2F6 and 13H9, showed high reactivity.

Although all the culture supernatants of 5 cell lines showed cross–reactivity against sonicated non–pathogenic *Listeria strains*, i.e., *L. seelingeri*, *L. welshimeri*, *L. grayi* and *L. ivanovii*, the culture supernatants of 2 cell lines (2F6 and 13H9) showed lower cross–reactivity against the sonicated mixture sample compared with those of the other 3 cell lines (Table 10). Moreover, the culture supernatants of the 2 cell lines strongly reacted with boiled LM 4b (Table 9). Based on these results, cell lines of 2F6 and 13H9 were selected for further cloning. Table 11 shows the reactivity of the culture supernatants of the 31 clones determined by SPR biosensor. Clone of 2F6–7 was found to produce MAb with high reactivity against boiled LM 4b and thus was selected for detection of boiled LM 4b and MAb was purified from the culture supernatant. The culture supernatants of the other clones derived from the cell line 2F6 had almost no reactivity against boiled or sonicated LM, thus cross–reactiv-

Table 6. Reactivity of culture supernatants of hybridoma cell lines raised against SE against various bacteria by SPR biosensor

Cell line	Signal change (RU)							
	Immobilization of antibody	HBS–EP	Boiled samples			Sonicated samples		
			SE	ST	Mixture–1*	SE	ST	Mixture–1*
1–4A	177.8	–5.3	20.1	–0.7	92.7	23.2	–0.9	58.7
1–4B	179.5	–6.1	19.6	–1.0	87.7	22.0	–1.2	58.0
1–5F	133.0	–1.9	10.6	1.2	46.0	14.8	–0.2	30.2
1–7D	108.6	–1.9	6.5	0.0	4.9	2.5	–0.7	23.2
1–8A	108.9	0.1	0.2	–0.4	–0.1	1.1	–0.5	2.5
1–8B	121.3	–0.8	0.9	0.1	–0.6	0.6	–0.8	3.5
1–11G	125.0	–1.4	0.7	–1.6	–0.3	13.1	0.0	3.6
2–7H	137.9	–1.3	–12.6	–0.9	4.3	28.1	–0.9	30.0
2–8H	57.5	–0.1	2.6	0.7	2.2	6.3	–0.6	5.5
3–2E	126.6	–1.0	–0.1	–0.4	–0.9	0.5	–0.5	1.6
3–2H	115.3	–0.8	1.1	0.3	–1.7	1.2	0.3	8.2
3–5E	184.6	–1.2	0.5	1.2	–0.5	1.2	–0.1	2.8
3–5H	238.3	–4.7	37.2	0.7	15.7	4.3	–0.8	80.3
3–7A	222.9	–3.1	25.5	1.0	11.5	7.4	0.1	95.5
3–7B	162.4	–2.4	–0.9	–0.1	–2.7	–0.3	–1.8	0.2
3–8F	104.8	–2.4	2.2	–1.2	–0.7	9.3	–0.7	–0.9
3–10F	105.7	–3.3	2.2	–0.1	–1.0	8.9	–1.0	2.6
4–3G	147.7	–2.9	–0.5	–0.8	–2.6	0.1	–0.4	1.6
4–4B	139.6	–2.1	0.1	–0.2	1.9	–1.0	–0.6	35.1
4–5A	110.7	–1.8	–0.5	–1.3	–0.6	–0.3	–1.2	1.1
4–6B	53.0	–0.7	–1.0	0.7	–0.2	–0.7	–2.1	–4.5
4–8A	128.3	–2.3	–0.4	0.1	0.8	–0.1	–2.5	–1.1
4–9D	230.6	–3.1	–0.2	–0.3	2.0	–0.1	–0.8	30.5

Table 6. (Continued).

Cell line	Signal change (RU)							
	Immobilization of antibody	HBS–EP	Boiled samples			Sonicated samples		
			SE	ST	Mixture–1*	SE	ST	Mixture–1*
4–10H	192.4	–0.9	0.3	–0.2	–0.3	1.0	–0.5	4.0
4–11C	196.0	–4.4	–0.5	–1.1	–1.0	–0.5	–2.0	2.4
5–2D	133.9	–5.0	–1.0	–2.0	–1.2	–1.1	–2.2	–1.9
5–6C	108.2	–2.5	0.5	–1.1	–1.0	10.3	–1.6	–2.8
5–6D	116.7	–1.9	–0.3	–1.2	–0.6	0.1	–1.1	–3.0

*Mixture–1: the mixture of bacterial suspension of EC, EA, KP, and BC in HBS–EP.
Cell concentrations of SE and ST were approximately 10^7 CFU/ml, mixture–1 was above 10^8 CFU/ml.

Table 7. Reactivity of the selected culture supernatants of hybridomas raised against SE against various bacteria by SPR biosensor

Clone	Signal change (RU)							
	Immobilization of antibody	HBS –EP	Boiled samples			Sonicated samples		
			SE	ST	Mixture–2*	SE	ST	Mixture–2*
1–4A–3A	527.7	–13.4	34.0	5.1	4.6	68.3	6.2	90.6
1–4A–8G	597.5	–10.9	53.0	26.3	24.5	95.2	22.5	173.4
1–4A–9D	690.0	–12.7	49.6	25.9	16.2	78.0	11.1	122.2
1–4B–2F	779.8	–17.7	42.5	25.2	10.8	72.2	7.7	97.0
1–4B–4C	932.4	–19.9	48.1	24.9	11.6	73.1	7.3	99.0
1–4B–4F	895.9	–22.4	35.4	15.9	3.6	60.6	0	71.3
1–11G–2	255.7	–11.7	0.4	–0.7	–5.2	42.7	7.6	36.1
1–11G–7	297.7	–7.0	1.0	0.5	–4.2	37.9	5.5	34.1
1–11G–8	379.4	–7.4	2.5	0.6	–3.6	44.2	5.4	32.2
2–8H–9B	651.3	–10.3	51.0	15.6	14.6	81.3	15.0	96.5
3–5H–1B	1172.3	–19.0	73.9	15.3	9.6	31.5	39.6	120.3
3–5H–3F	1238.2	–22.5	68.8	5.9	0.5	27.5	29.3	101.0
3–5H–5H	1050.5	–16.9	55.3	5.2	2.5	35.9	31.8	117.7
3–7A–7H	242.5	–14.7	–6.7	–8.6	–7.3	19.7	6.7	46.9
3–7A–8B	1178.7	–24.0	58.6	–0.3	0.4	28.3	25.5	148.1
3–7A–11E	110.9	–10.9	–2.3	–2.7	–3.9	20.8	5.4	47.5
3–8F–5B	449.6	–11.8	2.2	–7.1	–4.0	45.7	4.2	41.9
3–8F–6B	451.6	–10.8	1.8	–4.9	–3.9	47.4	4.9	39.2
3–8F–12C	371.8	–13.6	0.7	–6.9	–7.3	45.6	6.9	46.7
3–10F–2D	304.0	–6.8	2.0	–3.2	–4.4	37.5	9.0	38.5
3–10F–2F	445.8	–9.3	4.8	–5.4	–4.2	48.5	6.0	42.4
3–10F–3E	451.4	–7.7	3.9	–3.6	–2.2	51.7	6.2	40.7
5–6C–8E	398.7	–12.9	–1.1	–6.1	–3.9	33.5	3.6	39.5
5–6C–9E	455.8	–17.1	–1.4	–7.8	–4.6	34.4	1.9	37.7

*Mixture–2: the mixture of bacterial suspension of O157:H7, LM 1/2a, EC, EA, KP, and BC in HBS–EP.
Cell concentrations of SE and ST were approximately 10^7 CFU/ml, mixture–2 was approximately 10^8 CFU/ml.

ity of these clones against *L. innocua* and the mixture samples were not performed. The culture supernatants of the clones derived from the cell line 13H9 reacted strongly with sonicated LM 1/2a. Although the culture supernatants of these clones also reacted with sonicated *L. innocua*, the cross–reactivity of these clones against the sonicated mixture sample was very low. Among them, the culture supernatant of clone 13H9–2 showed the strongest response against sonicated LM 1/2a, and relative low reactivity to sonicated *L. innocua* and the mixture samples. Moreover, the clone–13H9–2–culture supernatant reacted strongly with boiled LM 4b without cross–reactivity against the other boiled samples. Hence, this clone was also selected for purification of anti–LM MAb.

Table 8. Reactivity of culture supernatants of hybridoma cell lines raised against LM 1/2a against LM 1/2a determined by ELISA

Cell line	Reactivity (A_{510})	Cell line	Reactivity (A_{510})
1C11	0.938	4H5	0.628
1F2	0.421	6F11	0.153
1F10	0.448	9B1	0.361
2A6	0.648	10C6	0.155
2A7	0.678	11D1	0.239
2A8	0.185	11F2	0.297
2A11	0.508	13H9	0.175
2F6	0.646	16A6	0.453
2G2	0.302	17E9	0.194
2G9	0.247	20B12	0.139
2G10	0.168	21E5	0.178
2G11	0.102	NC*	0.021
3F10	2.442	PC**	0.193

*NC: the blank control with the coating buffer only.
**PC: the positive control with 300–fold diluted antiserum of immunized mouse.

DISCUSSION

Many attempts have been made to produce MAbs against O157:H7 (Jin *et al.*, 2012; Li *et al.*, 2010; Ryu *et al.*, 2010; Yu *et al.*, 2007; Zhao and Liu, 2005), SE (Brooks *et al.*, 2012; Iankov *et al.*, 2001; Iankov *et al.*, 2004), and LM (Lin *et al.*, 2006; Lin *et al.*, 2009; Shim *et al.*, 2007). In those studies, the selection of a specific antibody was conducted via ELISA, in which the cells were attached to plates followed by the binding of candidate antibodies and a tagged secondary antibody. Antibody selected via ELISA method, however, may not prove adequate for direct binding to cells, due to its large size (Joung *et al.*, 2007). Joung *et al.* (2007) screened specific antibodies via direct binding to cells using an SPR biosensor and found that the screened antibody evidenced a significantly higher degree of sensitivity for direct detection of LM than the antibody selected via ELISA method.

In this study, MAbs against O157:H7, SE, and LM were screened by SPR biosensor in addition to indirect ELISA. MAbs obtained after screening by combined method showed a capacity to detect the target pathogens specifically by using SPR biosensor as well as ELISA method, while MAbs for LM showed cross–reactivity

Table 9. Reactivity of culture supernatants of hybridoma cell lines raised against LM 1/2a against various bacteria determined by SPR biosensor

Cell line	Signal change (RU)											
	Immobilization of antibody	HBS –EP	Boiled samples					Sonicated samples				
			LM 1/2a	*L. innocua*	LM 4b	LM 1/2b	Mixture*	LM 1/2a	*L. innocua*	LM 4b	LM 1/2b	Mixture*
1C11	852.4	–8.8	–4.8	–12.6	–7.2	–5.5	–1.9	8.8	–8.0	–4.8	–2.4	63.6
1F2	727.6	–8.9	–2.6	–3.8	10.9	–2.9	–3.3	218.1	136.6	188.0	172.1	41.1
1F10	458.3	–5.9	–1.5	–1.6	20.4	–2.9	–0.9	163.2	109.3	140.8	146.9	43.3
2A6	684.9	–7.4	–2.9	–3.9	20.1	–3.9	–3.8	94.4	45.1	28.2	25.9	13.8
2A7	677.7	–7.6	–1.1	–3.0	37.8	–4.5	9.0	151.9	95.5	160.0	159.5	54.0
2A8	73.9	–4.7	–3.2	–2.0	–1.9	–3.6	–4.1	3.0	3.6	2.6	3.0	16.3
2A11	281.2	–5.2	–2.6	–1.4	2.3	–2.3	–2.6	48.7	28.7	55.5	50.8	53.9
2F6	816.7	–8.7	–1.9	–3.5	20.6	–4.3	–0.1	221.4	140.6	239.0	224.2	32.9
2G2	617.4	–6.8	–2.2	–2.3	2.2	–3.6	–1.4	29.8	4.1	16.3	12.7	31.2
2G9	418.8	–9.6	–5.8	–3.1	–4.9	–5.0	–2.2	8.3	3.2	3.1	2.6	29.5
2G10	557.6	–8.7	–2.9	–2.6	5.5	–3.5	–5.7	86.6	60.6	87.5	88.6	24.2
2G11	167.5	–9.5	–6.4	–5.7	–5.6	–4.0	–4.7	–0.8	–2.1	1.5	0.6	21.5
3F10	135.8	–5.0	–3.3	–3.3	–2.9	–2.6	–2.7	3.2	0.9	1.5	1.1	22.5
4H5	508.6	–6.4	0.0	–0.5	8.5	–3.2	0.5	421.5	545.4	293.7	68.5	70.8
6F11	315.8	–8.4	–2.1	–3.8	12.8	–3.0	–3.3	3.8	2.2	5.2	4.6	24.8
9B1	945.7	–13.0	–5.7	–4.6	2.1	–6.2	–6.1	63.8	428.8	457.1	–4.4	21.9
10C6	341.2	–5.9	–1.0	–1.2	–3.7	–1.9	1.2	17.8	14.9	13.4	12.8	59.8
11D1	744.5	–8.2	1.1	–1.8	28.5	–3.3	18.5	56.2	14.8	32.5	34.3	64.5
11F2	596.8	–8.2	–2.9	–1.4	–3.5	–2.7	–3.0	13.2	6.9	5.6	6.0	28.6
13H9	738.1	–5.1	–3.4	–3.1	19.6	–3.0	–1.3	228.9	142.8	248.5	225.9	32.7
16A6	736.0	–6.7	–6.4	–5.8	–4.9	–4.3	–2.2	0.3	–0.9	–0.9	–1.8	3.8
17E9	471.9	–5.7	–0.3	–1.9	30.5	–5.0	27.3	42.1	44.9	36.9	36.4	53.0
20B12	360.6	–5.1	–1.4	–1.3	8.3	–3.7	–3.2	77.8	64.8	82.7	80.3	40.3
21E5	146.3	–9.0	–3.4	–2.3	–4.2	–3.7	–4.4	3.6	2.0	–1.8	1.9	22.1

*Mixture: the mixture of bacterial suspension of O157:H7, EC, EA, KP, SE, and BC in HBS–EP.
Cell concentrations of LM 1/2a, LM 1/2b, LM 4b, *L. innocua* and mixture were approximately 10^8 CFU/ml.

Table 10. Reactivity of the selected culture supernatants of hybridoma cell lines raised against LM 1/2a against non–pathogenic *Listeria* spp. by SPR biosensor

Cell line	Signal change (RU)						
	Immobilization of antibody	HBS–EP	*L. seelingeri*	*L. welshimeri*	*L. grayi*	*L. ivanovii*	Mixture*
1F2	792.2	–5.0	79.3	121.5	114.4	137.0	41.1
1F10	510.3	–2.5	85.5	133.6	138.8	138.8	43.3
2A7	719.8	–6.5	57.0	125.9	122.6	149.6	54.0
2F6	1013.7	–4.0	75.0	165.2	157.0	184.0	32.9
13H9	765.7	–4.1	87.6	172.0	171.9	192.5	32.7

*Mixture: the mixture of bacterial suspension of O157:H7, EC, EA, KP, SE, and BC in HBS–EP.
Cell concentrations of *L. seelingeri*, *L. welshimeri, L. grayi, L. ivanovii* and mixture were approximately 10^8 CFU/ml.

Table 11. Reactivity of the selected culture supernatants of hybridomas raised against LM 1/2a against various bacteria by SPR biosensor

Clone	Signal change (RU)									
	Immobilization of antibody	HBS–EP	Boiled samples				Sonicated samples			
			LM 1/2a	*L. innocua*	LM 4b	Mixture*	LM 1/2a	*L. innocua*	LM 4b	Mixture*
2F6–2	766.6	–2.7	–1.9		–1.4		0.1		–0.5	
2F6–3	505.7	0.0	0.0		2.0		5.6		2.8	
2F6–4	594.9	–0.2	–0.5	–	6.7	–	4.8	–	1.7	–
2F6–5	483.5	–2.1	–1.1		–1.1		1.0		0.4	
2F6–6	555.2	–1.3	–0.8		4.3		5.7		2.9	
2F6–7	701.3	–3.3	0.8	5.5	16.9	4.7	8.9	–2.5	–0.5	9.6
2F6–8	295.0	–4.6	0.9		–3.0		2.2		1.0	
2F6–9	458.6	–0.7	0.3		–0.1		6.8		3.0	
2F6–10	449.0	–0.7	0.9		0.2		8.3		3.6	
2F6–11	306.4	–5.7	–1.9		–3.1		2.1		1.6	
2F6–12	342.7	–1.6	–0.5		–0.2		5.4		2.3	
2F6–13	437.5	–3.6	–1.5	–	–1.5	–	1.3	–	2.8	–
2F6–14	384.7	–5.8	–1.4		–1.5		3.5		2.2	
2F6–15	121.6	–0.4	0.0		0.7		6.1		5.4	
2F6–16	315.7	–7.9	–2.6		–2.5		4.1		0.3	
2F6–18	546.1	–2.5	–0.5		1.7		9.0		3.8	
13H9–1	812.1	–0.1	0.8	–1.3	10.8	–4.8	242.1	196.5	49.3	2.3
13H9–2	718.6	–4.2	0.1	0.1	11.2	–3.3	224.9	172.7	46.6	2.7
13H9–3	525.4	–4.9	–1.6	1.5	5.7	–2.0	175.4	138.0	31.1	3.2
13H9–4	650.4	–4.5	–0.9	0.0	8.5	–2.9	211.7	169.2	45.9	6.6
13H9–5	517.0	–2.5	0.1	0.8	8.7	–1.8	169.3	133.5	32.0	6.1
13H9–6	646.7	–4.2	–0.2	0.9	9.8	–2.4	213.1	157.8	35.0	5.2
13H9–7	656.9	–5.0	–0.4	0.6	8.9	–3.5	207.1	164.9	41.7	4.0
13H9–8	574.1	–3.8	0.7	0.6	9.8	–2.6	190.6	144.7	33.5	3.5
13H9–9	612.0	–4.2	0.1	–0.3	10.0	–3.2	195.1	146.5	37.0	6.3
13H9–10	673.8	–3.8	0.1	0.8	10.4	–4.0	212.5	156.2	40.4	5.4
13H9–11	654.7	–6.3	–0.9	1.1	9.4	–3.8	216.6	163.1	38.7	5.1
13H9–12	558.8	–5.2	–0.7	1.0	8.8	–1.5	179.9	146.2	37.6	4.3
13H9–13	639.9	–4.0	–0.4	0.6	10.3	–2.9	203.2	159.7	36.2	3.0
13H9–14	583.2	–7.6	–1.7	0.5	8.1	–1.8	182.7	151.6	32.1	3.1
13H9–15	629.3	–6.7	–0.3	1.4	8.6	–1.5	167.0	165.1	31.6	3.2

–, not done. Cross–reactivity of these clones with *L. innocua* and mixture were not performed as they had almost no reactivity with boiled or sonicated LM samples.
*Mixture: the mixture of bacterial suspension of O157:H7, EC, EA, KP, SE, and BC in HBS–EP.
Cell concentrations of LM 1/2a, LM 4b, *L. innocua* and mixture were approximately 10^8 CFU/ml.

against boiled *L. innocua* or sonicated *L. innocua*. Cross–reactivity arises because the non–target bacterium shares an epitope in common with the target pathogen or because it has an epitope that is structurally similar to that of the target pathogen (Zhang *et al.*, 2014). In previous studies (Bhunia *et al.*, 1991; Bubert *et al.*, 1994; Kathariou *et al.*, 1994; Sølve *et al.*, 2000), a similar difficulty with a cross–reaction to *L. innocua* was also reported in the preparation of antibody specific to LM.

Further work is needed to determine detection sensitivity and specificity of the purified MAbs. The developed MAbs with high sensitivity and specificity will be also available for multichannel SPR biosensor. These MAbs are expected to be helpful in improving lower detection limits for O157:H7, SE, and LM in food samples by using SPR biosensor.

AUTHOR CONTRIBUTIONS

X. G. Zhang designed the study, performed the preparation of antigens, ELISA and SPR experiments, analyzed the data and wrote the paper. S. Tsuji, H. Kitaoka, and M. Tamai participated in the estimation of antibodies by ELISA and SPR. H. Kobayashi participated in the design of the study and performed a part of ELISA and SPR experiments. K. Honjoh participated in the design of the study. T. Miyamoto designed the study, supervised the work, wrote the paper and provided facilities and resources. All authors assisted in editing of the manuscript and approved the final version.

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