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Production of monoclonal antibodies to *Tropheryma whipplei* and identification of recognized Epitopes by 2-D electrophoresis and Mass Spectrometry

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ABSTRACT

Tropheryma whipplei, the agent of Whipple's disease, is a gram-positive rod-shaped bacterium that belongs to the group of actinobacteria. In order to produce monoclonal antibodies (MAbs) against this bacterium, we inoculated mice with two different strains, Slow2 and Endo5. We produced 13 and 10 MAbs against Slow2 and Endo5, respectively. Nine of the Slow2-MAbs and 7 of the Endo5-MAbs recognized a 58kDa epitope. In addition, three of other Endo5-MAbs detected a unique 84kDa epitope. These MAbs were species-specific as they did not react with a selection of 22 different bacterial species, but were not strain-specific as they did react with 6 other strains of *T. whipplei*. Two-dimensional gel electrophoresis (2-DE) was combined with mass spectrometry (MS) to identify the 58 kDa and 84 kDa epitopes recognized by MAbs. After trypsin in-gel digestion of the spot, the 58 kDa protein was identified as an ATP synthase F1 complex beta chain, whereas the 84 kDa protein was identified as a polyribonucleotide nucleotidyltransferase by MS with MALDI-TOF. In an *in vitro* model, one of these Mabs allowed good detection of *T. whipplei* in stool sample contrary to a rabbit polyclonal antibody which lead to high fluorescent background. In the prospective studies, the produced Mab will be tested for detection of *T. whipplei* in clinical samples, and the gene coding for identified 58 kDa and 84 kDa antigens will be tentatively cloned and then tested for its use in a diagnostic ELISA for Whipple's disease.

INTRODUCTION

Whipple's disease is a multisystemic bacterial infection which may involve any organ system in the body. This disease is known mainly as a chronic pathology involving the intestine. Malabsorption, diarrhea, and weight loss, and eventually associated with adenopathies and polyarthritis that correspond to the classical symptoms of Whipple's disease (4,7,17,22). Occasionally, it is also associated with cardiac manifestations such as myocarditis, pericarditis, and endocarditis or central nervous system involvement (21,31,38). Diagnosis of infection is usually based on classical histopathological examination of a duodenal biopsy specimen showing infiltration by large macrophages that contain periodic acid-Schiff-positive, non-acid-fast bacteria (1). The determination of the nucleotide sequence of the 16S rRNA gene of *Tropheryma whipplei* (32) the agent of Whipple's disease (14,40), then its isolation by cell culture, provided the basis for the development of species-specific diagnostic PCR systems (27,39). These PCR-based diagnostic methods have become standards for the diagnosis of Whipple's disease. Using a shell vial cell culture system, we first isolated the Whipple's disease bacterium from the cardiac valve of a patient with Whipple's disease-related endocarditis and successfully established a stable culture (28). Since then the isolation methods were improved and allowed us and others to isolate more *T. whipplei* strains (20). We first developed a specific microimmunofluorescence (MIF) assay with Labteck slide-grown bacteria (28). This technique presents several major drawbacks, but mostly loss of antigenicity of *T. whipplei* isolates after several subcultures. Considering the fact that Whipple's disease is rare, a sensitive screening test not requiring invasive specimens as a tool for patient's follow-up under antibiotic treatment would be extremely helpful. The need for standardization of diagnostic antigens is a strong rationale for the development of new serodiagnostic reagents. However, the immunodominant antigens of *T. whipplei* during infection are not well characterized. As a result, the ability of a single or multiple selected proteins to serve as an alternative to purified whole bacteria as antigens for serological

diagnostic tests is untested.

In a previous study, we had produced some monoclonal antibodies against Twist-Marseille strain *T. whipplei* (16). For unknown reason and even with several subcloning attempts, hybridomas producing Mabs were progressively lost. Moreover, since the separation based on a single physicochemical property is not sufficient, the immunodominant epitopes of the strain were not identified and characterized by general Western immunoblotting. In contrast, 2-DE blotting is a technique that combines two physicochemical properties, including p*I* and molecular mass. In this technique, the experimental conditions can be optimized according to the proteins of interest (25). It is possible to separate the components from each other only on combining two techniques, IEF and SDS-PAGE. Therefore, the combination of the high resolution electrophoresis (2-DE) with subsequent transfer onto a protein-binding membrane (blotting), immunological detection, and mass spectrometer (MS) is a powerful tool to identify and characterize immunodominant epitopes of *T. whipplei.*

In the present study, we first produced the monoclonal antibodies against the Slow2 and Endo5 strains of *T. whipplei,* and then identified and characterized the recognized epitopes with 2-DE blotting and MS.

MATERIALS AND METHODS

Preparation of antigen. *T. whipplei* strain Slow2-Marseille, which grown previously in 30 ml of minimal essential medium according to Raoult *et al* (28), was cultured on HEL cell monolayers in 150-cm^2 cell culture flasks. HEL cells infected with bacteria were harvested from forty 150-cm^2 flasks into 40 ml of phosphate-buffered saline (PBS). To which, trypsin (Gibco) was added at a final concentration of 5 mg ml^{-1} and the suspension was incubated at 30°C for 45 min. The suspension was then subjected to sonication (three times for 1 min, each time on ice), after which the unlysed cells were removed by centrifugation at 100 × *g* for 15 min. The

supernatant was layered onto a 25% (wt/vol) sucrose solution in PBS. After centrifugation at 9,000 × *g* for 30 min at 4°C, the pellet containing the bacteria was resuspended in 2 ml of PBS and carefully layered onto a 25-45% (wt/vol) Renografin step gradient (in PBS). This gradient was subjected to centrifugation at 130,000 × *g* for 1 h at 5°C. The bacteria were then harvested from the interface of the 25-45% Renografin gradient and washed twice in PBS. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the bacteria were resuspended in sterile distilled water at a final concentration of 1 mg ml^{-1}. Another *T. whipplei* strain Endo5 was cultured in axenic liquid medium as previously described (33) and washed twice in PBS.

Production of MAbs. The monoclonal antibodies (MAbs) were produced by inoculation of 6-8 week-old immunocompetent BALB/c mice with a total of 0.1 mg of purified strain Slow2-Marseille and Endo5 and CpG adjuvant, respectively, as described previously (9,15). The isotypes of the MAbs were determined with an ImmunoType Mouse Monoclonal Antibody (MAb) Isotyping kit with antisera to mouse immunoglobulin M (IgM), IgA, IgG1, IgG2a, IgG2b, and IgG3 (Sigma Chemical Co.). The specificities of the MAbs were tested by Western immunoblotting.

Specificity assay. Cross reaction was determined by MIF. The MAbs produced were tested against antigens from 6 other *T. whipplei* strains isolated in our laboratory (Twist, Dig7, Endo7, Dig9, Neuro1 and Neuro2) and 22 diverse bacterial strains that were also isolated in our laboratory from clinical samples, including *Actinomyces meyeri*, *Actinomyces viscosus*, *Actinomyces pyogenes*, *Nocardia asteroides*, *Propionibacterium acnes*, *Mycobacterium marinum*, *Mycobacterium avium*, *Bacillus cereus*, *Listeria monocytogenes*, *Corynebacterium* ANF group, *Corynebacterium striatum*, *Streptococcus bovis*, *Streptococcus agalactiae* (group B *Streptococcus*), *Clostridium perfringens*, *Clostridium bifermentans*, *Fusobacterium necrophorum*, *E. coli*, *Yersinia enterocolitica*, *Shigella sonnei*, *Shigella flexneri*, *Salmonella enterica*, and *Campylobacter jejuni.*

SDS-PAGE and Western blot study. SDS-PAGE and Western blotting were performed according to the method originally developed by Laemmli (14). Antigens were treated with proteinase K or boiled. Heat denaturation was performed by boiling the antigens at 100°C for 10 min. For Western blotting, the strips were incubated with diluted supernatants of MAbs (1:10 dilution) and polyclonal mouse *T. whipplei* antisera diluted in PBS (1:100 dilution) at room temperature for 1 h, and then washed 3 times with PBST.

Preparation of crude extracts for 2-D gel electrophoresis. The bacterial suspension was precipitated by using PlusOne 2-D Clean-Up Kit (Amersham Biosciences, Uppsala, Sweden) and resuspended directly in rehydration solution [7 M urea, 2 M thiourea, 4% (w/v) CHAPS]. The protein content of the solution was determined using a commercially available protein assay system that incorporated BSA as a standard (Bio-Rad, Hercules, CA, USA) (2).

2-DE blotting. Immobiline™ DryStrips (13 cm, pH 4-7, Amersham Biosciences, Uppsala, Sweden) were rehydrated overnight with 250 µg of proteins in rehydration solution supplemented with 2% (v/v) IPG buffer (pH 4-7) (Amersham Biosciences). IEF was carried out according to the manufacturer's protocol (Multiphor II system, Amersham Biosciences). Prior to electrophoresis in the second dimension, the strips had been equilibrated for 15 min in 10 ml of equilibration buffer [30% (v/v) glycerol, 2% (w/v) SDS, 6 M urea, 50 mM Tris/HCl, bromophenol blue, pH 8.8] containing 65 mM of DTT. This step was repeated once again using 10 ml of equilibration buffer supplemented with 100 mM of iodoacetamide. The strips so treated were then embedded in 0.5% agarose and the proteins resolved by 9-16% gradient SDS-PAGE (Bio-Rad Protean II xi chamber). Electrophoresis was performed at the constant voltage of 250 V until the bromophenol blue reached the end of gel. The molecular weight (M_r) was determined by running standard protein markers (LMW, Bio-rad, Hercules, CA). Gels were then processed either for silver staining (23) or for immunoblotting. For immunoblotting, the proteins were transferred onto nitrocellulose membranes (Trans-blot Transfer Medium, Pure Nitrocellulose

Membrane 0.45µm Bio-rad) by using a semi-dry transfer unit (Hoefer TE 77, Amersham Biosciences).

Digestion peptides and MALDI mass spectrometry analysis. The protein spots excised from silver-stained gels were destained and subjected to in-gel digestion with trypsin (Sequencing grade modified porcine trypsin; Promega, Madison, WI, USA) (35). The peptides obtained from protein digestion were dissolved in 10-20 µl of 0.1% TFA. Then, the peptide mixture was analyzed using an Ettan pro MALDI spectrometer (Amersham Biosciences) in positive ion reflector mode. For this, the sample (0.3 µl) of peptide mixture was co-crystallized in the presence of 0.5% TFA onto the MALDI target with an equal amount of matrix solution (3 mg/ml of α-cyano-4-hydroxycinnamic acid in 50 % acetonitrile). Alternatively, the peptide mixtures derived from protein digestion were desalted and concentrated using zip tips (Millipore Bedford, MA, USA) and deposited onto the MALDI target by elution with the matrix solution. Proteins were identified and assigned a number by Profound (ProteoMetrics, LLC, New York, NY) and Mascot (Matrix science Ltd, London, UK) software for comprehensive sequence databases (36).

Immunofluorescence detection of *T. whipplei* in stool samples. A healthy individual's stool sample, in which *T. whipplei* PCR detection was negative by previously described technique (30), was selected**.** This stool sample was diluted in PBS (20%, w/v) and mixed well. Four-hundred microliters of this sample were suspended in sterile distilled water and submitted to 1-min sedimentation. Supernatant was removed and aliquoted into two parts. To one part, a suspension of *T. whipplei* strain Endo5 suspended in PBS was added in order to obtain a concentration of 10^4 *T. whipplei* cells per ml of dilution. Two microliters from each part were deposited onto glass slides, air-dried and fixed with methanol for 5 min. Slides were stored at 4°C before use. For immunofluorescence assay, slides were saturated by the incubation with PBS-5% BSA at 37°C for 30 min, and then washed twice with PBS-0.1% Tween for 10 min and once with sterile distilled water for 5 min. Samples were incubated either with WS5H4 mouse

monoclonal antibody or with rabbit polyclonal serum at its respective 1: 100 or 1: 400 dilution in PBS-3% BSA-0.1% Tween for 30 min at 37°C. After a washing step as described above, bound antibodies were revealed with a FITC conjugated IgG goat anti-mouse or anti-rabbit (Immunotech, Marseille, France) diluted 1: 2000 times in PBS-3% BSA-0.1% Tween-0.2% Evans blue (BioMerieux, Marcy l'Etoile, France). For image scanning, slides were mounted with Fluoprep (BioMerieux, Marcy l'Etoile, France) after subsequent washing procedures and examined under an Olympus BX-51 epifluorescence microscope at × 100 magnification. In order to see the specificity of Mab, we also tested 15 stool samples prepared as mentioned above using WS5H4 mouse monoclonal antibody and compared the results with those obtained with rabbit polyclonal serum.

RESULTS

SDS-PAGE and Western blotting of *T. whipplei*. We obtained 13 MAbs against strain Slow2 and 10 MAbs against strain Endo5 (Table 1). Of these, 16 MAbs reacted with a 58 kDa antigen and 3 MAbs with an 84 kDa antigen. The MAbs WS6F5, WS1F6 and 6C3 recognized 134, 65 and 47 kDa antigens, respectively, whereas MAb 7H3 reacted simultaneously with 105- and 65-kDa protein bands (Figure 1).

2-D gel electrophoresis and Western blotting. To identify the epitopes recognized by the MAbs, Slow2 and Endo5 strain extracts were subjected to 2-DE and subsequent western blotting analysis. Figure 2 shows the typical electrophoregram of Slow2 and Endo5 extract components, which were obtained under the same experimental conditions, like molecular weight (M_r) 20-107 and *pI* 4.5-5.5, visualized by silver staining. After 2-DE, the proteins were transferred onto a nitrocellulose membrane, which was subsequently incubated with the MAbs. On Western blotting with MAbs WS3E5, WS3F9, WS1F6, WS4D11, WS2A3, WS5D1, WS1C6, WS5E5, WS5H4 and WS7G2, only one immunoreactive spot was detected at a 58 kDa protein (Figure

3A). MAbs WS6C3 and WS6F5 recognized the protein smear of about 47-48 kDa and 134 kDa, respectively, as well as the WS7H3 detected many spots ranging from 47 to 105 kDa (Figures 3B, 3C, 3D). It was thus extremely difficult to pick up spots except one that belong to WS7G2. In addition, the MAbs WE11F10, WE11B10 and 8H5 detected an epitope of 84 kDa protein, whereas other MAbs of Endo5 recognized the same spot that was noticed in the case of Slow2 at 58 kDa.

Identification of spot. The spots recognized by MAbs at 58 and 84 kDa were excised, digested with trypsin, and subjected to peptide sequencing by MALDI-TOF. Proteins were identified using the SwissProt database with Mascot search engine (www.matrixscience.com). In the identification of 58 kDa spot, sixteen peptides were obtained by mass spectrometry, and matched the ATP synthase F1 complex beta chain of *T. whipplei* strain Twist. The molecular weight and pI of this protein were recorded as 52.5 kDa and 5.1, respectively. On the other hand, seventeen peptides were obtained from 84 kDa spot, and matched the polyribonucleotide nucleotidyltransferase of *T. whipplei* strain Twist, which molecular weight was found as 81 kDa.

Specificity. The results of MIF showed that MAbs did not react either with HEL cells or with any of the 22 diverse bacterial strains tested. All MAbs reacted with the 6 other *T. whipplei* strains tested.

Immunofluorescence detection of *T. whipplei* in stool samples. For the 15 stool samples tested using polyclonal rabbit serum, high fluorescent background and numerous fluorescent bacteria were observed, whereas no fluorescent bacteria were observed with WS5H4 Mab. The same observation was made with the contaminated sample (Figures 5c, 5d). Bacteria with typical *T. whipplei* morphology were detected by Mab. On the contrary, the rabbit polyclonal serum reacted with many bacteria, most of which have no morphological features of *T. whipplei* (Figures 5a, 5b).

DISCUSSION

Since the clinical diagnosis of Whipple's disease is difficult and the isolation of the causative agent is time-consuming, the diagnosis of the disease is mainly based on the results of pathology and specific DNA detection. Although the serological diagnosis was encouraging among laboratory tests, this technique presents several drawbacks that render its routine use difficult (28). The identification and characterization of the immunodominant antigens could have important repercussions for developing novel diagnostic, prophylactic and therapeutic techniques for Whipple's disease. Moreover, the sequencing of epitope polypeptide will provide the foundation for cloning and expression of recombinant antigen to be used in an ELISA test.

The monoclonal antibody technique has proven to be a powerful tool in studying the antigenicity and virulence of microorganism (41). In the present work, we generated 13 and 10 MAbs that were as efficient as mouse polyclonal antibodies in recognizing *T. whipplei* strain Slow2 and Endo5, respectively by the MIF assay. These MAbs were demonstrated to be specific because they did not react either with 22 other pathogenic, phylogenetically closely related gram-positive bacteria or with common gastrointestinal pathogenic bacteria or with bacterial species that have been shown to be cross-reactive with the Whipple's diseases bacillus such as *S. agalactiae* and *S. flexneri* (6,11). The 58 and 84 kDa antigens appeared to be the immunodominant antigens, because most of MAbs found to be having strong reactivity to these antigens. The MAb 7H3 recognized two protein bands such as 105 and 65 kDa, in which the same epitope was probably present. Three antigens, 134, 65 and 47 kDa were recognized by only one MAb (Figure 1).

In a previous work, we had produced some monoclonal antibodies against *T. whipplei* strain Twist-Marseille. However, the immunodominant epitopes of the strain had not been identified and characterized, because the proteins were not separated well by SDS-PAGE. Results of the 2-D blotting indicated that MAb WS7G2 and WE11F10 were directed against only

one epitope located on the 58 and 84 kDa respectively, which were reproduced several times by other MAbs (WS4D11, WS5D1, WS1C6, WS5E5 and WS5H4, and WE11G10, WE11B10 and WE8H5). However, other MAbs (such as 6F5, 6C3, 7H3) recognized either a smear band or many spots. These results made it difficult for us to pick up the spots for MS. Therefore, only the 58 and 84 kDa antigenic spots were further analyzed by MS with MALDI-TOF. These proteins were identified, respectively, as an ATP synthase F1 complex beta-chain with 168 score and 46% sequence coverage and a polyribonucleotide nucleotidyltransferase with 130 score and 29% sequence coverage, which were matching respectively *T. whipplei* Twist strain isolated in Europe (29) and another strain from USA (19) in Mascot database. These results obtained from two strains from different geographical regions suggest that these epitopes are common to all *T. whipplei* strains and were confirmed by testing the corresponding MAbs to six other unrelated *T. whipplei* strains. Interestingly, the sizes estimated by SDS-PAGE were higher compared to the molecular mass determined by MALDI-MS, by which the molecular masses recorded were as low as 52 and 81 kDa. This can be explained by the fact, in general, that SDS-PAGE gives only a rough estimation of molecular mass.

The data presented in this paper demonstrate that 2-DE combined with MS constitutes a sensitive and powerful technique to identify the epitope of *T. whipplei* recognized by MAbs. The produced Mabs may be useful for better detection of *T. whipplei* in tissues or stools and the 58 and 84 kDa antigens recognized by our MAbs are good candidates for the development of an ELISA test using the recombinant antigens. In a previous work, we used serological proteomic approach for the identification of candidate antigen in Whipple's disease (12). The 58 and 84 kDa antigens identified herein were not detected. Than does not mean that these antigens are not immunogenic for humans because many proteins are present in the same area than these antigens and sera of patients and controls recognize many protein spots. Only production of the 58 and 84 kDa antigens (which is currently in progress) for testing with patients and control sera will

enable to address this issue.

Recently, the presence of *T. whipplei* in stool samples of patients with Whipple's disease was reported (8) and an isolate was obtained from stool sample (30). The MAb WS5H4 that recognize the 58kDa epitope was demonstrated in this study to be an efficient mean to detect *T. whipplei* in stool samples contrary to rabbit polyclonal serum that cross react with many other bacteria. In the future, this Mab will be used in our laboratory prospectively in combination with PCR amplification for the detection of *T. whipplei* in stool samples. This approach could help to differentiate, contrary to PCR, true digestive Whipple's disease form simple carriage without using an invasive procedure.

269 **Table 1.** Hybridomas obtained from inoculation by Slow2 and Endo5 strains.

270 NI: not identified; 1-D WB: one dimensional western blot; 2-D WB: two dimensional western

271 blot

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Hybridoma	Isotype	Obtained from		Size of recognized antigens		Epitope
		Slow 2	Endo 5	1-D WB	2-D WB	
WS3E5	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS3F9	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS1F6	IgG1	X		65 kDa	NI	NI
WS4D11	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS2A3	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS5D1	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS7H3	IgG1	X		105-65 kDa	Smear	NI
WS6F5	IgG1	X		134 kDa	Smear	NI
WS5H4	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS7G2	IgG1	X		58 kDa	58 kDa	ATP synthase F1 complex β chain
WS6C3	IgG1	X		47 kDa	Smear	NI
WS1C6	IgM		X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WS5E5	IgM		X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE7F6	IgG1		X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE10D11	IgG1		X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE11H11	IgG1		X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE11G10	IgG1		X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE8D5	IgG1		X	58 kDa	58 kDa	ATP synthase F1 complex β chain

WE9C1	IgG1	X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE9D4	IgG1	X	58 kDa	58 kDa	ATP synthase F1 complex β chain
WE8H5	IgG2a	X	84 kDa	84 kDa	polyribonucleotide nucleotidyltransferase
WE11B10	IgG2a	X	84 kDa	84 kDa	polyribonucleotide nucleotidyltransferase
WE11F10	IgG2a	X	84 kDa	84 kDa	polyribonucleotide nucleotidyltransferase

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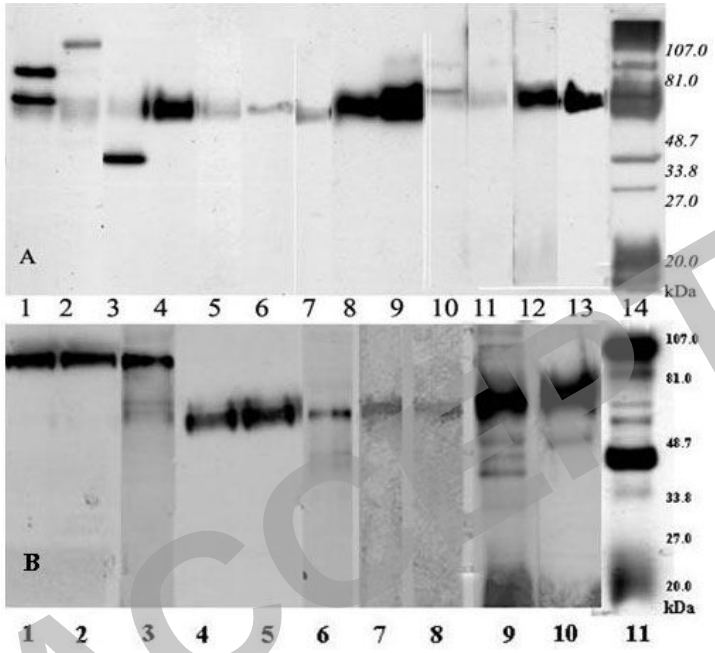
Figure 1. Immunoblots of antigens of *T. whipplei* with mono- and polyclonal antibodies. **(1A)** *T. whipplei* Slow-2 with its monoclonal antibodies. Lanes 1, MAb WS7H3; lane 2, MAb WS6F5; lane 3, MAb WS6C3; lane 4, MAb 7G2; lane 5, MAb WS5E5; lane 6, WS2A3; lane 7, MAb 3E5; lane 8, MAb WS5D1; lane 9, MAb 3F9; lane 10, MAb WS1F6; lane 11, MAb WS1C6; lane 12, MAb WS4D11; 13, MAb WS5H4; lane 14, polyclonal mouse antiserum. (**1B)** *T. whipplei Endo5* with its monoclonal antibodies. Lanes 1, MAb WE11F10; lane 2, MAb WE11B10; lane 3, MAb WE8H5; lane 4, MAb 11G10; lane 5, MAb WE8D5; lane 6, MAb WE9D4; lane 7, MAb WE7F6; lane 8, MAb WE9C1; lane 9, MAb WE11H11; lane 10, MAb WE10D11; lane 11, polyclonal mouse antiserum.

Figure 2. Two-dimensional gel of *T. whipplei* extract with Silver staining (The first one for Slow2, the second one for Endo5). Proteins were resolved in the first dimension over a p*I* gradient of 4.5 –5.5 followed by second dimension separation by SDS-PAGE in a 10% acrylamide gel. The prominent spots at 60 kDa and 84kDa (arrow), were cored from the gel and submitted for analysis by mass spectrometry. These spots corresponded to the 2-DE blotting

Figure 3. 2-D Western blot showing the reactivity of MAbs with Slow2 proteins. The monoclonal antibodies against Slow-2 were from the supernatant of hybridoma 7G2(A), 7H3(B), 6F5(C) and 6C3(D). The 7G2 antibody bound only one spot at 60 kDa and pI 5.1.

Figure 4. 2-D Western blot showing the reactivity of MAbs with Endo5 proteins. The monoclonal antibodies against Endo5 were from the supernatant of hybridoma 11G10(A) and 11F10(B). The 11F10 antibody bound a unique spot at 84 kDa and pI 5.3. The MAb WE11G10 recognized the same spot as WS7G2 did.

Figure 5. Immunofluorescence detection of *T. whipplei* by using WS5H4 Mab and rabbit polyclonal serum in artificially contaminated stools (5a and 5b, respectively) and in negative stool control (5c and 5d, respectively). Arrows indicate bacilli with *T. whipplei* typical morphology.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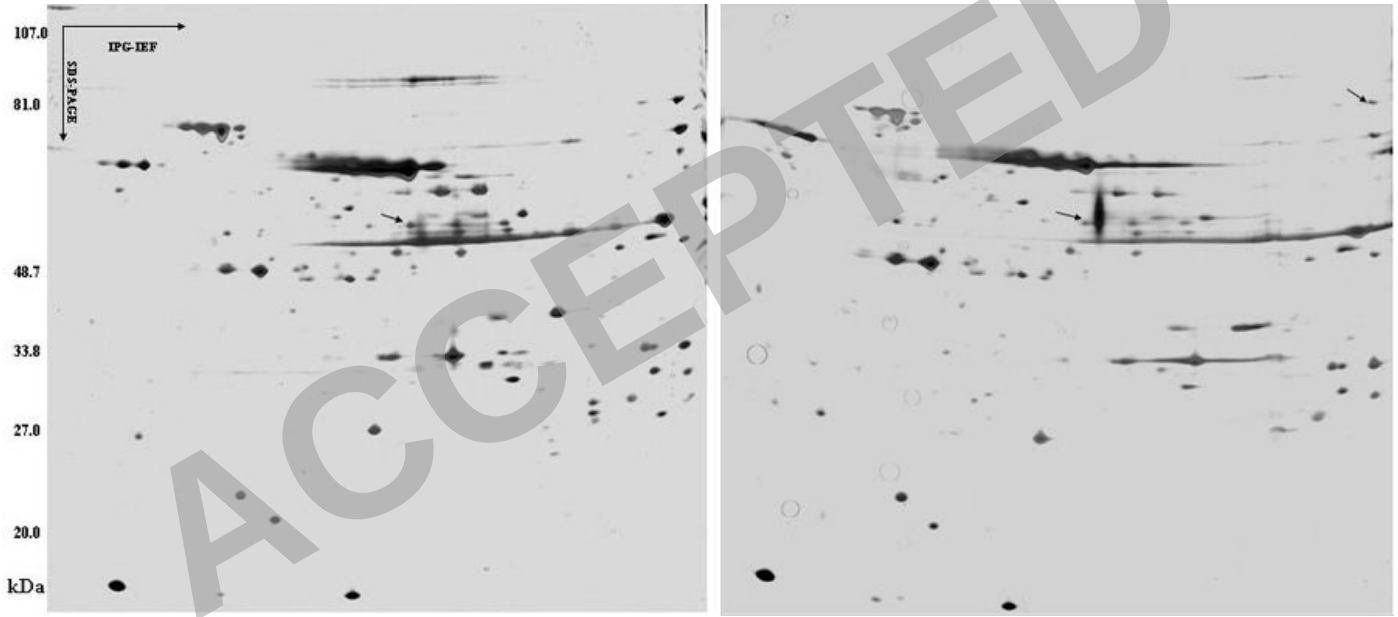

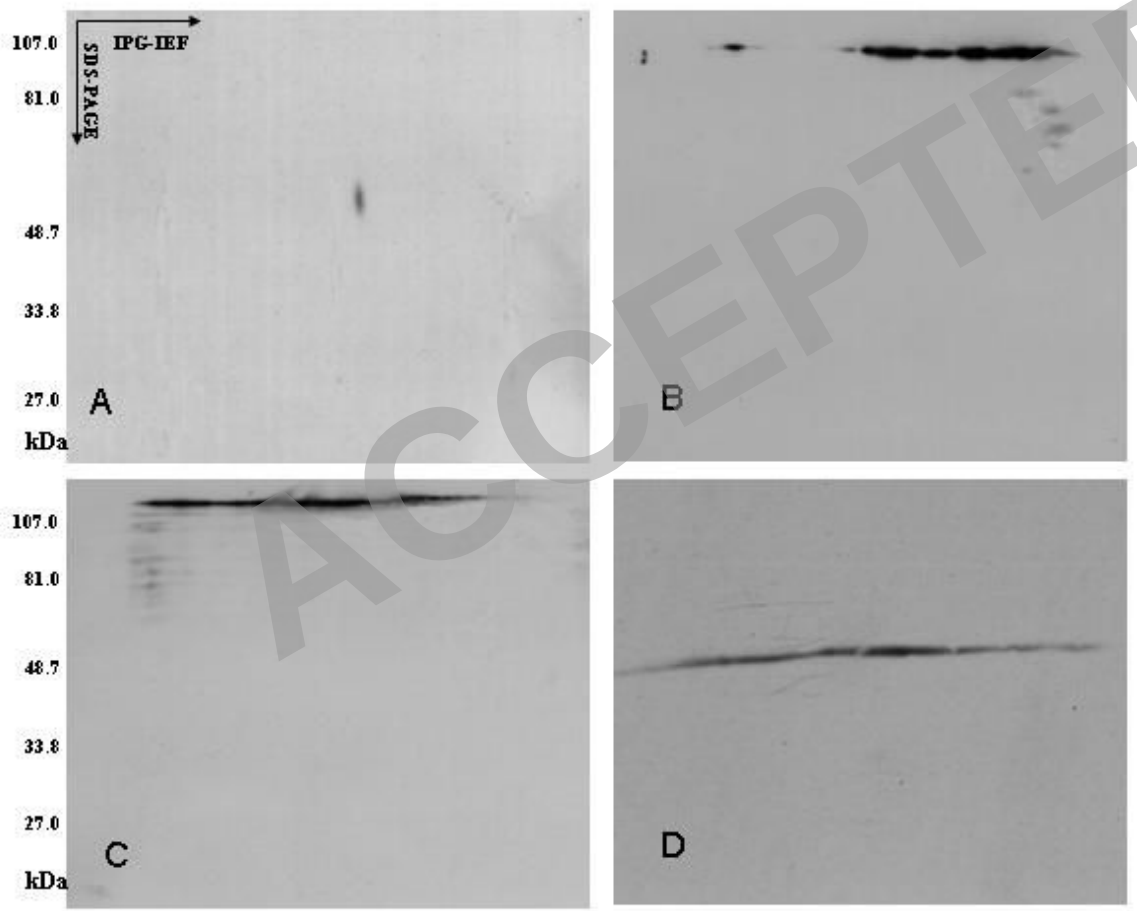
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