MASTITIS VACCINE ANTIBODIES (MASTIVAC I) PASIVELY PROTECT MICE AGAINST STAPHYLOCOCCUS AUREUS ISOLATES FROM USA, GERMANY AND ITALY

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ABSTRACT

Previously we described the development of a new *Staphylococcus aureus* vaccine denoted MASTIVAC I. The present study tested the ability of antibodies elicited by MASTIVAC I to protect mice against *S. aureus* isolates derived from infected bovine udders in Germany, the USA and Italy,. Variations were found among isolates but not between countries iusing the hemolytic slide-latex agglutination response on Baird Parker agar. No differences were found in the susceptibility tests and virulence determinations in mice. Cluster analysis performed by the unweighted pair-group method, using arithmetic averages (UPGMA), and calculation of the immunoblot relatedness with serum from MASTIVAC I- immune mice, revealed three groups with similarity coefficients of protection of 82-84%. The first 2 groups contained all *S. aureus* isolates without segregation regarding the origin of the strain. *Staphylococcus chromogenes* 36173/1 was separate in group 2 and group 3 included three *Staphylococcus haemoliticus* isolates with a coefficient of 76% from groups 1 and 2. Overall mortality rates of the groups vaccinated with MASTIVAC I and sham controls were 7.3 and 28.6%, respectively, and the morbidity rates were 21.4% and 46.4%, respectively. Between-group differences (vaccinated and control) were significant for mortality ($P < 0.001$), morbidity ($P < 0.05$), and mortality + morbidity ($P < 0.0001$) with variations among isolates.

S. aureus strains from the USA, Germany and Italy did not differ from the Israeli isolates in their antibody recognition and protection patterns. Moreover, the isolates possess crucial immunogenic determinants, which reacted with the antibodies elicited by the MASTIVAC I vaccine and probably will protect cows against these bacteria

Key Words: *Staphylococcus aureus*, mastitis, vaccine, dairy cow

INTRODUCTION

Staphylococcus aureus is still one of the major pathogens causing mastitis worldwide whose treatment necessitates the extensive use of antibiotics in dairy herds. The increasing public concern over food safety, expressed as the desire to minimize antibiotic residues in milk on the one hand, and the need to reduce somatic cell counts (SCC) on the other hand, vindicates our effort to combat *S. aureus* mastitis by vaccination. Nevertheless, previous attempts to develop a vaccine against *S. aureus* mastitis have failed to provide a substantial solution [3,5,13,16,19]. MASTIVAC I is a newly introduced vaccine (Patent no. IL122829, PTC/IL 98/00627, AU 746285, USA09/582692) designed to protect cows from *S. aureus* mastitis, which has been used commercially in Israel since 2004. The vaccine is composed of three field strains of *S. aureus*, which exhibit a broad spectrum of antigenic and immunogenic properties [9]. In controlled experiments, the vaccine was found effective in protecting cows challenged with a virulent field strain of *S. aureus* [10]. A large-scale field trial, lasting over 2 consecutive years and involved a total of 452 (vaccinated and control) Israeli-Holstein heifers in 7 dairy farms, resulted in 40% lower incidence of SCC in vaccinated cows in the first and second lactations than in unvaccinated control cows. Moreover, the vaccinated cows yielded 0.5 kg/d more milk than the unvaccinated ones [11]. For registering MASTIVAC I, a highly infected herd of 267 multiparous Israeli-Holstein cows, of which 22.1% were chronically infected with *S. aureus* mastitis, was vaccinated and monitored for a year under research regulations [12]. The field study again demonstrated the ability of MASTIVAC I to significantly reduce *S. aureus* new infection in vaccinated animals, all of which were uninfected at time of vaccination, and to cure vaccinated compared with placebo-treated *S. aureus* infected-animals. Although antibodies generated by MASTIVAC I recognized a wide range of Israeli

field strains of *S. aureus*, it was crucial to examine the capacity of the vaccine to recognize *S. aureus* isolates found in other dairy industries. This study therefore aimed to test the ability of antibodies elicited by MASTIVAC I to recognize (identify) *S. aureus* strains isolated from infected cattle in Germany, USA and Italy, and determine the protection provided by MASTIVAC I against challenge with these *S. aureus* strains in a mouse model.

MATERIALS AND METHODS

2.1. Mice

Female Swiss line of 6-8 weeks old mice were maintained in the animal facility of the Kimron Veterinary Institute. Three mice were housed in each cage under standard conditions of light (12/12 h light/dark) and temperature (22°C) and were fed standard laboratory chow and water ad lib. At the end of the experiments, the mice were euthanized with CO_2. Animal ethics approval was granted for all animal experiments by the Israeli ethics committee (Kimron Veterinary Institute Animal Ethics Committee).

2.2. Phenotypes of field isolates and susceptibility test.

2.2.1. *Bacterial Identification*

Twenty-eight staphylococci field isolates, comprising 10 from Germany and 9 from the USA, were provided by Boehringer (Ingelheim Vetmdica GmbH, Germany), and 8 isolates were provided by FATRO (Pharmaceutical Veterinary Industry, Ozzano, Italy). An aliquot of 10 µl from each sample was spread over blood agar plates (Hy-labs, Park Tamar, Rehovot, Israel) containing 5% washed sheep erythrocytes and incubated at 37°C for 24 h. The following tests were performed: Coagulase (tube test) (Anilab, Tal-Shachar, Israel) [6] slide latex agglutination test (BACTI Staph, Remel, Santa Fe Drive, Lenexa, KS) and selective media: Baird Parker (Difco, Laboratories, Detroit, MI). In the following step, isolates were identified by the ID-32-API STAPH test (BioMerieux S.A., France). Isolates were considered as being *S. aureus* if the isolate identification was > 98% with T > 65%. Phage typing was performed according to Blair and Williams [4], with phages issued by the International Reference Laboratory, Colindale, UK, as modified by Samra and Gadba [17].

2.2.2. *Susceptibility Test*

Antimicrobial susceptibility test (ATB) was performed in accordance with NCCLS guidelines [15] using commercial test disks (Beckton Dickinson, Le Pont de Claix, France) and the MIC test. Commercially available disks (Dispens-O-Disc, Susceptibility Test System, Difco) or BBL™ Sensi-Disc™ Antimicrobial Susceptibility Test Discs (Becton Dickinson, MD) were used as recommended, and the plates were incubated at 30°C for methicillin (5 µg/disk) and 37°C for other antibiotics, penicillin G (10 units/disk), erythromycin (15 µg/disk), cephalothin (30 µg/disk), neomycin (30 µg/disk), trimethoprim-sulfamethoxazol (SXT)(1.25-23.75 µg/disk). Susceptibility or resistance was interpreted according to the manufacturers' recommendations.

2.2.3. *Electrophoresis and immunohlotting*

One-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed [7]. The bacterial cells were disrupted with glass beads in a homogenizer (Braun Melsungen AG, Germany) for 10-15 min. The glass beads and the remaining bacteria were removed by centrifugation at 1,000 × *g* for 15 min, and the supernatant was filtered through 0.2-µm filters. Protein concentrations of the disrupted bacteria were determined with the Bio-Rad protein assay. The samples (antigens) were adjusted to a final protein concentration of 1 mg/ml, and 33 µl of each sample was mixed with 25 µl of 4 × NuPAGE sample buffer, 32 µl of ultrapure water and 10 µl of reducing agent. The sample mixtures were heated to 70°C for 10 min and loaded onto the gels at 30 µl per lane. The gel was 10% (Bis-Tris Gel with w/MOPS) (NOVEX, San Diego, CA), stained with colloidal blue. Molecular-weight markers for these gels were: See Blue Pre-Stained, 191-14 kDa for the 10% gel. For the immunoblot assay, a 0.2-µm nitrocellulose membrane was blocked with 3% casein and incubated with 1:100 dilutions of serum from mice that had been immunized with the MASTIVAC I (maximum dilution that gives a positive result in ELISA is 1:10.000). The blot was developed with goat anti-mouse IgG (H+L) alkaline peroxidase conjugate (1:1000), with a substrate of DAB (3,3-diaminobenzidine tetrahydrochloride) (ICN Pharmaceuticals, Inc., Costa Mesa, CA). Molecular-weight markers for these gels were: NOVEX Marker 12 Standard Bands (2.5-200 kDa).

Cluster analysis of the immunoblot was performed by the unweighted pair-group method, using arithmetic averages (UPGMA), and the calculation of their relatedness was based on the Dice coefficient [1].

2.3. Virulence vaccination and protection

Virulence and resistance to challenge after MASTIVAC I vaccination were determined in mice, with the German and US isolates in two sets of experiments.

2.3.1. *Virulence in a mouse model [9].*

Each of nineteen groups of nine mice was divided into three subgroups of three mice. The mice in each subgroup were inoculated intramuscularly in the left hind limb with one of the *S. aureus* strains with one of 3 doses of live bacteria (1×10^6 to 1×10^9 CFU). The mice were examined individually; the injected limb was visually inspected daily for visible erythrema and gangrene. Macroscopic inspections during 20 days of observation revealed either: (0) normal appearance; (1) morbidity – erythrema gangrene; or (2) mortality. Virulence was calculated as the CFU of bacteria that resulted in death (mortality) or erythrematous gangrene (morbidity) in 50% of the inoculated mice. Isolates whose virulence was not determined by these series of tests were retested as mentioned above, but the dosage of bacteria was increased to > 1×10^9 CFU/mouse or decreased to 1×10^5 CFU/mouse.

2.3.2. *Vaccination and protection*

Mice were vaccinated subcutaneously with MASTIVAC I at 0.2 mg/mouse in the right limb. Groups of 4 or 5 mice were vaccinated with MASTIVAC I and after 25 days they were challenged with one of the *S. aureus* isolates. Each vaccinated group was matched by a control group comprising the same number of mice. The level of challenge used for each of the isolates was that which resulted in virulence in 50% of the inoculated mice in the first series of tests.

2.4. Statistical Analysis

Data were analyzed with the JMP statistical software [18]. The analyzed parameters were: percentage mortality, percentage morbidity and percentage (mortality + morbidity). The group effect (vaccinated vs. control) was determined by applying a one-way ANOVA design in blocks (bacteria) with the linear model:

$$Y_{ij} = \mu + \alpha_i + B_j + c_{ij}$$

in which μ is the grand mean; α_i is the effect of the *i*th group; B_j is the variance between bacteria (random effect); and e_{ij} represent the random error.

RESULTS

3.1. Phenotyping the field isolates

Of the 28 staphylococci, one (USA 18) was found to be contaminated and was not identified. Isolate 36173/1 was identified as *S. chromogenes*, and isolates 937/6, VMTRC-AB019 and 931/1 were identified as S. *haemoliticus* by the coagulase and ID32 STAPH tests (Table I). All the remaining 23 identified *S. aureus* isolates were coagulase positive, 6/23 (26%) were not slide-latex agglutinated and 14/23 (61%) did not show a positive response on Baird Parker. Of the hemolysis reactions, 2 (8.7%) were non-hemolytic and 6 (26.1%) were α-hemolytic, all of which were from Germany; 6, of which 5 were from the USA, (26.1%) were β-hemolytic; 6, mostly from Italy, (26.1%) were (α + β) hemolytic; and 3 (13%) were (β + γ) hemolytic (Tab. I). All six German isolates, , were insensitive to any of the phages used, while the others showed a variety of patterns. All *S. aureus* isolates were sensitive to methicillin and cephalothin. All were sensitive to neomycin except for two isolates from Italy, All the Italian isolates were resistant to penicillin and all the German and American isolates were penicillin-sensitive. About half of the isolates from the three sources showed intermediate sensitivity to erythromycin and SXT (data not shown).

Antigenic patterns of the 27 staphylococcal isolates (from USA, Germany, Italy) and one Israeli *S. aureus* isolate (ZO3984), i.e., one of the three *S. aureus* strains included in the vaccine, were analyzed by electrophoresis and were further analyzed by immunoblotting with 1:100 dilutions of serum from mice immunized with MASTIVAC I. Fig. 1 shows the 1-dimension SDS-PAGE results and Fig. 2 shows the immunoblot results obtained with the same isolate. Cluster analysis by the unweighted pair-group method, using arithmetic averages (UPGMA), and calculation of the immunoblot relatedness (US and German isolates) revealed 3 groups (Fig. 3) with similarity coefficients of 82-84%. The first 2 groups contained *S. aureus* isolates from both countries, as well as isolates ZO3984 (Israel) and ATCC 29740, with no segregations to the source. In group 2, but separately, was found *S. chromogenes* 36173/1. Group 3 included the three *S. haemoliticus* isolates: 931/1, 937/6 and VMTRC-AB019 with a similarity coefficient of 76% with respect to both groups 1 and 2. The Italian isolates had similar immunoblotting patterns to those of groups 1 and 2.

3.2. Virulence in mice

The virulence of the *S. aureus* isolates varied among the German and US isolates (Tab. II). Virulence of ATCC 29740 was 5×10^7 CFU and that of most of the German isolates was similar, whereas about half of the US isolates were about half as virulent. The *S. chromogenes* and the three *S. haemoliticus* isolates did not cause clinical symptoms even when the highest dose (5×10^9 CFU) of bacteria was injected into the mice (data not shown).

Protection results of mice vaccinated with MASTIVAC I vaccine and then challenged with one of the 11 German and US *S. aureus* isolates are presented in Tab. III. The vaccine protected the mice as compared with the relevant control, against all isolates except 36220/3. Overall mortality rates of the vaccinated and control mice were 7.3 and 28.6%, respectively, and morbidity rates were 21.4 and 46.4%, respectively. The significance levels *P*[F] of the ANOVA effects (group and bacteria), the R^2 and the between-bacteria percentage of variance from the overall variance, for percentage mortality, percentage morbidity and percentage (mortality + morbidity) are presented in Tab. IV. The (vaccinated and control) differences between groups were significant for mortality ($P < 0.001$), morbidity ($P < 0.05$), and (mortality + morbidity) ($P < 0.0001$) with high variation among the isolates.

DISCUSSION

Under laboratory and field conditions, MASTIVAC I was found to have a wide range of recognition of Israeli *S. aureus* strains (9-11]. This was attributed to the unique features of the *S. aureus* strains in the vaccine as well as its realistic production method. In order to manufacture and use this vaccine to combat *S. aureus* mastitis in other countries, it was necessary to verify its ability to recognize *S. aureus* isolates from different target locations. Bacterial phenotypic and genotypic characterization is important for management and treatment. However, this provides only a rough estimate of the ability of antibodies to protect animals against infection. MASTIVAC I, which includes bacterial fragments of two *S. aureus* strains and the secretion of a third strain, is designed to recognize and protect against a wide range of *S. aureus* strains and also to partially CNS strains.

IgG_1 and IgG_2 antibody,isotypes, are the principal immunoglobulins of the mammary gland immune system, and are responsible for promoting polymorphonuclear phagocytosis [2,14]. The level of IgG in milk is low compared with that in the blood. However, IgG_1 is transferred to the mammary gland

continuously, regardless of infection with *S. aureus* [8], which renders the vaccinated cows better able to confront a new infection. Therefore, high affinity IgG1 antibodies that recognize a wide range of *S. aureus* strains and varieties are a crucial factor in protecting cows against *S. aureus* mastitis. In fact, the cluster analysis of the immunoblot relatedness revealed that the antibody response of MASTIVAC I recognized all *S. aureus* with a similarity coefficient of 82-84%. This assay also revealed that MASTIVAC I antibodies were able to recognize coagulate negative staphylococci (CNS), though to a lesser extent. This is important because, although the vaccine was directed to protect cows mainly from *S. aureus*, CNS comprise the group of Staphylococci that represent the most commonly found bacteria in dairy mastitis, mainly in its subclinical form.

The most important finding of this study is the significantly enhanced protection of mice vaccinated with MASTIVAC I vaccine. MASTIVAC I provided full protection from mortality to all but one (36220/3) of the vaccinated mice that were challenged with all of the US and German *S. aureus* strains tested. Moreover, it also provided significant protection against erythrematous gangrene, as indicated by comparison with the unvaccinated control mice. Similar results were obtained when mice were challenged with various Israeli *S. aureus* isolates [9].

In conclusion, *S. aureus* strains derived from USA, Germany and Italy did not differ in their antibody recognition and protection properties from the Israeli *S. aureus* isolates. Moreover, the isolates possess, with high probability, the crucial antigenic determinants that will react with MASTIVAC I vaccinal antibodies, and probably will protect cows against these bacteria.

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Table I. Phage type, coagulase, Baird Parker, agglutination and hemolysis patterns of 28 isolates from German, US and Italian mastitic cows.

	Isolate	Origin	Phage Type	Coagulase	B.P.	Agglutination	Hemolysis	Indentification (API)
1	M165	Ahlemer Inst	0 [0]	Pos	Neg	Pos	α	*S. aureus*
2	VMTRC-AB01	USA	29 [80|90|95]	Pos	Neg	Neg	β	*S. aureus*
3	VMTRC-AB03	USA	80|3/A|3/C|42E|92|94|95|96|D11⁻/HK2[29]	Pos	Neg	Pos	α + β	*S. aureus*
4	36220/3	Ahlemer Inst	0 [0]	Pos	Neg	Pos	α	*S. aureus*
5		Lufa Munster	0 [0]	Pos	Neg	Pos	α	*S. aureus*
6	VMTRC-AB06	USA	29|80|71|42E|75|90|92 [52|95]	Pos	Pos	Neg	β	*S. aureus*
7	VMTRC-AB02	USA	79|80|3/A|3/C|90|92|95	Pos	Neg	Neg	β + γ	*S. aureus*
8	36173/1	Ahlemer Inst	0 [0]	Neg	Neg	Neg	Neg	*S. chromogenes*
9	VMTRC-AB010	USA	52||80|42E|75|90|92	Pos	Neg	Neg	β	*S. aureus*
10	M1674	Ahlemer Inst	29|79|80|71|90|92|95|D11⁻/HK2 [52]	Pos	Neg	Neg	β + γ	*S. aureus*
11	ATCC 29740	USA	42E	Pos	Neg	Pos	β	*S. aureus*
12	VMTRC-AB08	USA	6|29|52|80|3/A|3/C|55|71|42E|54|75|88|89|90|92|95 [47|85]	Pos	Pos	Neg	β + γ	*S. aureus*
13	VMTRC-AB01I	USA	42E|75 [6|47|54]	Pos	Pos	Pos	β	*S. aureus*
14	36173/31	Ahlemer Inst	0 [0]	Pos	Neg	Pos	α	*S. aureus*
15		Lufa Munster	92	Pos	Neg	Pos	α	*S. aureus*
16	937/6	Ahlemer Inst	0 [0]	Neg	Neg	Neg	Neg	*S. haemoliticus*
17		Lufa Munster	0 [0]	Pos	Neg	Pos	α	*S. aureus*
19	VMTRC-AB019	USA	0 [0]	Neg	Neg	Neg	Neg	*S. haemoliticus*
20	931/1	Ahlemer Inst	0 [0]	Neg	Neg	Neg	Neg	*S. haemoliticus*
21	1645	Italy		Pos	Neg	Pos	α + β	*S. aureus*
22	954	Italy		Pos	Pos	Pos	β	*S. aureus*
23	845	Italy		Pos	Pos	Pos	α + β	*S. aureus*
24	598	Italy		Pos	Pos	Pos	α + β	*S. aureus*
25	1449	Italy		Pos	Pos	Pos	Neg	*S. aureus*
26	622	Italy		Pos	Pos	Pos	α + β	*S. aureus*
27	1425	Italy		Pos	Neg	Pos	Neg	*S. aureus*
28	1640	Italy		Pos	Pos	Pos	α + β	*S. aureus*

Table II. Virulence (CFU of bacteria result in mortality + morbidity of 50% of mice) derived from inoculation with several concentrations of various strains of *S. aureus* isolates from USA and Germany.

Bacterium	CFU/mouse	Mortality [a]	Morbidity [a]	Virulence
M165	1×10^7		0/3	5×10^7
	1×10^8		3/3	
	1×10^9	1/3	2/3	
VMTRC-AB01	1×10^6		0/3	5×10^7
	1×10^7		1/3	
	1×10^8		3/3	
VMTRC-AB03	3×10^6		0/3	5×10^8
	3×10^7		0/3	
	3×10^8		2/3	
36220/3	1×10^6		0/3	5×10^7
	1×10^7		2/3	
	1×10^8	2/3	1/3	
Lufa Munster (5)	3×10^5		0/3	5×10^7
	3×10^6		0/3	
	3×10^7		2/3	
VMTRC-AB06	1×10^7		0/3	$>1 \times 10^9$
	1×10^8		0/3	
	1×10^9		0/3	
VMTRC-AB02	5×10^5		0/3	1×10^7
	5×10^6		1/3	
	5×10^7	2/3	1/3	
VMTRC-AB010	1×10^7		0/3	$>1 \times 10^9$
	1×10^8		0/3	
	1×10^9		1/3	
M1674	2×10^6		0/3	1×10^7
	2×10^7		1/3	
	2×10^8		2/3	
ATCC 29740	5×10^5		0/3	5×10^7
	5×10^6		0/3	
	5×10^7	1/3	1/3	
VMTRC-AB08	1×10^7		0/3	1×10^9
	1×10^8		0/3	
	1×10^9	1/3	1/3	
VMTRC-AB011	5×10^6		0/3	1×10^8
	5×10^7		1/3	
	5×10^8	3/3		
36173/31	5×10^6		0/3	1×10^8
	5×10^7		1/3	
	5×10^8	1/3	1/3	
Lufa Munster (15)	5×10^5		0/3	5×10^7
	5×10^6		1/3	
	5×10^7	1/3	1/3	
Lufa Munster (17)	5×10^5		0/3	1×10^7
	5×10^6		1/3	
	5×10^7	2/3	1/3	

[a] Mice mortality during 20 days post inoculation.

Table III. Mortality and morbidity rates of MASTIVAC I vaccinated and control mice inoculated with *S. aureus* isolates from the USA and Germany.

Bacterium	CFU/mouse	Vaccinated (%)			Control (%)		
		#	mortality	morbidity	#	mortality	morbidity
M165	$1 \times i0^8$	5	0	60	5	40	60
VMTRC-AB01	3×10^7	4	0	0	4	25	25
36220/3	5×10^7	5	80	0	5	100	0
Lufa Munster (5)	$1 \times i0^8$	4	0	100	4	50	50
VMTRC-AB06	1×10^9	4	0	0	4	25	25
VMTRC-AB02	5×10^7	4	0	0	4	25	25
M1674	5×10^8	4	0	0	4	0	100
VMTRC-AB08	1×10^9	4	0	0	4	25	25
VMTRC-AB011	$1 \times i0^8$	4	0	0	4	0	50
36173/31	5×10^7	4	0	50	4	25	75
Lufa Munster (17)	5×10^7	4	0	25	4	0	75
Mean		7.3		21.4	28.6		46.4

Mice mortality during 20 days post inoculation.

Table IV. The significance level *P*[F] of the ANOVA effects (group and bacteria), the R square (R^2) and the percentage of variance between bacteria from the overall variance, for percentage mortality, percentage morbidity and percentage (mortality + morbidity) of inoculated mice with US or German *S. aureus* isolates post vaccinated with MASTIVAC I.

	Df	---------- *P*[F] ----------		
		% mortality	% morbidity	% (mortality + morbidity)
Group	1	0.0014	0.0493	<.0001
Bacteria	10	0.0006	0.1503	.0047
n		22	22	22
R^2		0.921	0.712	0.909
Variance between bacteria		81.43%	32.63%	71.24%

Figure 1. One-dimension SDS-polyacrylamide gel electrophoresis (10%) of disrupted *Staphylococcus* strains. 30 μg protein of disrupted *Staphylococcus* strains supernatant was applied to each lane. Lane 1: MW markers; Lane 2: *S. haemoliticus* 931/1; Lane 3: *S. haemoliticus* VMTR-AB19; Lane 4: *S.aureus* ZO3984 (Positive control, Israel); Lane 5: *S. aureus* Lufa Munster (17); Lane 6: *S. haemoliticus* 937/6; Lane 7: *S. aureus* Lufa Munster (15); Lane 8: *S. aureus* 36173/31; Lane 9: *S. aureus* VMTR-AB11; Lane 10: *S. aureus* VMTR-AB08; Lane 11: *S. aureus* ATCC29740.

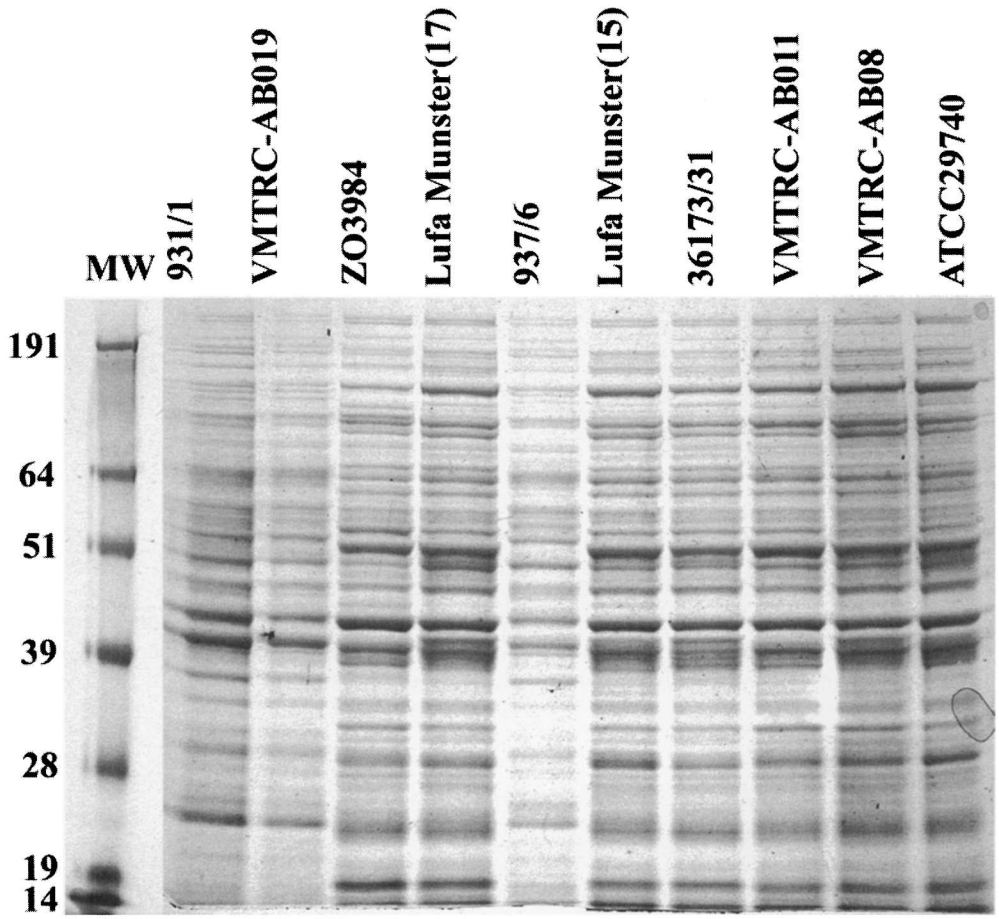

Figure 2. Immunoblot patterns of disrupted *Staphylococcus* strains with 1:100 dilutions of serum from mice immunized with **MASTIVAC I.** Lane 1: MW markers; Lane 2: *S. haemoliticus* 931/1; Lane 3: *S. haemoliticus* VMTR-AB19; Lane 4: *S. aureus* ZO3984 (Positive control, Israel); Lane 5: *S. aureus* Lufa Munster (17); Lane 6: *S. haemoliticus* 937/6; Lane 7: *S. aureus* Lufa Munster (15); Lane 8: *S. aureus* 36173/31; Lane 9: *S. aureus* VMTR-AB11; Lane 10: *S. aureus* VMTR-AB08; Lane 11: *S. aureus* ATCC29740.

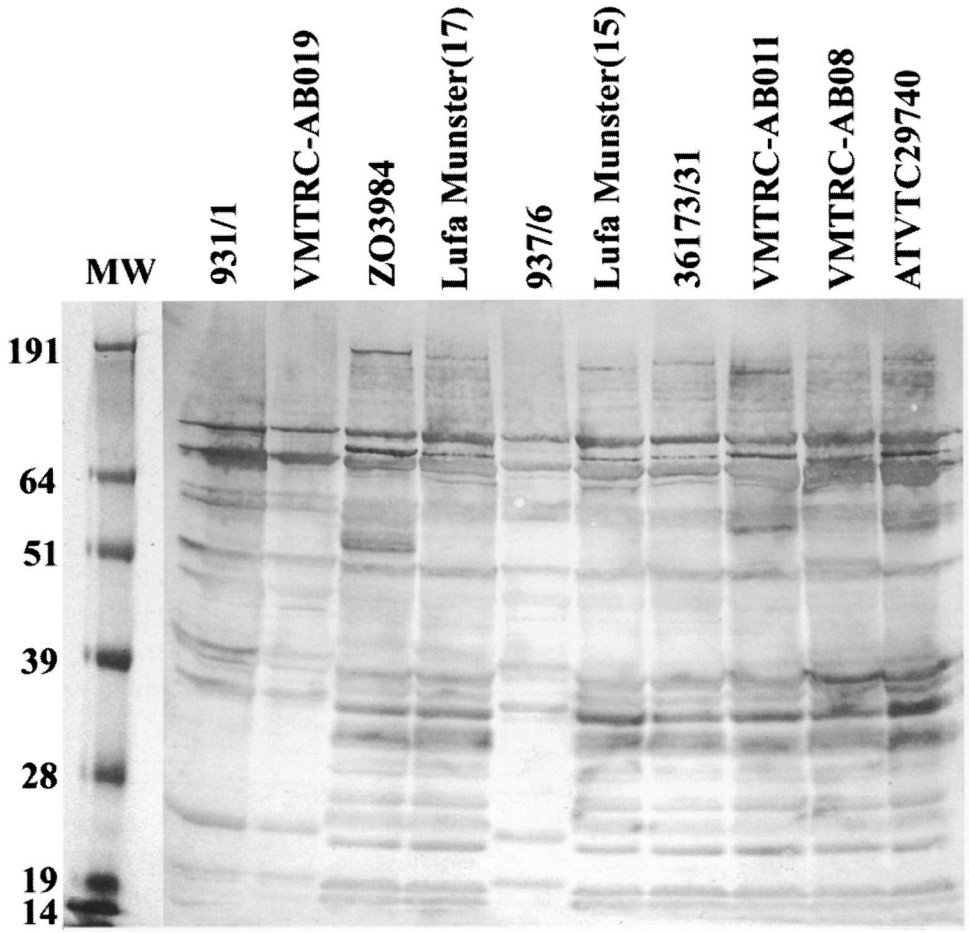

Figure 3. Cluster analysis performed by the unweighted pair-group method using arithmetic averages (UPGMA), and calculation of the immunoblot relatedness (US and German isolates).