

# Inhibition of Dermal MRSA Colonization by Microalgal Micro- and Nanoparticles

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## Key Words

Methicillin-resistant *Staphylococcus aureus* • Micro- and nanoparticles • Prevention of colonization • Animal skin

## Abstract

The aim of this study was to investigate the prevention of the dermal colonization of methicillin-resistant *Staphylococcus aureus* (MRSA) strains by the application of micro- and nanoparticles called Maresome<sup>TM</sup>. Maresome<sup>TM</sup> were prepared from selected microalgae by a novel emulsion technique. They contain lipids and all other components of the microalgae in an encapsulated form. It could be shown that Maresome<sup>TM</sup> prepared from a cyanobacterial strain of the order Nostocales (Bio33-Maresome<sup>TM</sup>) were able to inhibit the dermal colonization of different MRSA strains (North German Epidemic Strain, Col, N315) and even of the vancomycin-resistant strain MU50 in the models 'mouse ear' and 'cow udder teat'. Pretreatment of the skin with Maresome<sup>TM</sup> reduced the number of attached MRSA by 3–4 log units in comparison to the control. We assume that a prophylactic skin care with Maresome<sup>TM</sup> could complete the multibarrier anti-infectious strategy against MRSA.

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## Introduction

The occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains is a worldwide problem [1–3]. In Germany, presently more than 20% of nosocomial *S. aureus* isolates are MRSA [4, 5]. In other countries, e.g. Japan, France or the USA, the proportion of MRSA exceeds  $\geq 50\%$ . Colonized persons are the main reservoir for MRSA, and hand-to-hand contact is the main vector for transmission [4, 5].

A general concept for the prevention of MRSA infection requires establishment of a multibarrier anti-infectious strategy of hospital hygiene [5], including MRSA monitoring of patients at hospital admission [6], isolation and antiseptic sanitation and/or antibiotic treatment of colonized or infected patients and medical staff [7, 8].

In this paper a new approach to prevent the dermal colonization by MRSA by the use of novel microalgal preparations is presented. Microalgae possess a large variety of high-value compounds like antimicrobial lipids, polyunsaturated fatty acids, proteins, vitamins and minerals [9], and have a high capacity for water retention [10]. The importance of microalgae as source of new drugs and food supplements is strongly increasing [9]. The microencapsulation of the biomass of selected microalgae leads to micro- and nanoparticles with the trade name Maresome<sup>TM</sup> [11]. We investigated the effect of these particles

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**Table 1.** Formula of the cyanobacteria micro- and nanoparticles (Maresome™)

Content	Amount/ condition
Biomass (lipid composition in table 2), g	5.00
Emulsifying agent (Plantacare 2000®), g	0.05
Demineralized water, g	45.00
Cycles of homogenization	4
Working pressure, bar	600
Temperature, °C	25

on the colonization of *S. aureus* on the skin in two animal skin-derived test models (mouse ear and cow udder teats) and used as test organisms the following MRSA strains: North German Epidemic Strain (NES), Col, MU50 and N315. The NES was isolated in 1966 and has been disseminated widely in Europe and North America for an extended period of time [12]. The MRSA strain Col was isolated in the early 1960s from the operating room of a hospital in Colindale (UK) [13] and exhibits high methicillin resistance as well as resistance against tetracycline. The strain MU50 was isolated in 1996 from the pus of a Japanese male baby with a surgical wound infection which was resistant to vancomycin [14]. The MRSA N315 was isolated in a Japanese hospital from pharyngeal smear in 1982 [15]. The strains Col, MU50 and N315 are completely sequenced and frequently used in studies [16–18].

## Materials and Methods

### Microalgae

The microalgal strain Bio33 belongs to the species *Anabaena cylindrica* (Nostocales). It was isolated from a water sample collected from the Baltic Sea near Rügen Island (Germany) and established as laboratory culture in BG 11 medium  $\pm$  0.5% NaCl [11]. The strain is maintained in the culture collection of the Department of Pharmaceutical Biology, Institute of Pharmacy, Ernst Moritz Arndt University, Greifswald. Additionally, the cyanobacterium *Oscillatoria redekei* HUB 051 (provided by the Institute of Ecology, Humboldt University, Berlin) and commercially available strains of *Chlorella*, *Spirulina* and *Nostoc* (IGV Potsdam, Germany) were evaluated. For large-scale cultivation, the cyanobacteria were cultivated under continuous light in a glass column containing 40 liters of BG 11 medium under aeration with air and carbon dioxide. The concentration of carbon dioxide was regulated to maintain a pH of 8.5 [19]. The biomass was harvested after 5-week large-scale cultivation by centrifugation (4,500 rpm for 20 min), freeze dried and stored at  $-20^{\circ}\text{C}$  until use. The composition of the harvested biomass was adequately controlled using microscopic and chromatographic methods so that there was no batch-to-batch variation.

### Preparation of Micro- and Nanoparticles (Maresome™)

The preparation of micro- and nanoparticles was done according to PCT/DE 03/00747 [11]. A mixture of biomass and n-hexane was produced (1:10 v/v). The organic solvent was evaporated using a solvent rotation evaporator, Rotavapor® R-114 (Büchi AG, Uster, Switzerland). A presuspension of the biomass and a surfactant-water mixture were produced by a stirring machine (rotor-stator principle). This presuspension was homogenized by a high-pressure homogenizer (APV Deutschland GmbH, Unna, Germany) resulting in the preparation of Maresome™. The components and conditions are given in table 1. The particle size distribution was determined with a laser diffractometer (Malvern Mastersizer X, Malvern, UK) at a wavelength of 633 nm. The zeta potential was measured in NaCl  $10^{-3}$  M using a Zetasizer 4 (Malvern Instruments). For the preparation of Maresome™-containing ointments, 1 ml of the micro- and nanoparticle suspension was mixed into 1 g o/w emulsion (Heitland & Petre International GmbH, Celle, Germany). Maresome™-containing ointments were prepared with the biomasses of Bio33, *Oscillatoria*, *Chlorella*, *Spirulina* and *Nostoc*.

### Extraction and Chemical Investigations

The biomass of Bio33 was extracted successively with n-hexane, methanol (MeOH) and water for 3 h under stirring. The extracts were evaporated to dryness under reduced pressure in a rotavapor at  $40^{\circ}\text{C}$  and stored at  $-20^{\circ}\text{C}$  until use. Because the composition of lipids is important for the preparation of Maresome™, the fatty acids and fatty acid esters in the n-hexane extract of Bio33 were determined by gas chromatography with a GC80007 MD800 (Fisons Instruments, Manchester, UK; table 2).

### Test Organisms

The used MRSA test strains were NES (culture collection of the Friedrich Loeffler Institute of Medical Microbiology, University of Greifswald, reference strain), Col, MU50 and N315 (culture collection of the Institute of Microbiology, University of Greifswald). In addition a methicillin-susceptible *S. aureus* (MSSA) strain (ATCC 6538) and a coagulase-negative *Staphylococcus epidermidis* strain (SBUG 847) were tested (culture collection of the Institute of Microbiology, University of Greifswald).

For the preparation of the test suspensions, colonies were suspended in 0.9% NaCl solution to get an optical density of 0.33. The final concentration for each test is described below.

In order to verify the identity of staphylococci after treatment, selected isolates were characterized by the following assays:

- Staphaurex Plus test (Remel Europe Ltd., Dartford, UK) for selection of clumping-factor-positive staphylococci;
- API Staph system (Biomérieux Deutschland GmbH, Nürtingen, Germany) for biochemical identification of *S. aureus*;
- Mastalex-MRSATM test 185011 (Mast Diagnostica GmbH, Reinfeld, Germany) for identification of MRSA.

The enumeration of colonies was performed using the colony counter Scienceware® (Bel-Art Products, Pequannock, N.J., USA).

The test kits ID 32-Staph and API 50 CH (Biomérieux Deutschland GmbH) were used for the identification of *Staphylococcus caprae*, *Staphylococcus chromogenes*, *Staphylococcus xylos*, *Bacillus pumilus*, *Bacillus licheniformis* and *Micrococcus lylae* as constituents of the normal flora of cow udder skin.

For the agar diffusion test which was done according to the method of the European Pharmacopeia 2005 [20], the following

**Table 2.** Fatty acids of the microalgae Bio33 (relative composition)

Short name	Trivial name	Systematic name	Amount of fatty acid %
14:0	myristic acid	tetradecanoic acid	0.9
16:0	palmitic acid	hexadecanoic acid	48.8
16:1 ( $\Delta$ 7)	palmitoleic acid (isomer)	7- <i>cis</i> -hexadecanoic acid	3.7
16:2 ( $\Delta$ 7,10)		7,10- <i>cis</i> -hexadecadienoic acid	2.8
16:3 ( $\Delta$ 7,10,13)		7,10,13- <i>cis</i> -hexadecatrienoic acid	4.1
17:0	margaric acid	heptadecanoic acid	0.1
18:0	stearic acid	octadecanoic acid	0.7
18:2 ( $\Delta$ 9,12)	linoleic acid	9,12- <i>cis</i> -octadecadienoic acid	8.1
18:3 ( $\Delta$ 9,12,15)	linolenic acid	9,12,15- <i>cis</i> -octadecatrienoic acid	30.2
22:0	behenic acid	docosanoic acid	0.5
23:0		tricosanoic acid	0.1

strains were used: *S. aureus* (NES), *S. aureus* (ATCC 6538), *S. haemolyticus* 535 (Institute of Hygiene of Mecklenburg-Vorpommern, Germany), *S. epidermidis* 847 (Institute of Hygiene of Mecklenburg-Vorpommern, Germany), *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231). The diameter of the inhibition zone was given without the paper disk of 6 mm.

#### Mouse Ear Model

##### Treatment with Maresome™

For each test 1 ear of a living mouse (8 weeks old female BALB/C mouse, Charles River GmbH, Sulzfeld, Germany) was treated with Maresome™-containing ointment (about 4 mg) once per day for 3 days. The other ear of the same animal was treated with Maresome™-free ointment (control). After sacrificing the animals on day 4, the ears were cut off and fixed on clamps.

##### Direct Contamination

Five microliters of bacterial suspension [ $132 \pm 35$  colony-forming units (CFU) of NES/ $\mu$ l] were uniformly spread with a glass spatula on an area of 20 mm<sup>2</sup> of pretreated ears. The ears were incubated for 90 min at 30°C (incubator Jouan EB 53, Jouan GmbH, Unterhaching, Germany). After incubation, the ears were rubbed across the surface of Mueller-Hinton II agar plates (Becton Dickinson GmbH, Heidelberg, Germany) for 5 s. After that the plates were incubated aerobically for 48 h at 30°C. Thereafter the colonies were checked for MRSA and enumerated.

##### Skin-to-Skin Transmission (Simulation 'Donor' and 'Acceptor')

'Donor ears' were contaminated by spreading of 5  $\mu$ l NES suspension ( $132 \pm 35$  CFU of NES/ $\mu$ l) by a glass spatula on each ear. They were incubated for 90 min at 30°C. 'Acceptor ears' were pretreated with Maresome™-containing ointment (test) or with Maresome™-free ointment (control). Each acceptor ear was brought into close contact with one donor ear for 10 s. Former experiments with different contact times (5, 10, 20, 30, 60 s) had shown that the contact time has no significant influence on the number of colonies. Then the acceptor ears were incubated for 90 min at 30°C. Thereafter the acceptor ears were rubbed across the

surface of Mueller-Hinton II agar plates and incubated for 48 h at 30°C. The colonies were checked for MRSA and enumerated.

#### Cow Udder Teat Model

##### Direct Contamination

Cow udder teats were obtained from the abattoir at Anklam (Germany) directly after slaughter and kept on ice. The cow udder teats were disinfected with 70% propan-2-ol and fixed on clamps. In additional experiments the recovery rate of the bacteria from the skin was determined. For this purpose, an area of 10 × 10 mm was contaminated with 10  $\mu$ l of bacterial suspension ( $110 \pm 16$  CFU/ $\mu$ l). The viable MRSA on the cow udder teat were recovered quantitatively by rinsing of the bacteria with 500  $\mu$ l NaCl solution or by direct streaking of the contaminated skin on Mueller-Hinton II agar plates:  $124 \pm 88$  CFU were detected in the rinsing solution. After the direct streaking of the skin on the agar plate,  $570 \pm 125$  CFU/plate were enumerated. Because of the higher recovery rate with the direct streaking, we used this method for further assays.

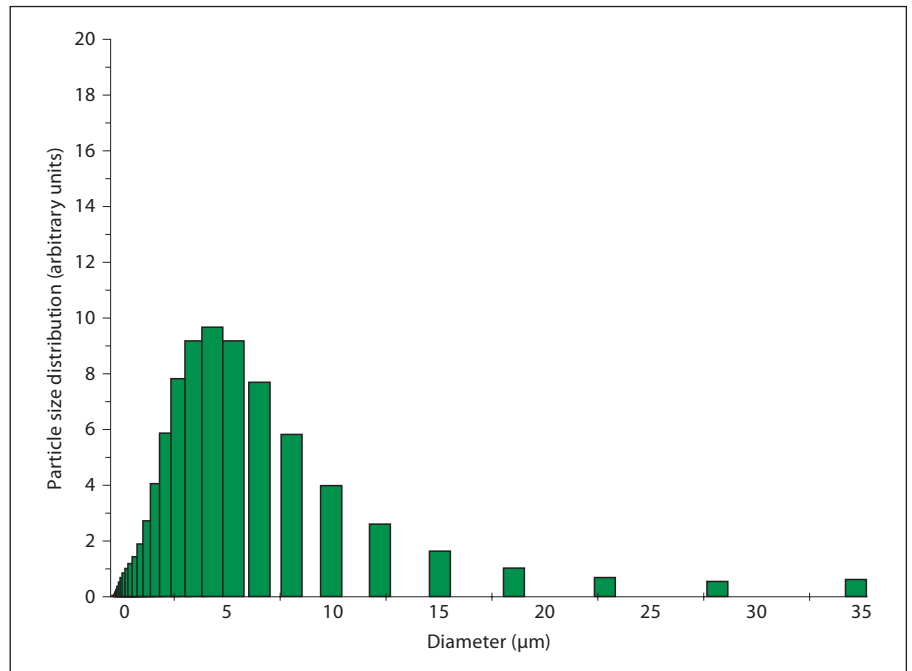
Two hours after disinfection, Maresome™ ointment (about 28 mg) was applied onto a marked area of the skin (10 × 10 mm). In controls, Maresome™-free ointment was used in the same way. Thirty minutes later, 10  $\mu$ l of bacterial suspension ( $110 \pm 16$  CFU/ $\mu$ l) were pipetted onto each area.

The contaminated cow udder teats were incubated for 90 min at 30°C. After this the teats were rubbed across the surface of Mueller-Hinton II agar plates. The plates were incubated for 48 h at 30°C. Thereafter the colonies were checked for MRSA and enumerated.

Comparison tests were done with algal extracts instead of Maresome™ and with Col, MU50, N315 and MSSA instead of NES.

##### Skin-to-Skin Transmission

'Donor cow udder teats' were prepared by spreading of 10  $\mu$ l MRSA suspension ( $110 \pm 16$  CFU/ $\mu$ l) by a glass spatula in a marked area (10 × 10 mm). The donor cow udder teats were incubated for 90 min at 30°C. 'Acceptor cow udder teats' were treated with Maresome™. The identical number of donor and acceptor teats was used. After 90 min, the acceptor was brought into



**Fig. 1.** Particle size distribution of the Maresome™ prepared using Bio33.

close contact with the donor for 10 s, after which the acceptors were incubated for 90 min at 30°C. After incubation, the acceptor teats were rubbed across the surface of Mueller-Hinton II agar plates (Becton Dickinson GmbH), and the plates were incubated for 48 h at 30°C. Thereafter the colonies were checked and enumerated for MRSA and MSSA.

#### Statistical Analysis

The data were analyzed using the  $\chi^2$  test.

## Results

### Characterization of the Maresome™

The Maresome™ suspensions are very stable in water. This is confirmed by the measured zeta potential (−38.8 mV) of Bio33-Maresome™, which is typical of repulsive negatively charged particles in suspensions [21]. Other microalgal Maresome™ have a lower zeta potential, for example *Chlorella*-Maresome™ (−23.5 mV) or *Spirulina*-Maresome™ (−32.7 mV). The particle size distribution of the Bio33-Maresome™ demonstrated in figure 1 confirms the good stabilization. The mean particle size is 5.3 µm, and the Maresome™ preserved their particle size even after a long storage time of 12 months. The mean particle sizes of *Oscillatoria*-, *Nostoc*- and *Chlorella*-Maresome™ are in the same order. The *Spirulina*-Maresome™ are smaller with a mean particle size of 750 nm.

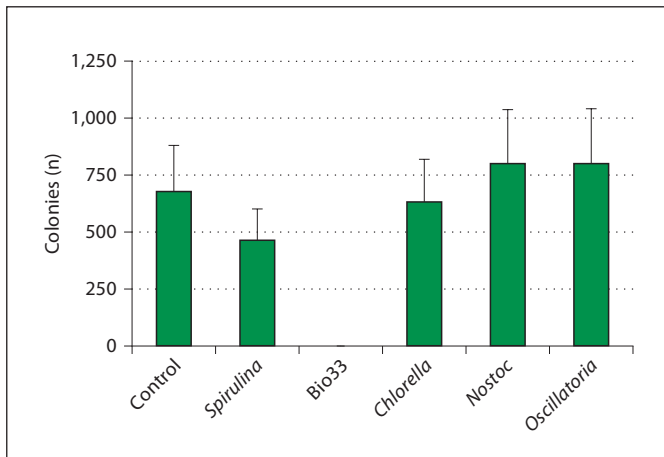
The degree of penetration was studied by applying fluorescein-labeled Maresome™ to cow udder skin and observing it by fluorescence microscopy (data not shown). Maresome™ penetrate only the upper layer of the skin.

Maresome™ are not active in the agar diffusion assay; a minimal inhibitory concentration could not be estimated.

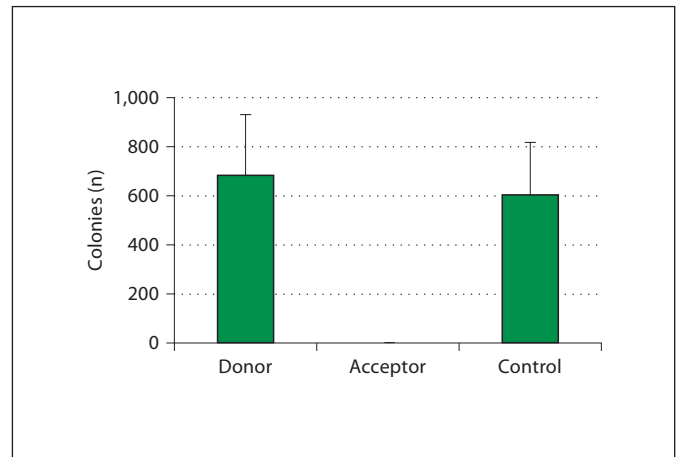
### Investigation of Bio33 Extracts (Fatty Acid Analysis and Antimicrobial Activity)

The lipid composition of the microalgae is of special importance for the preparation technique of the Maresome™ and for their possible activities. Therefore we analyzed the fatty acids of Bio33 by gas chromatography. The results are summarized in table 2. The quantitatively dominating fatty acids are the saturated fatty acid palmitic acid (C16:0; 48.8%), the polyunsaturated fatty acids linolenic acid [C18:3 ( $\Delta$ 9,12,15); 30.2%] and linoleic acid [C18:2 ( $\Delta$ 9,12); 8.1%]. The presence of a palmitoleic acid isomer [C16:1 ( $\Delta$ 7); 3.7%], of the unusual fatty acids C16:2 ( $\Delta$ 7,10; 2.8%) and C16:3 ( $\Delta$ 7,10,13; 4.1%) and of myristic acid (C14:0; 0.9%) is noteworthy.

The antimicrobial activity of extracts of the biomass of Bio33 in the agar diffusion test against all tested bacterial strains was very low (diameter of inhibition zone <3 mm). The extracts were not able to prevent dermal MRSA colonization in the mouse ear and cow udder teat model.



**Fig. 2.** Influence of Maresome™ prepared from biomass of Bio33 (n = 50), *Spirulina*, *Chlorella*, *Nostoc* and *O. redekei* (n = 6) on the colonization of NES in the model ‘mouse ear’ (direct contamination).



**Fig. 3.** Influence of Maresome™ prepared from biomass of Bio33 on the colonization of NES in the model ‘mouse ear’ (skin-to-skin transmission; n = 18).

#### Investigation of Maresome™ in the Mouse Ear Model

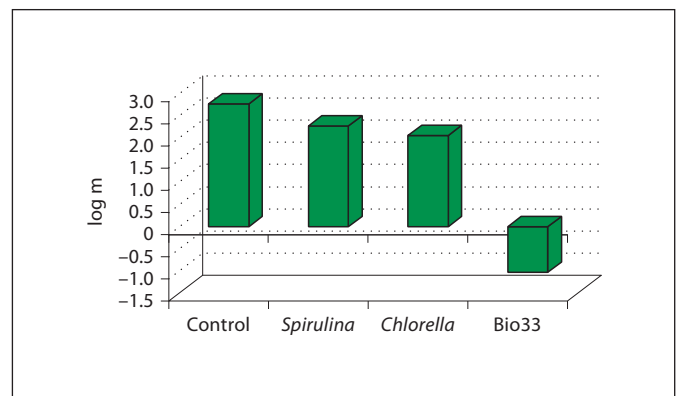
In the direct contamination assay, we found  $675 \pm 270$  CFU (n = 50) at the controls pretreated with Maresome™-free ointment. The pretreatment with Bio33-Maresome™ led to a significant decline in cultivability of the introduced NES on the mouse ear (significant,  $p = 0.001$ ). *Spirulina*-, *Oscillatoria*-, *Nostoc*- and *Chlorella*-Maresome™ only reduced the number of colonies (fig. 2).

In the skin-to-skin transmission assay, the mean number of colonies at the donor ears was  $683 \pm 308$  CFU (n = 50) after 48 h incubation. This corresponds to 31% of colonies determined directly on the agar plates instead of the mouse ears. After simulation of the hand-to-hand contact, the mean number of colonies at the acceptor control (pretreatment with Maresome™-free ointment) was  $601 \pm 214$  CFU (fig. 3). Just as in the direct contamination assay, the pretreatment of the acceptor with Bio33-Maresome™ led to a significant net decline in the number of introduced NES at the pretreated acceptor site ( $p = 0.001$ ).

#### Investigation of Maresome™ in the Cow Udder Teat Model

The results of the direct contamination assay in the cow udder teat model are demonstrated in figure 4.

Just as in the mouse ear model, only Bio33-Maresome™ inhibited nearly completely the growth of the NES ( $p = 0.001$ ). In 152 of 167 tests, we could not find any NES in the pretreated skin area. Only in 15 of 167 tests we found a small number of MRSA colonies. Therefore



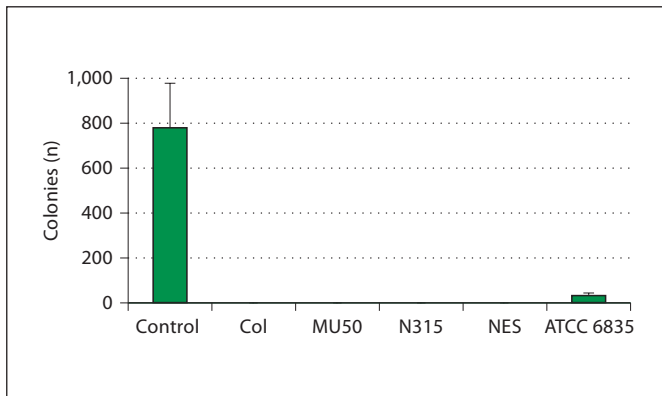
**Fig. 4.** Influence of Maresome™ prepared from biomass of *Chlorella* (n = 14), *Spirulina* (n = 14) and Bio33 (n = 167) compared to the control (n = 57) on the colonization of NES in the model ‘cow udder teat’ (direct contamination).

we calculated the mean number of expected colonies based on Poisson distribution. The mean number of expected colonies  $m$  was calculated by the following formula and is markedly  $<1$ :

$$m = -\ln \frac{\text{number of tests without detected colonies}}{\text{total number of tests}} = 0.09$$

In the controls (pretreatment with Maresome™-free ointment),  $685 \pm 165$  CFU of NES were determined.

Maresome™ prepared from *Chlorella* and *Spirulina* were not able to prevent the MRSA colonization (fig. 4).



**Fig. 5.** Influence of Maresome™ prepared from biomass Bio33 on the colonization of several MRSA strains (Col, MU50, N315, NES) and an MSSA strain (ATCC 6835) in the model 'cow udder teat' (direct contamination; n = 54).

The prevention of MRSA colonization by Bio33-Maresome™ was also detected for the 3 other multiresistant MRSA strains (fig. 5). The inhibition of the growth of the strains with a very broad resistance spectrum, including resistance to vancomycin (MU50), was remarkable.

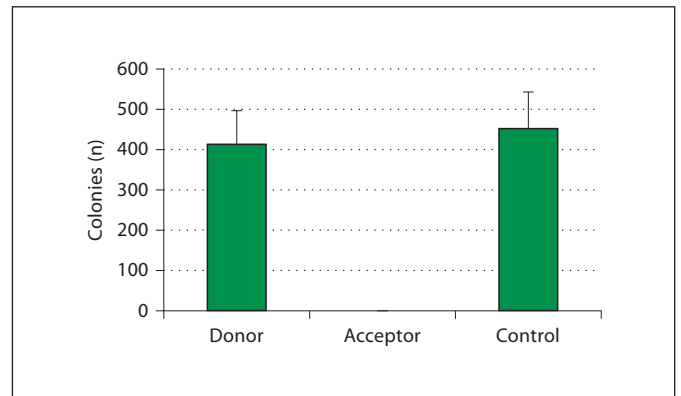
The effects against MSSA were weaker than against MRSA:  $33 \pm 22$  CFU were detected after pretreatment of the cow udder teat skin with Bio33-Maresome™. The colonization of MSSA was significantly prevented in approximately 70% of the samples.

In contrast to MRSA, the apathogenic species of the normal skin flora *S. caprae*, *S. chromogenes*, *S. xylois*, *M. lylae*, *B. pumilus* and *B. licheniformis* grew with only little inhibition after treatment with ointment containing Bio33-Maresome™ (results not demonstrated).

In the model 'cow udder teat: skin-to-skin transmission', the mean number of colonies at the donor was  $413 \pm 67$  CFU. The mean number of acceptor control was  $452 \pm 103$  CFU (fig. 6). Just as in the model 'mouse ear', the pretreatment of the acceptor with Bio33-Maresome™ completely inhibited the colonization of attached NES.

## Discussion

The presented results demonstrate that the micro- and nanoparticles, called Maresome™ and prepared from a microalgal strain belonging to the order Nostocales (Bio33), are able to prevent the colonization of different methicillin-resistant *S. aureus* strains on the skin in two animal-derived models. This effect is only attainable



**Fig. 6.** Influence of Maresome™ prepared from biomass Bio33 (n = 18) on the colonization of NES in the model 'cow udder teat' (skin-to-skin transmission).

with Bio33-Maresome™ and not with extracts of this algal strain. Maresome™ prepared from the other microalgae exhibited only low activity.

To explain the different effects of Maresome™ and extracts, the special properties of Maresome™ should be taken into consideration. In contrast to an extract, the particles contain the whole spectrum of substances present in the biomass. The matrix consists mainly of lipids. Due to their form and small size, lipid-containing Bio33 particles offer a very large surface to interact with bacteria. The penetration of the lipid particles depends on the particle size and other physicochemical properties [22]. Due to their size, only few particles can penetrate the outer layers of the stratum corneum. This is in correspondence with an investigation of Cross et al. [23], who described a minimal penetration of a sunscreen nanoparticle formulation. The main part of the Maresome™ forms a film on the outer layers of the stratum corneum and could interact there with the MRSA. Another aspect is the difference in the zeta potentials. The Bio33-Maresome™ have a much greater negative zeta potential ( $-38.8$  mV) than the biomass ( $-16.2$  mV) or other microalgal Maresome™ (e.g. with *Chlorella*  $-23.5$  mV). The strong negative zeta potential of the Bio33-Maresome™ leads to a high stability of the suspension system and a repulsion potential [21]. The direct interaction between bacteria and surfaces depends in the first step on the combination of attractive van der Waals forces and repulsive Coulomb forces. Satou et al. [24] demonstrated that the electrical repulsion forces strongly contribute to the adherence of cells with a high absolute zeta potential. The cell surface

of *S. aureus*, as in most bacteria, has a negative charge [25], which is probably influenced by the teichoic acids. Therefore it can be assumed that the adherence of *S. aureus* will be inhibited by the repulsion forces between negatively charged bacteria and the negatively charged Bio33-Maresome™ at the surface of the skin.

With respect to the effectiveness of Bio33-Maresome™ and the much smaller or lacking effects of the Maresome™ prepared from other microalgae, the different lipid composition could play a role. The antimicrobial effects of some fatty acids and esters are well known [26–30]. Strong inhibitors of *S. aureus* are e.g. lauric acid, capric acid, myristic acid, linoleic acid [29] and some isomers of palmitoleic acid [31]. In the investigations of Kelsey et al. [30], one strain of *S. aureus* was susceptible also to palmitic acid. They found that linoleic acid leads to a delay in the initiation of growth of the bacteria. The saturated myristic acid inhibited growth of bacteria through a decrease in the maximal amount of growth [30]. The effects of fatty acids might be caused mainly by their surfactant activity. Already small amounts of fatty acids change membrane fluidity, disturb signal transduction and reduce the production of virulence factors [32]. The direct biocide effect occurs only in higher concentrations [31]. Fatty acids can influence also the adhesion of microorganisms. While palmitoleic acid [C16:1 ( $\Delta$ 6)] at concentrations below 0.5 mg/ml had little effect on the growth of *C. albicans*, at concentrations above 0.1 mg/ml it is effective in preventing the attachment of pathogenic yeast cells to isolated sheets of mammalian stratum corneum [31].

Analysis of the fatty acids of Bio33 revealed remarkable differences to the fatty acid composition of *Spirulina platensis* [33] and the other used microalgae [34]. In contrast to the other microalgae, Bio33 contains many unusual fatty acids, especially isomers of palmitoleic acid and linolic acid, and the content of linolenic acid and myristic acid is higher than in the other microalgae.

The stronger effect of Bio33-Maresome™ on MRSA than on the bacteria of the normal skin flora could probably be ascribed to the stronger extracellular lipase activ-

ity of *S. aureus*. In other investigations we found that especially MRSA possess a considerable higher activity of extracellular lipases than coagulase-negative strains [unpubl. results]. This corresponds to other reports about the important role of lipase activity in facilitating bacterial colonization in nutrient-limited environments such as the human skin [35]. The higher lipase activity of MRSA could influence the release of antimicrobial fatty acids from Maresome™, and the composition of Maresome™ could also affect the lipase activity of MRSA. This should be investigated in future work.

Summarizing the results and the present knowledge, we assume that Bio33-Maresome™ have a multifactorial activity corresponding to the multifactorial process of adherence and colonization of *S. aureus* on the skin [36, 37].

For the elucidation of the effects of Maresome™ in a next step the global expression of MRSA under the influence of Maresome™ should be investigated. In addition anticolonization effects of ointments containing Bio33-Maresome™ against MRSA will be investigated in clinical studies. If the results are reproducible on human skin, a prophylactic skin care with Bio33-Maresome™ can complete the multibarrier anti-infectious strategy of hospital hygiene. A prophylactic skin care with Bio33-Maresome™ for medical personnel and for patients after hand disinfection is envisioned. Except for disinfection, the use of antiseptics e.g. for cleaning can thus be reduced. A prophylactic skin care with Maresome™ could therefore be a strategy to reduce costs for treatment of MRSA colonization and infection, respectively.

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