

2006

Methanogens as Ruminant-Specific Indicators of Fecal Pollution

Jennifer A. Ufnar

University of Southern Mississippi

Shiao Y. Wang

University of Southern Mississippi

R.D. Ellender

University of Southern Mississippi

Follow this and additional works at: https://aquila.usm.edu/mst_presentations

Recommended Citation

Ufnar, Jennifer A.; Wang, Shiao Y.; and Ellender, R.D., "Methanogens as Ruminant-Specific Indicators of Fecal Pollution" (2006).
Presentations. 4.

https://aquila.usm.edu/mst_presentations/4

This Poster is brought to you for free and open access by the Microbial Source Tracking at The Aquila Digital Community. It has been accepted for inclusion in Presentations by an authorized administrator of The Aquila Digital Community. For more information, please contact Joshua.Cromwell@usm.edu.



Methanogens as Ruminant-Specific Indicators of Fecal Pollution

Q489

For More Information Contact:
Dr. R.D. Ellender
University of Southern Mississippi
Department of Biological Sciences
118 College Drive #5018
Hattiesburg, MS 39406-0001
601-266-4720 or 601-266-4752(lab)

J. A. Ufnar¹, S. Y. Wang¹, H. Yampara-Iquise², C. A. Carson², D. F. Ufnar¹, R. D. Ellender¹;
¹University of Southern Mississippi, Hattiesburg, MS, ²University of Missouri, Columbia, MO

Abstract

Tracking the source of fecal pollution in surface waters has traditionally focused on the origin of enteric indicators including coliforms, enterococci, or *E. coli*. Recently, questions of genetic variability and environmental persistence have encouraged researchers to investigate additional animal specific indicators of fecal pollution. To date only eubacteria have been utilized as markers of human and animal-specific pollution. Here we report domestic ruminant-specific markers of fecal pollution utilizing methanogens found in the rumen. PCR primers for the *mcrA* gene of *Methanomicrobium mobile* (MMmcrA) and the *nifH* gene of *Methanobrevibacter ruminantium* (Mrmf-1) were designed, tested, and used to detect ruminant-specific pollution in fecal and environmental samples. The MMmcrA amplicons were detected in expected fecal and environmental samples (71% cow, 92% sheep, 50% goat, 100% cow lagoon samples, and 100% creek contaminated with cow lagoon waste), and were observed in only 5% of human and deer fecal samples. Mrmf-1 amplification was seen in 80% cow, 100% sheep, and 54% goat fecal samples; only 2% individual human samples were positive. No PCR amplification was observed when the MMmcrA and Mrmf-1 primers were tested against 47 bacterial stock cultures and fecal samples from 134 non-ruminant animals. More importantly, no amplification was observed in sewer samples using either primer pair. Sensitivity assays using MMmcrA primers demonstrated a detection limit of 0.01ng total DNA in bovine feces, 10ng in fecally contaminated surface water, and 5ng in cow lagoon samples.

Introduction

Fecal pollution of recreational waters from fecal and non-human sources can lead to human health risk of pathogen exposure and economic loss from closure of beaches and shellfish harvesting areas. Agricultural waste constitutes a significant portion of pollution in water lakes, rivers, and estuaries, and may pose human health risk due to domestic ruminant-associated pathogens. Cattle feed lots are a significant source of fecal pollution of surface waters by waste lagoon overflow or manure spreading. Plans for remediation of waterways require distinguishing fecal pollution from various hosts.

Most available techniques for fecal source differentiation utilize indicator eubacteria with dubious host specificity and variable survival times in the external environment. However, intestinally-derived methanogens are reportedly specific for particular host species, and we recently reported that the *nifH* gene of *Methanobrevibacter smithii* is a potentially valuable marker for sewage pollution.

Methanomicrobium mobile and *Methanobrevibacter ruminantium* are considered dominant methanogens in the rumen. Because these organisms have been found only in the intestinal tract of ruminants and are unable to survive in the outside environment we examined their use as possible domestic ruminant-specific markers of fecal pollution. The *mcrA* gene of *Methanomicrobium mobile* and the *nifH* gene of *Methanobrevibacter ruminantium* were targeted due to methanogen specificity. The *mcrA* gene codes for the α -subunit of methyl coenzyme-M reductase, which is responsible for catabolism of methyl-coenzyme M and coenzyme-B to heterodisulphide and methane during methanogenesis. The methanogen *nifH* operon, although conserved in methanogens and prokaryotes, have been phylogenetically grouped with the pseudo-Nif clusters, corresponding to Group IV *nifH* homologs found only in methanogens. These *nifH* genes do not code for a functional nitrogenase and have significant sequence divergence from the other groups. The specificity of the PCR assays for detection of the *mcrA* and *nifH* genes was examined by analyzing domestic ruminant feces (bovine, ovine, and goat), non-domestic ruminant feces (deer), sewage, non-ruminant feces (human, pig, dog, horse, turkey, chicken, and goose). DNA from eubacteria, various methanogens, and bacteria isolated from the environment were also studied. Environmental samples collected from a dairy farm waste lagoon and creek contaminated with lagoon effluent were analyzed to determine the specificity of the PCR assay in detecting recently discharged fecal contamination into surface waters.

Materials and Methods

Primer Specificity

Sampling and DNA Extraction:

Primer specificity was tested against the type strains of each species (positive controls) and 12 additional methanogen genera as negative controls for each assay (27 methanogen species/strains total; Table 1). Cultures were applied to Whatman FTA cards and prepared for PCR by washing 3X (5 min) with FTA Purification Reagent and 2X (5 min) with TE-1 buffer.

The specificity of the MMmcrA primers was further determined by testing 548 Gram (+) and Gram (-) environmental bacteria as negative controls. Water samples from sewage lagoons in Hattiesburg, MS, Mississippi coastal sewage lift stations, and Mississippi Department of Environmental Quality coastal sampling sites (MDEQ Stations 8 & 9) were processed by filtering through a 47mm, 0.45 μ m filter and grown on Gram (+) and Gram (-) selective media. Whole-cell PCR was conducted on the environmental isolates from each media using the MMmcrA amplification conditions below.

Human and animal feces (Table 2) were collected and processed in Hattiesburg, MS and Columbia, MO. DNA was extracted from fecal samples using the UltraClean Soil DNA Extraction kit and the Powersoil DNA Kit (MO BIO Labs, Carlsbad, California) following the manufacturer instructions. Extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). DNA from human and animal fecal samples was extracted at the University of Missouri campus using the BIO 101 Fast DNA Spin Kit for soil (Q-Biogene, Inc., Carlsbad, CA) and quantitated using a BioSpec-mini DNA/RNA/protein analyzer (Shimadzu, Scientific Instruments, Inc., Bohemia, NY).

Sewer samples (500 ml) were collected from seven different sewers in Gulfport, MS each week for a period of two months in 2004-2005 (Table 3). Water samples (500 ml) were collected from four different coastal sampling stations for a three-month interval and tested using the MMmcrA assay (Table 3). Each sample was prefiltered through a 3 μ m cellulose acetate filter (Pall Corporation, West Chester, PA) and concentrated onto a 0.2 μ m Supor[®]-200 filter (Pall Corporation). The bacteria were dislodged and centrifuged for 15 min at 13,000 x g. The DNA from pelleted cells was extracted and used in subsequent PCR reactions. Sewer samples were processed at the University of Missouri campus by concentrating 40 mL aliquots by centrifugation (2,052 X g for 15 min at 4°C), resuspending the pellet in 2 mL residual supernatant, and extracting the DNA using the BIO 101 FastDNA Spin Kit for Soil (Q-Biogene, Inc.).

DNA from coastal sediments was tested with the MMmcrA assay (Table 3). Sediment cores were taken at four Mississippi Department of Environmental Quality (MDEQ) sampling stations using a sterile conical-shaped 50 ml centrifuge tube (Sarstedt, Newton, North Carolina). DNA was extracted from surface sediment samples and 6 and 12-inch sediment samples using the UltraClean Soil DNA Extraction Kit (MO BIO).

PCR Conditions:

Whole cell PCR for both the MMmcrA and the Mrmf-1 assays was performed on overnight cultures of Gram (+) and Gram (-) eubacteria (10⁹ bacteria/ml). Ten μ L PCR reactions were performed for whole cell eubacteria and with DNA extracted from fecal samples. Each reaction contained: 1X PCR Buffer (New England Biolabs, Ipswich, MA), 0.1% BSA, 200 μ M dNTP (USB Corporation), 0.5U Taq Polymerase (New England Biolabs), 0.5 μ M primer, 1 μ L template cells (approximately 1 x 10⁹ cells \cdot ml⁻¹) or 1 μ L DNA template. A lower detection limit using the MMmcrA assay in bovine, ovine, and goat feces was established by adding 50ng, 20ng, 10ng, 5ng, 1ng, and 0.1ng fecal DNA to a 20 μ L PCR reaction. Environmental samples (sewer, water, and sediment) were amplified using 20 μ L PCR reactions containing 1X PCR Buffer (New England Biolabs), 0.1% BSA, 200 μ M dNTP (USB Corporation), 0.5U Taq Polymerase (New England Biolabs), 0.5 μ M each primer, and varying concentrations of environmental DNA.

FTA Card DNA extracts were amplified in 50 μ L reactions containing 1X PCR Buffer (New England Biolabs), 0.1% BSA, 200 μ M dNTP (USB Corporation), 2.5U Taq Polymerase (New England Biolabs), 0.5 μ M primer, and the 2.0 mm washed punch. The cycling conditions for the MMmcrA assay were an initial denaturation of 2 min at 92°C and 30 cycles of: denaturation for 1 min at 92°C, annealing for 30 sec at 60°C, and elongation for 1 min at 72°C. A final elongation was performed for 6 min at 72°C. The cycling conditions for the Mrmf-1 assay were an initial denaturation of 2 min at 92°C and 30 cycles of: denaturation for 30s at 92°C, annealing for 15sec at 62°C, and elongation for 30s at 72°C. A final elongation was performed for 6 min at 72°C.

Validation of MMmcrA Method

Two samples each (500 ml) from a bovine waste lagoon and an adjacent creek contaminated with lagoon water were collected and tested using the MMmcrA assay (Table 3). Creek water was collected approximately 1/4 mile downstream from the point where the lagoon effluent emptied into the creek. Each sample was centrifuged at 10,000 X g for 15 minutes and the resulting pellet used directly for DNA extraction using the MO BIO Power Soil DNA Extraction Kit (MO BIO). Varying amounts of total DNA from the bovine waste lagoon (130ng, 100ng, 50ng, 20ng, 10ng, 5ng, and 1ng) and adjacent creek (80ng, 20ng, 10ng, 5ng, and 1ng) were added to the PCR cocktail to determine the presence or absence of the *mcrA* gene.

Acknowledgments

This work was supported by the NOAA OHH Program, Grant No. NA04OAR4600216; USEPA Gulf of Mexico Program, Grant No. MX96401204-0; and USEPA Gulf of Mexico Program, Grant No. MX96429505.

Results

The MMmcrA primers amplified a 212bp product; the Mrmf-1 primers amplified a 336bp product. The MMmcrA and Mrmf-1 primer pairs produced the expected amplicons in only *M. mobile* and *M. ruminantium* respectively; no products were amplified in any other microorganisms tested (Table 1). Tests of fecal DNA showed that 71% of bovine fecal samples were positive for *M. mobile* (Figure 1). Among human and animal fecal samples tested, 71% of bovine fecal samples, 92% of sheep feces, 50% of goat feces, 5% deer, and 5% human fecal samples produced the expected amplicon with the MMmcrA primers (Table 2). No products were amplified using the MMmcrA primers with DNA extracts of other non-ruminant feces or sewer samples (Tables 2&3). Preliminary results for the Mrmf-1 primer pair showed amplification in only cow, sheep, and goat fecal samples (80%, 100%, and 54% respectively). No amplification was observed in non-domestic ruminant fecal samples or sewer samples (n=16).

Environmental sampling of non-polluted surface waters using the MMmcrA primers produced no amplicons in marine water (n=25) or creek samples (n=13). Samples collected from a bovine waste lagoon and a creek polluted with lagoon waste were positive using the MMmcrA and Mrmf-1 primer pairs. Using the MMmcrA primer pair, the minimal amount of DNA required to produce visible amounts of the expected amplification product were 0.01ng in cow fecal DNA, 5ng in cow waste lagoon samples, and 10ng in samples collected from a creek polluted with cow waste (Figures 1&2).

Table 1. Species tested for MMmcrA and Mrmf-1 assay specificity

Species Negative for <i>M. mobile mcrA</i> gene and <i>nifH</i> gene of <i>Methanobrevibacter ruminantium</i>	Species positive for <i>M. mobile mcrA</i> gene	Species positive for <i>M. ruminantium nifH</i> gene
<i>Staphylococcus aureus</i> ATCC 29213, <i>Staphylococcus aureus</i> ATCC 12228, <i>Staphylococcus aureus</i> ATCC 10561, <i>Staphylococcus aureus</i> ATCC 10562, <i>Staphylococcus aureus</i> ATCC 10563, <i>Staphylococcus aureus</i> ATCC 10564, <i>Staphylococcus aureus</i> ATCC 10565, <i>Staphylococcus aureus</i> ATCC 10566, <i>Staphylococcus aureus</i> ATCC 10567, <i>Staphylococcus aureus</i> ATCC 10568, <i>Staphylococcus aureus</i> ATCC 10569, <i>Staphylococcus aureus</i> ATCC 10570, <i>Staphylococcus aureus</i> ATCC 10571, <i>Staphylococcus aureus</i> ATCC 10572, <i>Staphylococcus aureus</i> ATCC 10573, <i>Staphylococcus aureus</i> ATCC 10574, <i>Staphylococcus aureus</i> ATCC 10575, <i>Staphylococcus aureus</i> ATCC 10576, <i>Staphylococcus aureus</i> ATCC 10577, <i>Staphylococcus aureus</i> ATCC 10578, <i>Staphylococcus aureus</i> ATCC 10579, <i>Staphylococcus aureus</i> ATCC 10580, <i>Staphylococcus aureus</i> ATCC 10581, <i>Staphylococcus aureus</i> ATCC 10582, <i>Staphylococcus aureus</i> ATCC 10583, <i>Staphylococcus aureus</i> ATCC 10584, <i>Staphylococcus aureus</i> ATCC 10585, <i>Staphylococcus aureus</i> ATCC 10586, <i>Staphylococcus aureus</i> ATCC 10587, <i>Staphylococcus aureus</i> ATCC 10588, <i>Staphylococcus aureus</i> ATCC 10589, <i>Staphylococcus aureus</i> ATCC 10590, <i>Staphylococcus aureus</i> ATCC 10591, <i>Staphylococcus aureus</i> ATCC 10592, <i>Staphylococcus aureus</i> ATCC 10593, <i>Staphylococcus aureus</i> ATCC 10594, <i>Staphylococcus aureus</i> ATCC 10595, <i>Staphylococcus aureus</i> ATCC 10596, <i>Staphylococcus aureus</i> ATCC 10597, <i>Staphylococcus aureus</i> ATCC 10598, <i>Staphylococcus aureus</i> ATCC 10599, <i>Staphylococcus aureus</i> ATCC 10600, <i>Staphylococcus aureus</i> ATCC 10601, <i>Staphylococcus aureus</i> ATCC 10602, <i>Staphylococcus aureus</i> ATCC 10603, <i>Staphylococcus aureus</i> ATCC 10604, <i>Staphylococcus aureus</i> ATCC 10605, <i>Staphylococcus aureus</i> ATCC 10606, <i>Staphylococcus aureus</i> ATCC 10607, <i>Staphylococcus aureus</i> ATCC 10608, <i>Staphylococcus aureus</i> ATCC 10609, <i>Staphylococcus aureus</i> ATCC 10610, <i>Staphylococcus aureus</i> ATCC 10611, <i>Staphylococcus aureus</i> ATCC 10612, <i>Staphylococcus aureus</i> ATCC 10613, <i>Staphylococcus aureus</i> ATCC 10614, <i>Staphylococcus aureus</i> ATCC 10615, <i>Staphylococcus aureus</i> ATCC 10616, <i>Staphylococcus aureus</i> ATCC 10617, <i>Staphylococcus aureus</i> ATCC 10618, <i>Staphylococcus aureus</i> ATCC 10619, <i>Staphylococcus aureus</i> ATCC 10620, <i>Staphylococcus aureus</i> ATCC 10621, <i>Staphylococcus aureus</i> ATCC 10622, <i>Staphylococcus aureus</i> ATCC 10623, <i>Staphylococcus aureus</i> ATCC 10624, <i>Staphylococcus aureus</i> ATCC 10625, <i>Staphylococcus aureus</i> ATCC 10626, <i>Staphylococcus aureus</i> ATCC 10627, <i>Staphylococcus aureus</i> ATCC 10628, <i>Staphylococcus aureus</i> ATCC 10629, <i>Staphylococcus aureus</i> ATCC 10630, <i>Staphylococcus aureus</i> ATCC 10631, <i>Staphylococcus aureus</i> ATCC 10632, <i>Staphylococcus aureus</i> ATCC 10633, <i>Staphylococcus aureus</i> ATCC 10634, <i>Staphylococcus aureus</i> ATCC 10635, <i>Staphylococcus aureus</i> ATCC 10636, <i>Staphylococcus aureus</i> ATCC 10637, <i>Staphylococcus aureus</i> ATCC 10638, <i>Staphylococcus aureus</i> ATCC 10639, <i>Staphylococcus aureus</i> ATCC 10640, <i>Staphylococcus aureus</i> ATCC 10641, <i>Staphylococcus aureus</i> ATCC 10642, <i>Staphylococcus aureus</i> ATCC 10643, <i>Staphylococcus aureus</i> ATCC 10644, <i>Staphylococcus aureus</i> ATCC 10645, <i>Staphylococcus aureus</i> ATCC 10646, <i>Staphylococcus aureus</i> ATCC 10647, <i>Staphylococcus aureus</i> ATCC 10648, <i>Staphylococcus aureus</i> ATCC 10649, <i>Staphylococcus aureus</i> ATCC 10650, <i>Staphylococcus aureus</i> ATCC 10651, <i>Staphylococcus aureus</i> ATCC 10652, <i>Staphylococcus aureus</i> ATCC 10653, <i>Staphylococcus aureus</i> ATCC 10654, <i>Staphylococcus aureus</i> ATCC 10655, <i>Staphylococcus aureus</i> ATCC 10656, <i>Staphylococcus aureus</i> ATCC 10657, <i>Staphylococcus aureus</i> ATCC 10658, <i>Staphylococcus aureus</i> ATCC 10659, <i>Staphylococcus aureus</i> ATCC 10660, <i>Staphylococcus aureus</i> ATCC 10661, <i>Staphylococcus aureus</i> ATCC 10662, <i>Staphylococcus aureus</i> ATCC 10663, <i>Staphylococcus aureus</i> ATCC 10664, <i>Staphylococcus aureus</i> ATCC 10665, <i>Staphylococcus aureus</i> ATCC 10666, <i>Staphylococcus aureus</i> ATCC 10667, <i>Staphylococcus aureus</i> ATCC 10668, <i>Staphylococcus aureus</i> ATCC 10669, <i>Staphylococcus aureus</i> ATCC 10670, <i>Staphylococcus aureus</i> ATCC 10671, <i>Staphylococcus aureus</i> ATCC 10672, <i>Staphylococcus aureus</i> ATCC 10673, <i>Staphylococcus aureus</i> ATCC 10674, <i>Staphylococcus aureus</i> ATCC 10675, <i>Staphylococcus aureus</i> ATCC 10676, <i>Staphylococcus aureus</i> ATCC 10677, <i>Staphylococcus aureus</i> ATCC 10678, <i>Staphylococcus aureus</i> ATCC 10679, <i>Staphylococcus aureus</i> ATCC 10680, <i>Staphylococcus aureus</i> ATCC 10681, <i>Staphylococcus aureus</i> ATCC 10682, <i>Staphylococcus aureus</i> ATCC 10683, <i>Staphylococcus aureus</i> ATCC 10684, <i>Staphylococcus aureus</i> ATCC 10685, <i>Staphylococcus aureus</i> ATCC 10686, <i>Staphylococcus aureus</i> ATCC 10687, <i>Staphylococcus aureus</i> ATCC 10688, <i>Staphylococcus aureus</i> ATCC 10689, <i>Staphylococcus aureus</i> ATCC 10690, <i>Staphylococcus aureus</i> ATCC 10691, <i>Staphylococcus aureus</i> ATCC 10692, <i>Staphylococcus aureus</i> ATCC 10693, <i>Staphylococcus aureus</i> ATCC 10694, <i>Staphylococcus aureus</i> ATCC 10695, <i>Staphylococcus aureus</i> ATCC 10696, <i>Staphylococcus aureus</i> ATCC 10697, <i>Staphylococcus aureus</i> ATCC 10698, <i>Staphylococcus aureus</i> ATCC 10699, <i>Staphylococcus aureus</i> ATCC 10700, <i>Staphylococcus aureus</i> ATCC 10701, <i>Staphylococcus aureus</i> ATCC 10702, <i>Staphylococcus aureus</i> ATCC 10703, <i>Staphylococcus aureus</i> ATCC 10704, <i>Staphylococcus aureus</i> ATCC 10705, <i>Staphylococcus aureus</i> ATCC 10706, <i>Staphylococcus aureus</i> ATCC 10707, <i>Staphylococcus aureus</i> ATCC 10708, <i>Staphylococcus aureus</i> ATCC 10709, <i>Staphylococcus aureus</i> ATCC 10710, <i>Staphylococcus aureus</i> ATCC 10711, <i>Staphylococcus aureus</i> ATCC 10712, <i>Staphylococcus aureus</i> ATCC 10713, <i>Staphylococcus aureus</i> ATCC 10714, <i>Staphylococcus aureus</i> ATCC 10715, <i>Staphylococcus aureus</i> ATCC 10716, <i>Staphylococcus aureus</i> ATCC 10717, <i>Staphylococcus aureus</i> ATCC 10718, <i>Staphylococcus aureus</i> ATCC 10719, <i>Staphylococcus aureus</i> ATCC 10720, <i>Staphylococcus aureus</i> ATCC 10721, <i>Staphylococcus aureus</i> ATCC 10722, <i>Staphylococcus aureus</i> ATCC 10723, <i>Staphylococcus aureus</i> ATCC 10724, <i>Staphylococcus aureus</i> ATCC 10725, <i>Staphylococcus aureus</i> ATCC 10726, <i>Staphylococcus aureus</i> ATCC 10727, <i>Staphylococcus aureus</i> ATCC 10728, <i>Staphylococcus aureus</i> ATCC 10729, <i>Staphylococcus aureus</i> ATCC 10730, <i>Staphylococcus aureus</i> ATCC 10731, <i>Staphylococcus aureus</i> ATCC 10732, <i>Staphylococcus aureus</i> ATCC 10733, <i>Staphylococcus aureus</i> ATCC 10734, <i>Staphylococcus aureus</i> ATCC 10735, <i>Staphylococcus aureus</i> ATCC 10736, <i>Staphylococcus aureus</i> ATCC 10737, <i>Staphylococcus aureus</i> ATCC 10738, <i>Staphylococcus aureus</i> ATCC 10739, <i>Staphylococcus aureus</i> ATCC 10740, <i>Staphylococcus aureus</i> ATCC 10741, <i>Staphylococcus aureus</i> ATCC 10742, <i>Staphylococcus aureus</i> ATCC 10743, <i>Staphylococcus aureus</i> ATCC 10744, <i>Staphylococcus aureus</i> ATCC 10745, <i>Staphylococcus aureus</i> ATCC 10746, <i>Staphylococcus aureus</i> ATCC 10747, <i>Staphylococcus aureus</i> ATCC 10748, <i>Staphylococcus aureus</i> ATCC 10749, <i>Staphylococcus aureus</i> ATCC 10750, <i>Staphylococcus aureus</i> ATCC 10751, <i>Staphylococcus aureus</i> ATCC 10752, <i>Staphylococcus aureus</i> ATCC 10753, <i>Staphylococcus aureus</i> ATCC 10754, <i>Staphylococcus aureus</i> ATCC 10755, <i>Staphylococcus aureus</i> ATCC 10756, <i>Staphylococcus aureus</i> ATCC 10757, <i>Staphylococcus aureus</i> ATCC 10758, <i>Staphylococcus aureus</i> ATCC 10759, <i>Staphylococcus aureus</i> ATCC 10760, <i>Staphylococcus aureus</i> ATCC 10761, <i>Staphylococcus aureus</i> ATCC 10762, <i>Staphylococcus aureus</i> ATCC 10763, <i>Staphylococcus aureus</i> ATCC 10764, <i>Staphylococcus aureus</i> ATCC 10765, <i>Staphylococcus aureus</i> ATCC 10766, <i>Staphylococcus aureus</i> ATCC 10767, <i>Staphylococcus aureus</i> ATCC 10768, <i>Staphylococcus aureus</i> ATCC 10769, <i>Staphylococcus aureus</i> ATCC 10770, <i>Staphylococcus aureus</i> ATCC 10771, <i>Staphylococcus aureus</i> ATCC 10772, <i>Staphylococcus aureus</i> ATCC 10773, <i>Staphylococcus aureus</i> ATCC 10774, <i>Staphylococcus aureus</i> ATCC 10775, <i>Staphylococcus aureus</i> ATCC 10776, <i>Staphylococcus aureus</i> ATCC 10777, <i>Staphylococcus aureus</i> ATCC 10778, <i>Staphylococcus aureus</i> ATCC 10779, <i>Staphylococcus aureus</i> ATCC 10780, <i>Staphylococcus aureus</i> ATCC 10781, <i>Staphylococcus aureus</i> ATCC 10782, <i>Staphylococcus aureus</i> ATCC 10783, <i>Staphylococcus aureus</i> ATCC 10784, <i>Staphylococcus aureus</i> ATCC 10785, <i>Staphylococcus aureus</i> ATCC 10786, <i>Staphylococcus aureus</i> ATCC 10787, <i>Staphylococcus aureus</i> ATCC 10788, <i>Staphylococcus aureus</i> ATCC 10789, <i>Staphylococcus aureus</i> ATCC 10790, <i>Staphylococcus aureus</i> ATCC 10791, <i>Staphylococcus aureus</i> ATCC 10792, <i>Staphylococcus aureus</i> ATCC 10793, <i>Staphylococcus aureus</i> ATCC 10794, <i>Staphylococcus aureus</i> ATCC 10795, <i>Staphylococcus aureus</i> ATCC 10796, <i>Staphylococcus aureus</i> ATCC 10797, <i>Staphylococcus aureus</i> ATCC 10798, <i>Staphylococcus aureus</i> ATCC 10799, <i>Staphylococcus aureus</i> ATCC 10800, <i>Staphylococcus aureus</i> ATCC 10801, <i>Staphylococcus aureus</i> ATCC 10802, <i>Staphylococcus aureus</i> ATCC 10803, <i>Staphylococcus aureus</i> ATCC 10804, <i>Staphylococcus aureus</i> ATCC 10805, <i>Staphylococcus aureus</i> ATCC 10806, <i>Staphylococcus aureus</i> ATCC 10807, <i>Staphylococcus aureus</i> ATCC 10808, <i>Staphylococcus aureus</i> ATCC 10809, <i>Staphylococcus aureus</i> ATCC 10810, <i>Staphylococcus aureus</i> ATCC 10811, <i>Staphylococcus aureus</i> ATCC 10812, <i>Staphylococcus aureus</i> ATCC 10813, <i>Staphylococcus aureus</i> ATCC 10814, <i>Staphylococcus aureus</i> ATCC 10815, <i>Staphylococcus aureus</i> ATCC 10816, <i>Staphylococcus aureus</i> ATCC 10817, <i>Staphylococcus aureus</i> ATCC 10818, <i>Staphylococcus aureus</i> ATCC 10819, <i>Staphylococcus aureus</i> ATCC 10820, <i>Staphylococcus aureus</i> ATCC 10821, <i>Staphylococcus aureus</i> ATCC 10822, <i>Staphylococcus aureus</i> ATCC 10823, <i>Staphylococcus aureus</i> ATCC 10824, <i>Staphylococcus aureus</i> ATCC 10825, <i>Staphylococcus aureus</i> ATCC 10826, <i>Staphylococcus aureus</i> ATCC 10827, <i>Staphylococcus aureus</i> ATCC 10828, <i>Staphylococcus aureus</i> ATCC 10829, <i>Staphylococcus aureus</i> ATCC 10830, <i>Staphylococcus aureus</i> ATCC 10831, <i>Staphylococcus aureus</i> ATCC 10832, <i>Staphylococcus aureus</i> ATCC 10833, <i>Staphylococcus aureus</i> ATCC 10834, <i>Staphylococcus aureus</i> ATCC 10835, <i>Staphylococcus aureus</i> ATCC 10836, <i>Staphylococcus aureus</i> ATCC 10837, <i>Staphylococcus aureus</i> ATCC 10838, <i>Staphylococcus aureus</i> ATCC 10839, <i>Staphylococcus aureus</i> ATCC 10840, <i>Staphylococcus aureus</i> ATCC 10841, <i>Staphylococcus aureus</i> ATCC 10842, <i>Staphylococcus aureus</i> ATCC 10843, <i>Staphylococcus aureus</i> ATCC 10844, <i>Staphylococcus aureus</i> ATCC 10845, <i>Staphylococcus aureus</i> ATCC 10846, <i>Staphylococcus aureus</i> ATCC 10847, <i>Staphylococcus aureus</i> ATCC 10848, <i>Staphylococcus aureus</i> ATCC 10849, <i>Staphylococcus aureus</i> ATCC 10850, <i>Staphylococcus aureus</i> ATCC 10851, <i>Staphylococcus aureus</i> ATCC 10852, <i>Staphylococcus aureus</i> ATCC 10853, <i>Staphylococcus aureus</i> ATCC 10854, <i>Staphylococcus aureus</i> ATCC 10855, <i>Staphylococcus aureus</i> ATCC 10856, <i>Staphylococcus aureus</i> ATCC 10857, <i>Staphylococcus aureus</i> ATCC 10858, <i>Staphylococcus aureus</i> ATCC 10859, <i>Staphylococcus aureus</i> ATCC 10860, <i>Staphylococcus aureus</i> ATCC 10861, <i>Staphylococcus aureus</i> ATCC 10862, <i>Staphylococcus aureus</i> ATCC 10863, <i>Staphylococcus aureus</i> ATCC 10864, <i>Staphylococcus aureus</i> ATCC 10865, <i>Staphylococcus aureus</i> ATCC 10866, <i>Staphylococcus aureus</i> ATCC 10867, <i>Staphylococcus aureus</i> ATCC 10868, <i>Staphylococcus aureus</i> ATCC 10869, <i>Staphylococcus aureus</i> ATCC 10870, <i>Staphylococcus aureus</i> ATCC 10871, <i>Staphylococcus aureus</i> ATCC 10872, <i>Staphylococcus aureus</i> ATCC 10873, <i>Staphylococcus aureus</i> ATCC 10874, <i>Staphylococcus aureus</i> ATCC 10875, <i>Staphylococcus aureus</i> ATCC 10876, <i>Staphylococcus aureus</i> ATCC 10877, <i>Staphylococcus aureus</i> ATCC 10878, <i>Staphylococcus aureus</i> ATCC 10879, <i>Staphylococcus aureus</i> ATCC 10880, <i>Staphylococcus aureus</i> ATCC 10881, <i>Staphylococcus aureus</i> ATCC 10882, <i>Staphylococcus aureus</i> ATCC 10883, <i>Staphylococcus aureus</i> ATCC 10884, <i>Staphylococcus aureus</i> ATCC 10885, <i>Staphylococcus aureus</i> ATCC 10886, <i>Staphylococcus aureus</i> ATCC 10887, <i>Staphylococcus aureus</i> ATCC 10888, <i>Staphylococcus aureus</i> ATCC 10889, <i>Staphylococcus aureus</i> ATCC 10890, <i>Staphylococcus aureus</i> ATCC 10891, <i>Staphylococcus aureus</i> ATCC 10892, <i>Staphylococcus aureus</i> ATCC 10893, <i>Staphylococcus aureus</i> ATCC 10894, <i>Staphylococcus aureus</i> ATCC 10895, <i>Staphylococcus aureus</i> ATCC 10896, <i>Staphylococcus aureus</i> ATCC 10897, <i>Staphylococcus aureus</i> ATCC 10898, <i>Staphylococcus aureus</i> ATCC 10899, <i>Staphylococcus aureus</i> ATCC 10900, <i>Staphylococcus aureus</i> ATCC 10901, <i>Staphylococcus aureus</i> ATCC 10902, <i>Staphylococcus aureus</i> ATCC 10903, <i>Staphylococcus aureus</i> ATCC 10904, <i>Staphylococcus aureus</i> ATCC 10905, <i>Staphylococcus aureus</i> ATCC 10906, <i>Staphylococcus aureus</i> ATCC 10907, <i>Staphylococcus aureus</i> ATCC 10908, <i>Staphylococcus aureus</i> ATCC 10909, <i>Staphylococcus aureus</i> ATCC 10910, <i>Staphylococcus aureus</i> ATCC 10911, <		