# Surface Display of a Functional Single-Chain Fv Antibody on Staphylococci

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Two different host-vector expression systems designed for cell surface display of chimeric receptors on *Staphylococcus xylosus* and *Staphylococcus carnosus* have been evaluated for surface display of a mouse immunoglobulin G1(κ) [IgG1(κ)] anti-human IgE single-chain Fv (scFv) antibody fragment. To achieve surface anchoring of the chimeric receptors containing the scFv, the cell surface attachment regions from *Staphylococcus aureus* protein A were used in both expression systems. The different chimeric receptors could be recovered from cell wall extracts of both *S. xylosus* and *S. carnosus*, and surface localization was demonstrated by taking advantage of a serum albumin-binding reporter region present within the two types of receptors. In addition, the two different recombinant staphylococci carrying hybrid receptors containing the scFv were demonstrated to react with the antigen, which was human IgE, in whole-cell enzyme-linked immunosorbent assays. This is the first report of an antibody fragment expressed in a functional form anchored to the surface of gram-positive bacteria. The potential use of recombinant gram-positive bacteria as whole-cell diagnostic devices or alternatives to filamentous phages for surface display of scFv libraries is discussed.

Bacterial surface display, in which heterologous peptides and proteins are exposed on the surface of recombinant bacteria, has become an increasingly important study objective in microbiology, molecular biology, and immunology (9, 25). For example, heterologous surface display has indeed proven to be useful in elucidating the mechanisms for surface targeting (15, 41–43). Immunological research has used surface display to determine the epitope specificity of monoclonal antibodies and for the purpose of developing live bacterial vaccine vehicles (9, 25). In gram-negative bacteria, various surface proteins have been investigated in this context, including different outer membrane proteins (1, 3, 7), lipoproteins (17), fimbriae (18), and flagellum proteins (28). Lipoprotein-assisted surface display has also been accomplished in *Mycobacterium bovis* BCG (46). Various gram-positive bacteria, such as staphylococci (16, 29, 30, 38), streptococci (15, 34, 35, 37), and enterococci (15), have recently started being investigated for surface display purposes. For gram-positive bacteria, cell surface anchoring has been achieved by using C-terminal regions of different gram-positive receptors, including *Staphylococcus aureus* protein A (16, 29, 30, 38), the *Streptococcus pyogenes* M6 protein (34, 35), and the *S. pyogenes* fibronectin-binding protein (15). The most common application for bacterial surface display is the development of live bacterial vaccine delivery systems, since the cell surface display of heterologous antigenic determinants has been considered advantageous in order to induce antigen-specific antibody responses when live recombinant cells are used for immunization (9, 29, 44, 46).

Single-chain Fv (scFv) antibody fragments have also been expressed and anchored to the surface of *Escherichia coli* (6, 8). Fuchs and coworkers (8) fused an scFv to the N terminus of the peptidoglycan-associated lipoprotein (PAL), while Francisco and coworkers (6) used a hybrid of the outer membrane lipoprotein (Lpp) and the outer membrane protein A (OmpA) for surface display of an scFv. Fluorescein-marked antigens were used to verify the functionality of the scFvs (6, 8), and fluorescence-activated cell sorting (FACS) could be used to enrich scFv-displaying bacteria from an excess of control cells (6). These results opened the discussion of whether *E. coli* cells with surface-displayed hybrid receptors would be an alternative to the rapidly developing phage technology for selection of peptides or recombinant antibody fragments from large libraries (24, 25). In fact, the surface display of a dodecamer peptide library in the flagellin of *E. coli* was recently reported (26). However, gram-positive bacteria exhibit certain properties which potentially make them more suitable for such applications. First, the surface receptors of gram-positive bacteria seem to be more permissive for the insertion of extended sequences of foreign proteins than the different gram-negative receptors (5). Second, in the gram-negative systems, both translocation through the cytoplasmic membrane and correct integration into the outer membrane are required (24) for surface display, while in the gram-positive systems, translocation through a single membrane is enough to achieve proper surface exposure of the heterologous polypeptide. Third, considering the practical handling of the bacteria as whole-cell diagnostic devices or in "panning" experiments to select specific clones from large libraries, gram-positive bacteria have the additional advantage of being more rigid because of the thicker cell wall, which thus allows various laboratory procedures without extensive cell lysis (20, 45).

Two different surface expression systems for staphylococci have recently been developed (16, 38), one designed for targeting of heterologous proteins to the surface of *Staphylococcus xylosus* (16), and the second suitable for surface display of hybrid receptors on *Staphylococcus carnosus* (38). Both of these bacteria are described as nonpathogenic (10, 30), and the latter is indeed used for meat fermentation applications (10, 23).

Here, the surface display of an scFv antibody on *S. xylosus* and *S. carnosus* has been investigated. The gene fragment

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TABLE 1. Strains and plasmids

| Strain | Plasmid | Reference |
|---|---|---|
| *E. coli* RRIΔM15 | ptrpBBscFv | 33 |
| *S. xylosus* KL117 | None | 39 |
|  | pSEmp18ABPXM | 29 |
|  | pSEαIgEABPXM | This study |
| *S. carnosus* TM300 | None | 10 |
|  | pSPPmABPXM | 38 |
|  | pSPPαIgEABPXM | This study |

encoding the scFv, which was constructed from a mouse hybridoma producing a monoclonal antibody reactive to human immunoglobulin E (IgE), was introduced into the two expression vectors designed for surface display on *S. xylosus* and *S. carnosus*. The expressed hybrid receptors containing the scFv antibody were characterized both after extraction and affinity purification and on the intact recombinant bacteria.

## MATERIALS AND METHODS

**Bacterial strains and plasmid vectors.** The strains and plasmids used in this study are listed in Table 1.

**Preparation and transformation of protoplasts.** The preparation and transformation of protoplasts were performed as described earlier by Götz and coworkers (11, 12).

**DNA constructions.** The construction of a mouse IgG1(κ) anti-human IgE scFv antibody fragment from a mouse hybridoma has been described earlier (33). This single-chain antibody construct was amplified by PCR with the oligonucleotide 5'-GGGGTCGACTCAGGTGAAGCTGCAGGAGTC-3' as the upstream primer and 5'-GGGAAGCTTTTTATTTCCAGCTTGGTCCC-3' as the downstream primer, with noncomplementary sequences introducing recognition sites for the endonucleases *Sal*I and *Hin*dIII, respectively. A generated PCR fragment was restricted with the appropriate enzymes, ligated with plasmid pUC19 (47) previously restricted with the same enzymes, and subjected to solid-phase DNA sequencing (19) with the A.L.F. DNA sequencer system (Pharmacia Biotech, Uppsala, Sweden). A verified clone was *Sal*I-*Hin*dIII subcloned to the expression vector pSEmp18ABPXM (29), used in *S. xylosus*, or pSPPmABPXM (38), used in *S. carnosus*. The vectors, obtained, designated pSEαIgEABPXM and pSPPαIgEABPXM, respectively, expressing the recombinant receptors were used to transform staphylococcal protoplasts.

**Rabbit antiserum.** A rabbit antiserum reactive with the serum albumin-binding protein (ABP) (32, 38) present within the chimeric receptors was generated as described by Hansson and coworkers (16).

**Extraction and affinity purification of recombinant receptors.** The extraction and purification of receptors, an assay which simplifies the analysis of the hybrid receptors produced since the samples can be concentrated by affinity purification, were performed essentially as described earlier by Samuelson and coworkers (38). Wild-type and recombinant *S. xylosus* cells harboring the expression vector pSEαIgEABPXM as well as wild-type and recombinant *S. carnosus* cells harboring the expression vector pSPPαIgEABPXM were grown overnight in tryptic soy broth (30 g/liter; Difco) supplemented with yeast extract (5 g/liter; Difco) and chloramphenicol (10 mg/liter) for the recombinant cells. Samples were diluted 1:50 and grown at 37°C to an $A_{578}$ of ≈1. The cells were harvested and washed twice in phosphate-buffered saline (PBS, pH 7.3) and resuspended in 5 ml of a modified SMMP medium (12) composed of 7.5 parts SMM (1 M sucrose, 0.04 M maleic acid, 0.04 M MgCl$_2$ [pH 6.5]) and 2.5 parts 7% Penassay broth (Difco). Lysostaphin (Sigma) was added to a final concentration of 25 μg/ml and incubated for 45 min at 37°C. The cell wall proteins were separated from the protoplast protein fraction by centrifugation twice at 7,000 × $g$ for 20 min. The supernatant containing solubilized receptors was diluted 1:20 in Tris-buffered saline (TST; 25 mM Trizma base-HCl [pH 8], 0.2 M NaCl, 1 mM EDTA, 0.05% Tween 20) before being subjected to affinity chromatography on a human serum albumin (HSA)-Sepharose column for purification of the hybrid receptors. Relevant eluted fractions were pooled and lyophilized prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% polyacrylamide) analysis under reducing conditions. The gel was subjected to an immunoblot analysis with polyclonal rabbit serum reactive with the ABP region of the protein.

**ELISA to detect scFv binding activity of extracted receptors.** Samples from overnight cultures of recombinant *S. xylosus* cells (harboring pSEmp18ABPXM or pSEαIgEABPXM) and *S. carnosus* cells (harboring pSPPmABPXM or pSPPαIgEABPXM) were diluted 1:100 in 30 ml of the medium described above and grown at 37°C to an $A_{578}$ of ≈1. The cells were washed twice in PBS, and the recombinant receptors were extracted by lysostaphin treatment (final concentration, 50 μg/ml) in 2.5 ml of modified SMMP for 1 h at 37°C for degradation of

the cell wall. The protoplasts were pelleted at 4,000 rpm in an Eppendorf centrifuge for 20 min and at 4,500 rpm for 10 min to obtain a protoplast-free solution containing solubilized recombinant receptors. The supernatant was diluted 1:1 in PBS containing 0.1% gelatin, and the solution was loaded in a 96-well microtiter plate which had been incubated with human IgE (or human IgG as a negative control) (10 and 100 μg/ml, respectively, in 0.1 M NaHCO$_3$ [pH 9.2]) for 2 h at 37°C, fixed in methanol for 5 min, and blocked with 0.1% gelatin in PBS with 0.05% Tween 20 [PBST, pH 7.3]) for 1 h at 30°C. The wells were washed three times in PBST prior to incubation for 1 h at 30°C with a polyclonal rabbit anti-ABP serum diluted 1:1,000 in PBS containing 0.1% gelatin. After the wells had been washed three times in PBST, they were incubated for 1 h at 30°C with a goat anti-rabbit IgG-alkaline phosphatase conjugate (Sigma) diluted 1:40,000 in PBS with 0.1% gelatin. The wells were washed three times in PBST, and after a final wash in substrate buffer (1 M diethanolamine-HCl [pH 9.8], 0.5 mM MgCl$_2$), 75 μl of substrate buffer and 75 μl of substrate solution (*p*-nitrophenylphosphate [Sigma]) were added. The change in absorbance at 405 nm after 30 min was measured in an enzyme-linked immunosorbent assay (ELISA) reader (SLT EAR 340AT; SLT-Labinstruments, Grödig, Austria).

**Colorimetric assay to investigate the surface display of ABP-containing receptors.** The colorimetric assay was performed essentially as described earlier by Samuelson and coworkers (38). Briefly, wild-type and recombinant *S. xylosus* and *S. carnosus* cells were grown overnight in the broth mentioned above, and samples were diluted 1:100 and grown at 37°C to an $A_{578}$ of ≈1. The cells were harvested and washed twice in PBST. One milliliter of cell suspension, diluted in PBST to an $A_{578}$ of ≈1, was incubated with biotinylated HSA (biotinylated with D-biotinoyl-ε-aminocaproic acid *N*-hydroxysuccinimide ester [Boehringer] according to the supplier's recommendations) at a final concentration of 2 μg/ml for 30 min at 25°C. The cells were washed twice in PBST before being resuspended in 1 ml of PBST containing 0.5 U of streptavidin-alkaline phosphatase (Boehringer) and incubated for 15 min at 25°C. The mixtures were washed once in PBST and once in substrate buffer (see above) before the different cell types were resuspended in substrate buffer. Four aliquots of 100 μl from each cell type were loaded in a microtiter plate before 100 μl of the substrate solution *p*-nitrophenylphosphate was added. The change in $A_{405}$ was measured for 5 min in an ELISA reader.

**Enzymatic assay to characterize the cell surface displayed scFv hybrid receptors.** The enzymatic assay was essentially carried out as described above with the antigen (human IgE) recognized by the scFv biotinylated and biotinylated IgG as a negative control.

Recombinant *S. xylosus* cells harboring plasmids pSEmp18ABPXM or pSEαIgEABPXM and recombinant *S. carnosus* cells harboring pSPPmABPXM or pSPPαIgEABPXM were cultured as described above at 37°C to an $A_{578}$ of ≈1. The cells were harvested, washed twice in PBST, and diluted in PBST to an $A_{578}$ of ≈1. One-milliliter fractions were incubated with biotinylated IgE or biotinylated IgG (biotinylated as above) at final concentrations of 3 μg/ml for 30 min at 25°C. The bacteria were washed twice in PBST before resuspension in 1 ml of PBST containing 0.5 U of streptavidin-alkaline phosphatase (Boehringer) and incubated at 25°C for 15 min. The cells were washed twice in PBST and once in substrate buffer before resuspension of the different cells in substrate buffer. Six aliquots of 100 μl from each cell type were loaded in a microtiter plate before adding 100 μl of the substrate solution, *p*-nitrophenylphosphate. The change in $A_{405}$ was measured for 30 min in an ELISA reader. The entire experiment was repeated four times to verify reproducibility.

**Nucleotide sequence accession numbers.** The GenBank accession numbers for the expression vectors pSEmp18ABPXM and pSPPmABPXM are U38693 and U15516, respectively.

## RESULTS

**Expression vectors for surface display of scFv receptors.** A gene fragment encoding a mouse hybridoma-derived single-chain Fv reactive with human IgE (α-IgE-scFv) was amplified by PCR from a previously described construct (33), which was functionally expressed in *E. coli*. The gene fragment encoding the scFv was inserted into the two general expression vectors pSEmp18ABPXM (29) and pSPPmABPXM (38), designed for surface display on *S. xylosus* and *S. carnosus*, respectively. The two resulting expression vectors were designated pSEαIgEABPXM (Fig. 1A) and pSPPαIgEABPXM (Fig. 1B), respectively. The two vectors differ in that the *S. xylosus* vector, pSEαIgEABPXM (Fig. 1A), takes advantage of the promoter and signal sequence from *S. aureus* protein A (SpA), while the *S. carnosus* vector, pSPPαIgEABPXM (Fig. 1B), utilizes the promoter, signal sequence, and propeptide sequence (PP) from a *Staphylococcus hyicus* lipase gene construct (22), optimized for expression in *S. carnosus*. The lipase propeptide, which is processed in its homologous host, *S. hyicus* (2), but not in *S.*
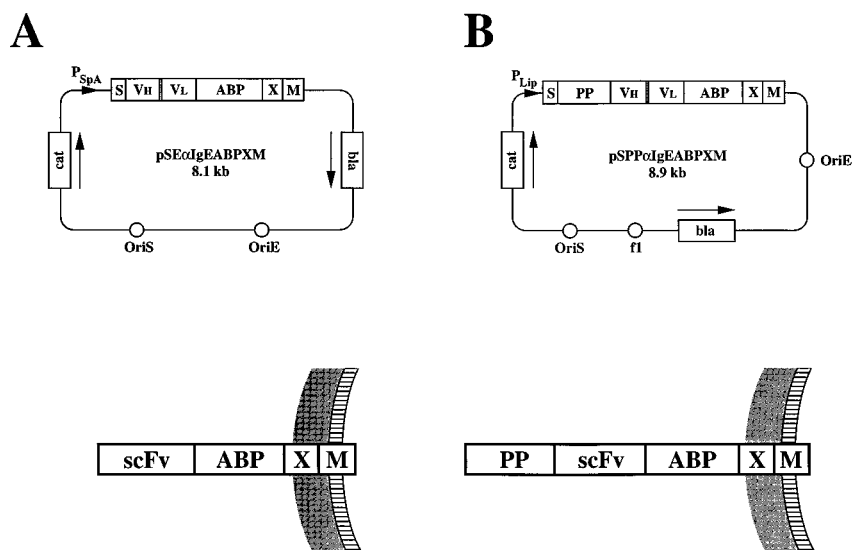
FIG. 1. Expression vectors and encoded gene products. Abbreviations: bla, β-lactamase gene; cat, chloramphenicol acetyltransferase gene; f1, origin of replication for phage f1; OriE, origin of replication from *E. coli*; OriS, origin of replication from *S. aureus*; $p_{SpA}$, promoter region from the SpA gene; $p_{Lip}$, *S. hyicus* promoter region designed for *S. hyicus* lipase production in *S. carnosus*. (A) Expression vector pSEαIgEABPXM, suitable for surface display in *S. xylosus*. The processed gene fusion product scFv-ABP-XM is illustrated as anchored to the cell surface. (B) Expression vector pSPPαIgEABPXM, suitable for surface display in *S. carnosus*. The processed gene fusion product PP-scFv-ABP-XM is illustrated as anchored to the cell surface. Note that the propeptide (PP) from the *S. hyicus* lipase is not processed in *S. carnosus*.

*carnosus* (10), has been shown to be essential for secretion of heterologous gene fusion products from *S. carnosus* (4, 22) using the lipase signal peptide. The two *E. coli-Staphylococcus* shuttle vectors, pSEαIgEABPXM (Fig. 1A) and pSPPαIgEABPXM (Fig. 1B), have the following features in common: (i) the origin of replication for *E. coli* and the β-lactamase gene giving ampicillin resistance for transformed *E. coli* cells, (ii) an origin of replication that is functional in *S. aureus* and the chloramphenicol acetyltransferase gene for staphylococcal expression, (iii) a gene fragment encoding ABP from streptococcal protein G (29, 32, 38), and (iv) gene fragments encoding the cell wall anchoring regions X and M from SpA. The C-terminal surface-anchoring region of SpA consists of a charged repetitive region (X), postulated to interact with the peptidoglycan cell wall (14), and a region common to gram-positive cell surface-bound receptors (M), containing an LPXTG motif, a hydrophobic region, and a short charged tail (5). It has been demonstrated that the latter tripartite region is required for cell surface anchoring (42, 43), and it has been shown that cell wall sorting is accompanied by proteolytic cleavage within the LPXTG motif, between the threonine and glycine residues, and subsequent covalent linking of the surface receptor to the cell wall (27, 41). In addition, the *S. carnosus* vector pSPPαIgABPXM carries the origin of replication for phage f1. The ABP region has been introduced to fulfill three different functions. First, it is positioned adjacent to the cell wall to act as a spacer protein to increase the accessibility of surface-expressed peptides (29). Second, because of its albumin-binding capacity, hybrid receptors extracted from the cell wall can be affinity purified on HSA columns (38). Third, the albumin-binding protein can be generally utilized as a reporter molecule to analyze the surface accessibility of expressed chimeric receptors (38).

**Extraction, affinity purification, and characterization of the chimeric receptors.** In order to investigate whether the assembled gene constructs could be expressed as coherent fusion proteins and to study the localization of the hybrid receptors,

recombinant *S. xylosus* and *S. carnosus* cells harboring the constructs shown in Fig. 1, with wild-type *S. xylosus* and *S. carnosus* cells as controls, were grown to the same cell density, harvested, and subjected to lysostaphin treatment to release cell wall-bound proteins. The protein fractions from the cell walls of wild-type and recombinant *S. xylosus* and *S. carnosus* cells were subjected to affinity purification on HSA-Sepharose and subsequently analyzed by SDS-PAGE and immunoblotting with an ABP-reactive antiserum (Fig. 2). The extracted and affinity-purified fusion proteins from cultivations of pSEαIgEABPXM-transformed *S. xylosus* (Fig. 2, lane 2) and pSPPαIgEABPXM-transformed *S. carnosus* (Fig. 2, lane 4) were recognized by the ABP antiserum, while the two wild-type cultivations were not (Fig. 2, lanes 1 and 3). The size of the hybrid receptor encoded by the recombinant *S. xylosus* cells (72 kDa) corresponded well with the size of the full-length band (Fig. 2, lane 2), while the hybrid receptor from the *S.*
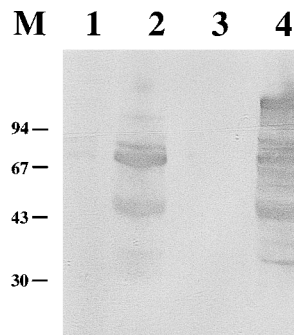


FIG. 2. Immunoblotting analysis after SDS–12% PAGE with ABP-reactive antibodies of staphylococcal cell wall extracts subjected to HSA affinity purification. Lane 1, wild-type *S. xylosus*; lane 2, *S. xylosus* harboring pSEαIgEABPXM; lane 3, wild-type *S. carnosus*; lane 4, *S. carnosus* harboring pSPPαIgEABPXM. Approximate molecular masses (in kilodaltons) are given in the left margin.
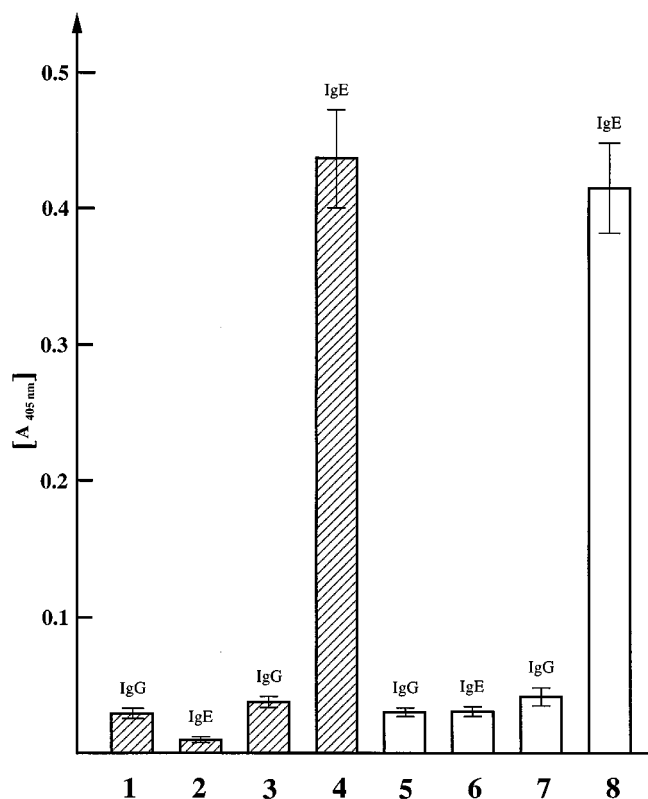
FIG. 3. Results from the ELISA to determine IgE reactivity in cell wall extracts from recombinant staphylococci. Microtiter plates were coated with human IgE (or human IgG as a control) and incubated with the cell wall extracts. The plates were developed by the addition of a primary rabbit anti-ABP serum and a secondary alkaline phosphatase-labeled goat anti-rabbit monoclonal antibody. Bars indicate the $A_{405}$ for cell wall extracts from *S. xylosus* cells (hatched bars) transformed with pSEmp18ABPXM (bars 1 and 2) or pSEαIgEABPXM (bars 3 and 4) and *S. carnosus* cells (open bars) transformed with pSPPmAB-PXM (bars 5 and 6) or pSPPαIgEABPXM (bars 7 and 8) for their reactivity to IgE and IgG (negative control). The analyzed antigens, human IgG or IgE, are indicated above the bars.

*carnosus* expression system (94 kDa) migrated as a somewhat larger protein (Fig. 2, lane 4). However, such abnormal migration has been described earlier for gene products containing the lipase propeptide (10, 38). The existence of smaller bands indicates that a certain degradation has occurred, but it is difficult to determine whether this degradation is due to proteolysis during cultivation or if it can be attributed to the extraction procedure.

To investigate whether hybrid receptors were also present in the culture supernatants, the growth medium fractions were collected and loaded on HSA-Sepharose. No detectable amounts of the receptors were obtained, as judged by subsequent SDS-PAGE or immunoblotting analyses (data not shown). These results demonstrate that the hybrid receptors were properly expressed and localized to the cell walls of the recombinant *S. xylosus* and *S. carnosus* cells. Furthermore, the extracted hybrid receptors display serum albumin-binding capacity, since full-length fusion proteins could be recovered by HSA affinity chromatography.

To investigate whether the hybrid receptors were reactive with human IgE, indicating a correct folding of the scFv moiety, cell wall extracts from cultivations of recombinant *S. xylosus* and *S. carnosus* cells harboring the constructs shown in Fig. 1 were analyzed in a microtiter plate format (Fig. 3) with

human IgE as the antigen. *S. xylosus* cells harboring the general vector pSEmp18ABPXM (29), displaying ABP-XM on the cell surface, and *S. carnosus* cells containing the pSPPmABPXM vector (38), displaying a PP-ABP-XM fusion, were used as control cells. A positive response to IgE was found for both *S. xylosus* and *S. carnosus* harboring pSEαIgEABPXM and pSPPαIgEABPXM, respectively (Fig. 3, bars 4 and 8). Cell wall extracts from recombinant *S. xylosus* and *S. carnosus* which did not express the α-IgE scFv receptors were negative in their IgE reactivity (Fig. 3, bars 2 and 6). None of the cell wall extracts from recombinant staphylococci showed any reactivity to human IgG (Fig. 3, bars 1, 3, 5, and 7). These results demonstrate that the α-IgE scFv moiety of the extracted hybrid receptors had the ability to fold properly to bind the human IgE antigen. However, the surface accessibility and functionality of the hybrid receptors on intact recombinant staphylococci remained to be proved.

**Characterization of the surface accessibility and functionality of the scFv receptors.** Since the hybrid receptors could be affinity purified by using their serum albumin-binding capacity, a detection method based on ABP as a reporter protein for successful surface display could be used (38). The results summarized in Fig. 4 showed a positive response for *S. xylosus*
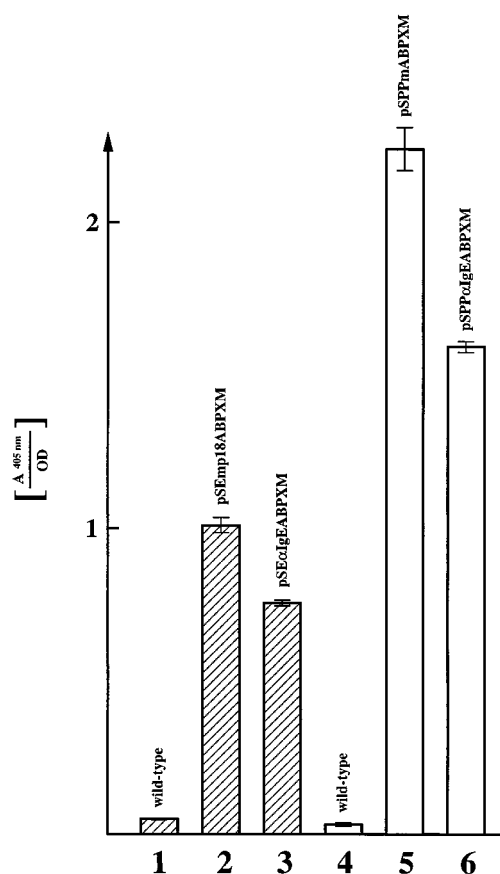


FIG. 4. Histogram of the results from a colorimetric assay for detection of surface-displayed receptors containing ABP. Wild-type and recombinant *S. xylosus* and *S. carnosus* cells were incubated with biotinylated HSA for binding to successfully exposed receptors containing ABP. Subsequent additions of a streptavidin-alkaline phosphatase conjugate and a chromogenic substrate allow monitoring of a color shift. Hatched bars, $A_{405}$ for *S. xylosus* cells: wild type (bar 1), pSEmp18ABPXM transformed (bar 2), and pSEαIgEABPXM transformed (bar 3); open bars, *S. carnosus* cells: wild type (bar 4), pSPPmABPXM transformed (bar 5), and pSPPαIgEABPXM transformed (bar 6).
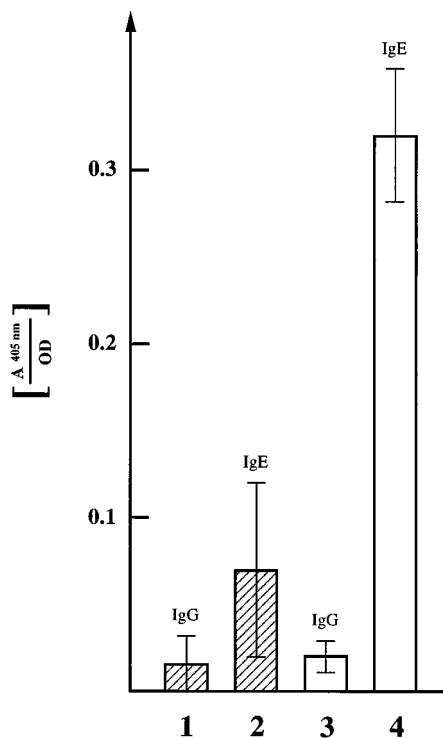
FIG. 5. Histogram of whole-cell reactivity to human IgE (with human IgG as a negative control). Recombinant staphylococcal cells were grown to the same cell density, harvested, and allowed to react with biotinylated human IgE or biotinylated human IgG as a control. A streptavidin-alkaline phosphatase conjugate was added, and the color shift after addition of substrate was monitored. The background responses to IgE (or IgG) of the recombinant *S. xylosus* and *S. carnosus* cells expressing hybrid receptors without the scFv were subtracted from the responses of the *S. xylosus* and *S. carnosus* cells expressing hybrid receptors containing the α-IgE scFv (see text for details). Bars indicate the $A_{405}$ response for *S. xylosus* cells (hatched bars) and *S. carnosus* cells (open bars) for their reactivity to IgE (bars 2 and 4) and IgG (bars 1 and 3).

harboring plasmid pSEmp18ABPXM or pSEαIgEABPXM (Fig. 4, bars 2 and 3, respectively) and *S. carnosus* harboring plasmid pSPPmABPXM or pSPPαIgEABPXM (Fig. 4, bars 5 and 6, respectively). Wild-type *S. xylosus* and *S. carnosus* cells were, as expected, found to be negative (Fig. 4, bars 1 and 4). The somewhat lower values obtained for the recombinant staphylococci encoding hybrid receptors containing the scFv region (Fig. 4, bars 3 and 6) than for those lacking this region (Fig. 4, bars 2 and 5) indicate a slightly lower surface density or accessibility for these hybrid receptors. Nevertheless, these data demonstrate that at least the serum albumin-binding region of the hybrid receptors, containing the scFv antibody, was accessible on the cell surface of both recombinant *S. xylosus* and *S. carnosus* cells.

To investigate whether the cell surface-exposed hybrid receptors scFv-ABP-XM and PP-scFv-ABP-XM (Fig. 1) contained functional surface-anchored scFv antibodies, recombinant staphylococci expressing the hybrid receptors were analyzed for their capacity to bind to human IgE. The four recombinant staphylococci investigated in the serum albumin-binding assay (Fig. 4) were also analyzed for IgE binding in an enzymatic assay, performed in a microtiter plate format (Fig. 5). Although the reactivity to IgE was much higher for the staphylococcal cells carrying hybrid receptors containing the α-IgE scFv, both recombinant *S. xylosus* and *S. carnosus* with chimeric receptors lacking the scFv region showed a significant, unexpected background binding to human IgE. The val-

ues for the reactivities to IgE for bacteria carrying the scFv and bacteria transformed with parental vectors were 0.838 ± 0.027 and 0.786 ± 0.023, respectively, for *S. xylosus* and 0.954 ± 0.013 and 0.635 ± 0.025, respectively, for *S. carnosus*. Six independent identical samples were compared each time, and the entire experiment was repeated four times with highly reproducible results. However, this type of background binding to human IgG was not found (data not shown). Nevertheless, the IgE-binding capacity of recombinant *S. xylosus* and *S. carnosus* carrying hybrid receptors containing the α-IgE scFv was clearly demonstrated (Fig. 5). *S. xylosus* and *S. carnosus* cells with surface-exposed scFv receptors specifically bound human IgE (Fig. 5, bars 2 and 4), while the same cells showed no binding to human IgG (Fig. 5, bars 1 and 3).

## DISCUSSION

We have described how an scFv antibody fragment can be expressed in a functional form as anchored to the cell surface of two different gram-positive bacteria, *S. xylosus* and *S. carnosus*. Although *S. carnosus* has been used as a host for recombinant protein production, with secretion of the gene product into the culture medium (10, 21), e.g., for the expression of scFvs (36, 40), the present study is the first example of surface display of a functional antibody fragment on gram-positive bacteria. In our case, the antigen for the scFv was human IgE, and both recombinant *S. xylosus* and *S. carnosus* with surface-displayed α-IgE scFv receptors were demonstrated to bind human IgE significantly more efficiently than control cells (Fig. 5).

One practical application of this type of recombinant bacteria would be to use them as "whole-cell monoclonal antibodies" in different diagnostic tests. The described strategy could prove to be a straightforward and cost-effective way of producing "monoclonal antibodies" for diagnostic purposes. Furthermore, recombinant staphylococci could be evaluated as an alternative to filamentous phages for affinity selection (panning) of peptide or antibodies from large libraries. An obvious bottleneck would be the frequency of transformation when introducing plasmid DNA into gram-positive bacteria. However, transformation frequencies of $10^5$ to $10^6$ transformants per μg of DNA have been reported for *S. carnosus* (12), which indicates that significantly large libraries of recombinant staphylococci with surface-displayed variants of peptides or antibody fragments could be envisioned. Fluorescence-activated cell sorting could then be a powerful tool in the selection of bacterial clones carrying the desired peptide or antibody variant, since recombinant staphylococci have proven to be easy to analyze by FACS technology (29, 38). For certain applications, it might be possible to use strains with high mutation frequencies to obtain in vivo evolution (13) to further improve the combinatorial power of the bacterial cell library. Also, non-immunoglobulin scaffolds could potentially be used for the presentation of randomized segments surface displayed on gram-positive bacteria. Particularly interesting would be combinatorial libraries based on receptor derivatives from gram-positive bacteria, such as the one described by Nord and co-workers (31), utilizing a domain from SpA as scaffold, which thus should be suitable for display on staphylococcal surfaces. The concept described in this study might also find applications within the field of biofilter development. Ligands selected as being specific for a certain compound could be surface displayed on bacteria, which then could be prepared in a biofilter format for specific capture. Alternatively, enzymes could be surface exposed in attempts to develop novel microbial bio-

catalysts. Both of these strategies could be evaluated for specific detoxification of, e.g., wastewater.

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