Surface display on staphylococci: a comparative study

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Abstract Two different host-vector expression systems, designed for cell surface display of heterologous receptors on *Staphylococcus xylosus* and *Staphylococcus carnosus*, respectively, were compared for the surface display of four variants of a 101 amino acid region derived from the G glycoprotein of human respiratory syncytial virus (RSV). Surface localization of the different chimeric receptors was evaluated by a colorimetric assay and by fluorescence-activated cell sorting. It was concluded that the *S. carnosus* system was better both in the ability to translocate inefficiently secreted peptides and in the number of exposed hybrid receptors. The potential use of the described staphylococci as live bacterial vaccine vehicles or alternatives to filamentous phages for surface display of protein libraries is discussed.

Key words: Surface display; *Staphylococcus xylosus*; *Staphylococcus carnosus*; Staphylococcal protein A; Respiratory syncytial virus; Fluorescence-activated cell sorting

1. Introduction

Bacterial surface display, where heterologous peptides and proteins are exposed on the surface of recombinant bacteria, has recently become an increasingly important objective in microbiology, molecular biology and immunology [1,2]. Gram-negative bacteria, such as *Escherichia coli* and *Salmonella* spp. [3–8], and more recently certain Gram-positive bacteria have been investigated for surface display purposes. The *Mycobacterium bovis* strain, bacillus Calmette-Guerin (BCG) [9], as well as staphylococci [10–14] and streptococci [15–18] have been investigated. Most thoroughly evaluated are the staphylococcal systems which have been utilized for surface display of various heterologous proteins [10–14]. The different surface display systems have been extensively used to express heterologous antigenic determinants on the bacterial cells for the purpose of developing live bacterial vaccine vehicles, and the cell-surface presentation has for certain systems been considered advantageous to induce an antibody response to the exposed antigens using live recombinant bacteria for immunization [1,6,12,19]. Also other types of proteins have been surface-displayed on bacteria. The expression of functional single-chain antibodies on the surface of *E. coli* [20,21] and staphylococci [14] has opened the discussion whether this strategy would be an alternative to the rapidly developing phage technology, for the selection of peptides or recombinant antibody fragments from large libraries [2,22]. In fact, the surface display of a dodecamer peptide library in the flagellin of *E. coli* was recently reported [23]. The use of enzyme-coated bacteria as novel biocatalysts has also been envisioned, since enzymes with retained activity have been surface displayed on *E. coli* [24,25].

Gram-positive bacteria exhibit certain properties which potentially make them more suitable for such applications: (i) the surface receptors of Gram-positive bacteria seem to be more permissive for the insertion of extended sequences of foreign proteins, as compared to the different Gram-negative receptors [26], (ii) in the Gram-negative systems, both translocation through the cytoplasmic membrane and correct integration into the outer membrane are required 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, and (iii) considering the practical handling of the bacteria, Gram-positive bacteria have the additional advantage of being more rigid, due to the thicker cell wall, which thus allows various laboratory procedures without extensive cell lysis [14].

Two of the most promising Gram-positive bacteria include the staphylococcal host-vector systems which have recently been developed [10,12], one designed for targeting of heterologous proteins to the surface of *S. xylosus*, and the second suitable for surface display of hybrid receptors on *S. carnosus* [13]. Both these bacteria are described as non-pathogenic [11,27], and are indeed being used in starter cultures for meat-fermentation applications [28,29]. In previous studies both these types of staphylococci carrying heterologous antigenic determinants have been suggested as live bacterial vaccine vehicles [11–13], but comparative studies have been lacking.

We have in this study investigated the surface expression on *S. xylosus* and *S. carnosus*, respectively, of four different variants of a 101 amino acid sequence derived from the major glycoprotein (G protein) of human Long strain RSV [30]. This antigen has been described as a major candidate for a subunit RSV vaccine [31]. The ability of the two staphylococcal systems to achieve proper surface exposure of the G protein variants has been evaluated by studying the surface localization of chimeric receptors on the different recombinant staphylococcal cells.

2. Materials and methods

2.1. Construction of the surface display vectors

Gene fragments encoding two variants, G_{nat} and G_{cys}, of a 101 amino acid fragment of the RSV G protein (amino acids 130–230 [30]) were constructed by solid phase gene assembly of synthetic oligonucleotides as described earlier [32,33]. Briefly, the gene assembly was initiated by immobilizing 5'-biotinylated oligonucleotides to streptavidin-coated paramagnetic beads (Dynabeads M-280 Streptavi-

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din, Dynal AS, Oslo, Norway), and complementary overlapping oligonucleotides were thereafter added in a stepwise and directed manner to generate the extended DNA constructs [32,33]. The gene fragments encoding G_{nat} and G_{cys} were assembled and ligated to a plasmid vector pRIT28 [34] to verify the nucleotide sequences by applying *Taq* DNA polymerase cycle sequencing on an automated DNA sequencer (373A, Applied Biosystems, Foster City, CA). G_{nat} encompasses the native amino acids 130–230 of the G protein of human RSV [30], while in G_{cys} two cysteine codons at positions 173 and 186 were substituted for serine codons. Two additional modified versions of the G_{cys} gene constructs were generated using PCR-based site-directed in vitro mutagenesis as previously described [12]. A gene fragment encoding a modified G protein fragment, denoted G_{sub}, where codons for phenylalanine at positions 163, 165, 168 and 170 were substituted for serine codons, was generated by a PCR strategy [12]. A second variant, G_{del}, of the G protein-encoding gene fragment was generated by an alternative PCR mutagenesis strategy [12], where a sequence was deleted which encoded nine amino acids including the four phenylalanines (DFHFEVFNF). The resulting fragments, G_{sub} and G_{del}, were subcloned to plasmid pRIT28 and a correct nucleotide sequence was verified by *Taq* DNA polymerase cycle sequencing, as above. Four expression vectors, $pSEG_{nat}ABPXM$, $pSEG_{cys}ABPXM$, $pSEG_{sub}ABPXM$ and $pSEG_{del}ABPXM$, designed for surface display on *S. xylosus*, were constructed by a *Bam*HI-*Hin*dIII subcloning of the four G protein-encoding gene fragments from their respective pRIT28 clones, into the mp18 linker of the general *S. xylosus* surface-display vector pSEmp18ABPXM [12]. To construct the four expression vectors, $pSPPG_{nat}ABPXM$, $pSPPG_{cys}ABPXM$, $pSPPG_{sub}ABPXM$ and $pSPPG_{del}ABPXM$, designed for surface display on *S. carnosus*, the gene fragments encoding the G protein variants fused to the serum albumin binding protein (ABP) [13] were *Bam*HI-*Xho*I-excised from the corresponding *S. xylosus* constructs and ligated to the general *S. carnosus* surface-display vector pSPPmABPXM [13].

2.2. Preparation and transformation of protoplasts

The preparation and transformation of protoplasts were performed as described earlier by Götz and coworkers [35].

2.3. Rabbit antisera

A rabbit antiserum reactive with the serum ABP [13,36] present within the chimeric receptors was generated as described by Hansson and coworkers [10]. A G protein-reactive rabbit antiserum was generated as described by Nguyen and coworkers [12]. This antiserum, which reacted strongly in enzyme-linked immunosorbent assays (ELISAs) with all four variants of the recombinant G protein fragments (data not shown), was used in the FACScan experiment. No cross-reactivity between the ABP and G protein-reactive antisera could be detected (data not shown).

2.4. Immunogold-electron microscopy analysis

S. xylosus and *S. carnosus* cells, wild type and those transformed with plasmid $pSEG_{sub}ABPXM$ or $pSPPG_{sub}ABPXM$, respectively, were subjected to an immunogold-electron microscopy analysis as described earlier [13], using an anti-ABP rabbit serum [10]. Briefly, the bacteria were incubated with rabbit anti-ABP or preimmune serum and subsequently with 10 nm colloidal gold conjugated to protein A (Aurion, Gent, Belgium). The suspension was washed and fixed using 3% glutaraldehyde. The bacteria were pelleted in melting agar, dehydrated with alcohol and embedded according to standard procedures [10]. Ultrathin sections of the material were stained with uranylacetate and lead-citrate and examined in a Philips EM 400 transmission electron microscope.

2.5. Colorimetric assay to investigate the surface display of ABP-containing receptors

This assay was performed essentially as described earlier by Samuelson and coworkers [13]. Briefly, wild type and recombinant *S. xylosus* and *S. carnosus* cells, respectively, were grown overnight, and samples were diluted 1:100 and grown at 37°C to $A_{580nm} \approx 1$. The cells were harvested and washed twice in PBS with 0.05% Tween 20 (PBST, pH 7.3). 1 ml of cell suspension, diluted in PBST to $A_{580nm} \approx 1$, 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 15 min at 25°C. The cells were washed twice in PBST before being resuspended in 1 ml of PBST containing 0.5 units streptavidin-alkaline phosphatase (Boehringer) and incubated for 15 min at 25°C. The mixtures were washed once in PBST and once in substrate buffer (1 M diethanolamine-HCl, pH 9.8, 0.5 mM $MgCl_2$), before resuspending the different cell types in substrate buffer. Four 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_{405nm} was measured for 5 min in an ELISA reader.

2.6. FACScan analysis of the staphylococcal surface display

This assay was performed essentially as described earlier by Samuelson and coworkers [13]. Briefly, wild-type and recombinant *S. xylosus* and *S. carnosus* cells, respectively, were grown at 37°C to $A_{580nm} = 8–9$. The bacteria were resuspended in 0.1% sodium azide in PBS to a final concentration of $A_{580nm} = 1$. Aliquots of 30 μl of the stock suspension were added to each conic well of a 96 well microtiter plate and sedimented by centrifugation for 10 min at $550 \times g$ at 4°C. After removing the supernatant, the bacterial suspension was incubated for 30 min with 150 μl of G protein-reacting rabbit antiserum diluted 1:1000 in PBS. The cells were washed twice in PBS and were subsequently incubated 30 min with 150 μl of fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit IgG (Sigma) at a dilution of 1:100 in PBS. The cells were washed twice, resuspended in 1 ml of PBS and analyzed on the basis of fluorescence intensity on a FACScan flow cytometer (Becton Dickinson, Sunnyvale, CA), using 488 nm as excitation wavelength at 15 mW for the argon ion laser, with saline as sheath buffer. Data for 6000–10 000 cells were collected in List Mode by a LYSYS II software (Becton Dickinson). The fluorescent light was collected through a band pass interference filter, with emission detection between 515 and 545 nm. The threshold trigger was set on side scatter to eliminate background noise and to select intact bacteria. The cell suspension fluorescence distribution was represented by fluorescence histograms and median fluorescence was given for each histogram.

3. Results and discussion

3.1. Expression vectors for surface display of RSV G protein variants on S. xylosus and S. carnosus

Two general expression vectors, pSEmp18ABPXM [12] and pSPPmABPXM [13], designed for surface display on *S. xylosus* and *S. carnosus*, respectively, were recently described [12,13]. In this study we have evaluated the two described systems for surface display by expressing four different protein fragments, G_{nat}, G_{cys}, G_{sub} and G_{del} (Fig. 1), derived from the G protein of human RSV [30], and thereby compared the different recombinant staphylococcal systems in their ability to translocate the G protein variants for exposure in an accessible form on the bacterial surface.

The native RSV G protein consists of 298 amino acids, with a predicted molecular mass of 32.6 kDa. We decided to investigate the production of a segment, comprising amino acids 130–230, of the G protein of Long strain RSV [30]. The choice of fragment was based on deletion mutant analyses of the RSV G protein [37] and identification of protective and neutralizing epitopes [38]. The selected segment of the G protein contains four cysteine residues, of which Cys-176 and Cys-182 have been suggested to form a disulfide bridge essential for the reaction with monoclonal antibodies which are able to confer passive protection upon RSV challenge [38,39]. Four synthetic gene fragments were assembled, encoding variants of the described region of the G protein, all with codons selected to be suitable for bacterial production [12,32,33]. The first constructed gene fragment encoded the non-engineered G protein sequence (aa 130–230) and was denoted G_{nat}. In a second construct, G_{cys}, the codons for the two cysteine residues not included in the predicted essential loop formation (Cys-173

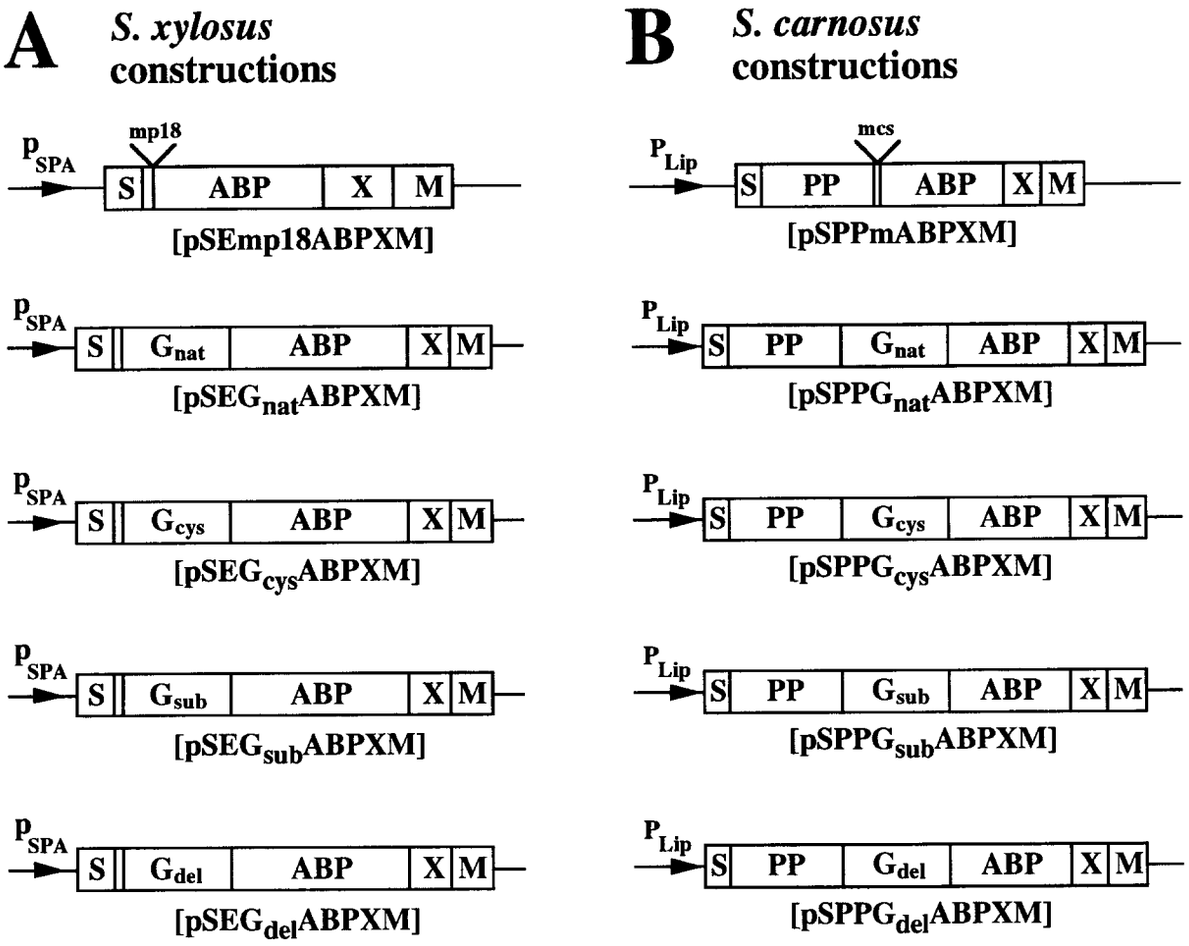

Fig. 1. The expression cassettes for the general expression vectors and plasmids encoding one of the four different RSV G protein derivatives, designed for surface display on (A) *S. xylosus* and (B) *S. carnosus*. Abbreviations: p_{SPA}, promoter region from *S. aureus* protein A; p_{Lip} promoter region from the *S. hyicus* lipase gene construct; mcs, multiple cloning site.

and Cys-186), were substituted for serine codons. We also designed two alternative G protein segments in which a hydrophobic region preceding the four cysteines was engineered. In G_{sub} the four phenylalanine codons were substituted for serine codons and in G_{del} the region encoding the four phenylalanine residues was deleted [12].

Four different expression vectors for *S. xylosus* and four vectors for *S. carnosus*, encoding G protein variants, have thus been constructed (Fig. 1). The two vector systems differ in that the *S. xylosus* vectors take advantage of the promoter and signal sequence from *S. aureus* protein A (SpA), while the *S. carnosus* vectors utilize the promoter, signal sequence, and propeptide sequence (PP) from a *S. hyicus* lipase gene construct [40], optimized for expression in *S. carnosus*. The lipase propeptide, which is processed in its homologous host, *S. hyicus* [41], but not in *S. carnosus* [27], has been shown to be essential for secretion of heterologous gene fusion products from *S. carnosus* [40,42] using the lipase signal peptide. The two *E. coli*-staphylococcus shuttle vector systems (Fig. 1) 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 functional in *S. aureus* and the chloramphenicol acetyltransferase gene for staphylococci expression, (iii) a gene fragment encoding a serum ABP from streptococcal protein G [12,13,32], (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 [43], and a region common for Gram-positive cell surface bound receptors (M) containing an LPXTG motif, a hydrophobic region and a short charged tail [26]. It has been demonstrated that the latter tripartite region is required for cell surface anchoring [44,45] and it has been shown that the 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 [46,47]. In addition, the *S. carnosus* vectors carry the origin of replication for phage f1. The ABP region has been introduced to fulfil three different functions: (i) it is positioned adjacent to the cell wall to act as a 'spacer protein' to increase the accessibility of surface expressed peptides [12], (ii) due to its albumin-binding capacity, hybrid receptors extracted from the cell wall can be affinity purified on human serum albumin (HSA) columns [13], and (iii) the ABP can be generally utilized as a reporter molecule to analyze the surface accessibility of expressed chimeric receptors [13].

3.2. Characterization of the recombinant staphylococci

Traditional methods to analyze surface receptors on intact

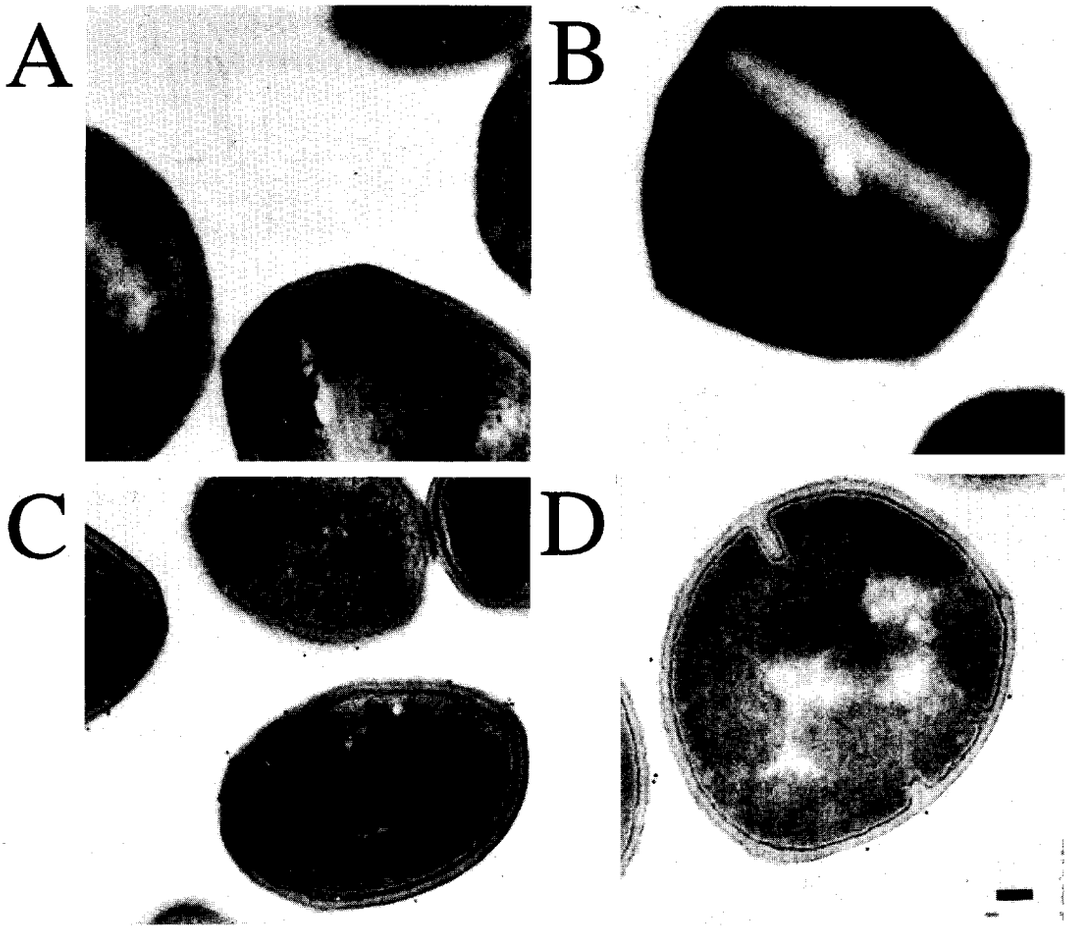

Fig. 2. Ultrathin sections of immunogold electron microscopy of *S. xylosus* and *S. carnosus* cells. Reaction of ABP rabbit antiserum is analyzed by the presence of 10 nm colloidal gold particles in association with wild type *S. xylosus* (A) and *S. carnosus* (B) cells, as well as with pSEG$_{sub}$ABPXM transformed *S. xylosus* (C) and pSPPG$_{sub}$ABPXM transformed *S. carnosus* (D) cells. Space bar, 100 nm.

cells include immunofluorescence and immunogold assays. Both these types of analysis are sufficient in giving qualitative answers concerning whether a certain receptor molecule is present or not on a cell surface, but fail to give any quantitative information. In Fig. 2, wild type *S. xylosus* (Fig. 2A) and *S. carnosus* (Fig. 2B) are compared in an immunogold electron microscopy assay to recombinant *S. xylosus* (Fig. 2C) and *S. carnosus* (Fig. 2D) transformed with plasmids pSEG$_{sub}$ABPXM and pSPPG$_{sub}$ABPXM, respectively. The recombinant bacterial cells are thus expected to carry chimeric receptors; G$_{sub}$-ABP-XM for *S. xylosus* and PP-G$_{sub}$-ABP-XM for *S. carnosus*. The four types of staphylococcal cells were probed with ABP-reacting rabbit antiserum and then stained with a protein A-gold conjugate. When ultrathin sections of the cells were analyzed in electron microscopy, the surface display of the hybrid receptors containing ABP was visualized by the colloidal gold particles bound to the outer surface of the cell wall of recombinant bacteria (Fig. 2C,D). As expected, the ABP receptor could not be detected on wild type *S. xylosus* or *S. carnosus* (Fig. 2A,B). Preimmune sera did not stain any of the cells (data not shown).

Based on the fact that the hybrid receptors contain the ABP region, a straightforward detection method utilizing ABP as a reporter protein for successful surface display was recently devised [13]. A sandwich concept was employed where biotinylated HSA was allowed to bind to recombinant staphylococcal cells with an exposed receptor containing ABP. A streptavidin-alkaline phosphatase conjugate was added and the color shift, after addition of substrate, was monitored. This convenient enzymatic assay, which can be performed in an ELISA plate format, is rapid and has been shown to give highly reproducible results [13,14]. The entire assay can after optimization [14] be performed within 2 h. Here, this assay has been used to evaluate recombinant *S. xylosus* and *S. carnosus* cells (Fig. 3), transformed with constructs encoding G$_{nat}$, G$_{cys}$, G$_{sub}$ and G$_{del}$, respectively. Wild type staphylococci as well as *S. xylosus* and *S. carnosus* transformed with the parental vectors pSEmp18ABPXM [12] and pSPPmABPXM [13], thus carrying ABP receptors, were included as controls. Hybrid receptors were found to be accessible on the surface of *S. xylosus* cells expressing either of the two G fragments, G$_{sub}$ and G$_{del}$, where the hydrophobic region was engineered (Fig. 3). In contrast, all recombinant *S. carnosus* constructs reacted positive indicating the presence of ABP-containing receptors, suggesting that this expression system, taking advantage of the signal peptide and propeptide from a *S. hyicus* lipase, gives a more efficient translocation. The hybrid receptors containing the G$_{nat}$ and G$_{cys}$ fragments, which could not be translocated by the *S. xylosus* system, were found to be present on the corresponding *S. carnosus* cells (Fig. 3). Furthermore, the

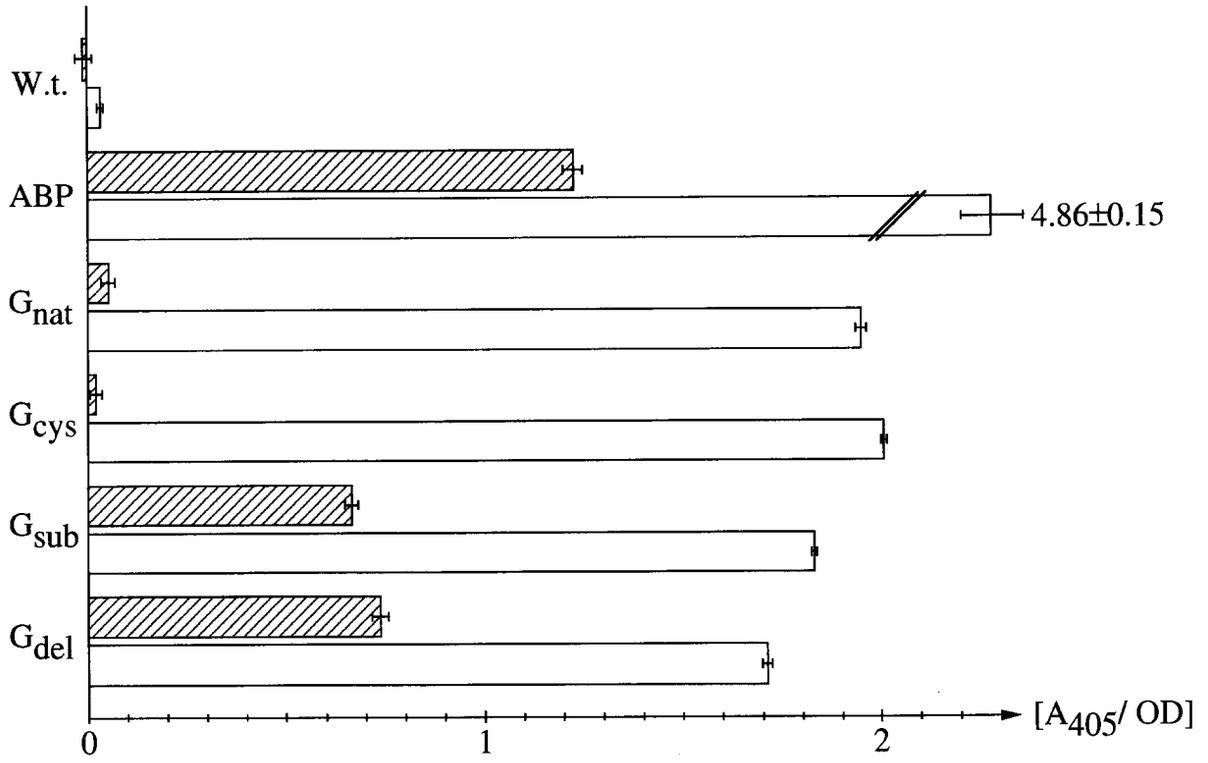

Fig. 3. Histogram representation of the results from a colorimetric assay for detection of surface displayed receptors containing ABP. Wild type and recombinant *S. xylosus* or *S. carnosus* cells, respectively, were incubated with biotinylated HSA for binding to surface-exposed receptors containing ABP. Subsequent additions of a streptavidin-alkaline phosphatase conjugate and a chromogenic substrate allows monitoring of a color shift. Bars indicate the A_{405nm} response for *S. xylosus* (hatched bars) and *S. carnosus* cells (open bars). In the left margin the type of cells analyzed is indicated: w.t., wild type bacteria; ABP, bacteria transformed with the parental vectors, pSEmp18ABPXM (*S. xylosus*) and pSPPmABPXM (*S. carnosus*).

higher color signal obtained from all the different recombinant *S. carnosus* clones indicates that a higher number of heterologous receptors is surface exposed per bacterium by this system (Fig. 3). As expected, the staphylococcal cells transformed with the parental vectors reacted positive and the wild type bacteria negative in this assay (Fig. 3).

3.3. FACScan analysis for detection of the G protein receptors

The wild type and recombinant staphylococci which were subjected to the colorimetric assay described above were further analyzed in a fluorescence-activated cell sorter (FACScan) assay (Fig. 4), where the accessibility of the G protein fragments was investigated. The bacterial cells were probed with a primary rabbit antiserum reactive with the RSV G protein-derived portion of the hybrid receptors, and thereafter fluorescently stained using a FITC-labelled secondary antibody. The FACScan results (Fig. 4) demonstrated accessibility to the G fragments on the same recombinant *S. xylosus* cells that reacted positive in the ABP-based colorimetric assay (Fig. 3), verifying that the G_{nat} and G_{cys} hybrid receptors were not properly surface exposed. For *S. carnosus*, three out of the four G protein fragments were found to be accessible on the corresponding cells. Only the *S. carnosus* cells carrying the G_{nat} receptor showed unexpected low reactivity in the FACScan assay (Fig. 4). This interesting discrepancy, a positive signal in the ABP assay but low FACS reactivity, suggests that the ABP part of the receptor is exposed on the cell surface while the G_{nat} region is retained in an inaccessible form during the translocation process. As expected, wild type *S. xylosus* and *S. carnosus* as well as *S. xylosus* and *S. carnosus* transformed with the parental vectors, pSEmp18ABPXM and pSPPmABPXM, respectively, did not react with the RSV G protein-reactive rabbit antisera (Fig. 4).

The higher fluorescence reactivity for the *S. carnosus* cells carrying the G_{sub} and G_{del} receptors, as compared to *S. xylosus* cells carrying the corresponding receptors, supports the results from the ABP assay indicating a higher surface density of hybrid receptors on the recombinant *S. carnosus* cells (Fig. 4). Furthermore, the results demonstrate that the G protein fragments are accessible on recombinant *S. carnosus*, despite the fact that a rather large propeptide (207 amino acids) is located outside the G protein fragments. This accessibility is of course of importance when utilizing the recombinant staphylococci for immunogen delivery in vaccination approaches.

3.4. Concluding remarks

We have in this study investigated the surface expression on staphylococci of four versions of a 101 amino acid region of the G protein from human RSV. Two host-vector systems, designed for *S. xylosus* and *S. carnosus*, respectively, have been compared for their capacity to surface display hybrid receptors containing the different G protein variants. It could be concluded that the *S. carnosus* system was better, since (i) a G protein variant, G_{cys}, that could not be translocated by the *S. xylosus* system was found to be surface exposed on the

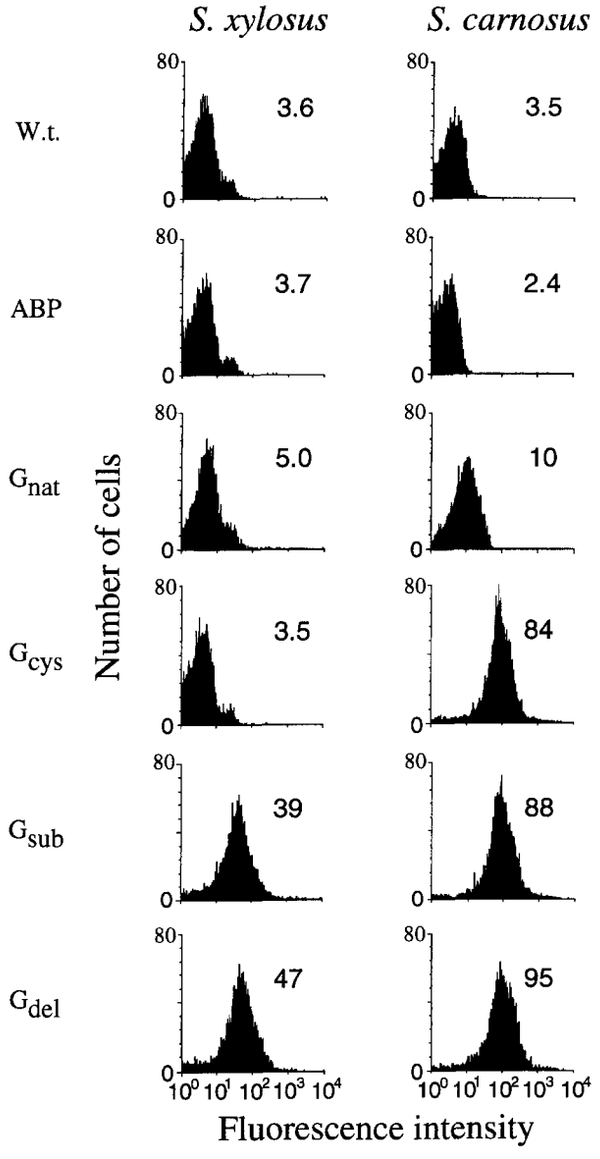

Fig. 4. Flow cytometry analysis (FACScan) of wild type and recombinant *S. xylosus* and *S. carnosus* cells probed with rabbit serum, reactive with the RSV G protein-derived part of the recombinant receptors, and stained with FITC labeled goat anti-rabbit serum. The histograms show nonstained cells displayed to the left and cells which expose G protein fragments in an accessible form on their surface shifted to the right. In the left margin the analyzed cell type is indicated. The median fluorescence reactivity is given for each histogram. Note that the X-axis has the same scale in all histograms.

corresponding *S. carnosus* cells and (ii) the *S. carnosus* system also expressed a significantly higher number of hybrid receptors on the bacterial surface, which obviously would be of importance when investigating such bacteria in the context of vaccine delivery. The results were assessed by FACScan analysis and by a colorimetric assay taking advantage of an albumin-binding protein present within the hybrid receptors. In fact, recent data suggest that the *S. carnosus* system gives approximately three times higher surface density of heterologous proteins and that the actual number of surface receptors exceeds 1000 receptors per cell both for *S. xylosus* and *S. carnosus* (Andréoni et al., unpublished data). Interestingly, the *S. carnosus* system did not seem to have any obvious drawbacks, either in surface accessibility or in number of receptors, from the large propeptide (207 aa) which is not processed in *S. carnosus*. Quite the opposite, the propeptide might be advantageous in the translocation of protein fragments which are difficult to secrete (e.g. G_{cys}). A potential disadvantage with the *S. carnosus* system could however be that proteins which are sensitive to N-terminal fusions for retained structure or function are not likely to be correctly surface displayed. For that reason it would be of interest to minimize or replace, or alternatively find a way to process, the propeptide. The latter might be achieved by coexpression of the protease responsible for the processing in the native host, *S. hyicus* [41]. Taken together, we find the described staphylococcal surface display systems, and thus particularly the *S. carnosus* system, of interest for surface expression of various proteins including (i) antigens to create vaccine vehicles, (ii) enzymes to develop novel microbial biocatalysts, (iii) different ligands in approaches towards new types or biofilters. Moreover, perhaps the most interesting application of the described systems would be to evaluate recombinant staphylococci as alternatives to filamentous phages for affinity selection (panning) of peptide or antibodies from large libraries. Also non-immunoglobulin scaffolds could potentially be utilized 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 coworkers [48], utilizing a domain from staphylococcal protein A as scaffold, which thus should be suitable for display on staphylococcal surfaces.

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