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Comparison of Autotransporter and Ice Nucleation Protein as Carrier Proteins for Antibody Display on The Cell Surface of *Escherichia coli**

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Abstract Antibody surface display technology is critical for novel antibody screening and antibody affinity maturation. Currently most frequently used display methods are phage display and yeast display. Although *Escherichia coli* (*E. coli*) is easily cultured and genetically manipulated, thus is potentially an excellent display host, the display technology based on *E. coli* has not been widely used. One of the problems is lack of efficient display of antibodies on the surface of *E. coli*. Many proteins have been tested as display carriers in outer membrane display in *E. coli*. Display systems based on autotransporter protein (AT) and ice nucleation protein (INP) is the most extensively studied. Another problem is unstable survival rates of *E. coli* when antibody is displayed. In this study, we systematically examined display level, antigen-binding affinity of displayed antibody and survival rate of *E. coli* using Ag43β (β domain of Antigen43, an AT protein) and INPNC (fragment of N-terminal and C-terminal of INP) as carrier proteins and T7, *lac*, araBAD as promoters for antibody expression. We found that the antigen-binding ability of the Ag43β based display was superior to that of the INPNC based system. As expected, T7, *lac* and araBAD promoters drove high, medium and low expressions of antibody. The host survival rate using T7 promoter was extremely low (INPNC: 0.0033%, Ag43β: 0.02%, the host bearing araBAD promoter had the highest survival rate (INPNC: 37.80%, Ag43β: 90.23%), and the *lac* based system had a survival rate of 2.04% (INPNC) and 13.27% (Ag43β). Balancing the antigen-binding abilities, antibody expression levels and survival rates, a system using Ag43β as carrier protein and *lac* as promoter is the best choice for antibody display on *E. coli*.

Key words cell surface display, autotransporter, INP, scFv, survival rate
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Recombinant antibodies are increasingly used in many applications such as clinical diagnosis and therapeutics [1], which require antibodies with high antigen affinity and specificity [2]. To achieve this purpose, researchers invented many techniques to display engineered antibody fragments or full IgG on the surface of phage[3], yeast[4] or bacteria[5-6] for affinity maturation and improvement of other features for antibody applications. Display on bacteriophage is the most widely used protein library-screening method, and many important antibodies were obtained from it[7-8], some of which are used in clinic treatment[9-10]. Yeast display is the most used cell display system. Unlike phage, yeast cells are big enough for analysis and sort by flow cytometry. Flow cytometry is a particularly powerful tool used in library screening as it confers a high speed sorting (>10^4 cells per second) based on highly precise and quantitative multiparameters[11]. The disadvantage is that it is very difficult to construct a yeast antibody display library larger than 10^9, considerably smaller than the sizable phage display library (3×10^{11})[12].

Compared to yeast, *Escherichia coli* provides a much larger library due to the high DNA

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transformation efficiency. Georgiou's group developed an APEx (anchored periplasmic expression) system to display scFv antibody library on the inner membrane of *E. coli* and successfully screened for antibody mutants with pmol/L affinity [13-14]. However, this technique requires preparation of spheroplasts because the scFv antibodies are displayed on the inner membrane and not freely accessible on the bacterial cell surface. As preparation of spheroplasts simply kills the bacterial cells, antibody genes needs to be cloned from isolated spheroplasts and retransform *E. coli* with the newly cloned genes.

Outer membrane display system can retain the integrity and viability of the bacterial cells, and thus sorted cells can be cultured and immediately subjected to additional rounds of screening. This feature helps isolating specific antibodies from a library significantly more conveniently. Many proteins have been used as displaying carriers, such as OmpA [15], flagellin [16], ice nucleation protein (INP) [5] and autotransporters [17]. Among them display systems based on autotransporter proteins (ATs) and INP are excellent systems for the display of large and complex proteins [18]. Although both of them can display scFv antibody [5-6], there has been no report comparing their suitability for antibody display.

In this study, we systematically examined the effects of carrier proteins (the autotransporter Ag43β and the ice nucleation protein INPNC) and promoters (T7, *lac* and araBAD) on the expression level, display level and antigen-binding ability when ATscFv (an anti-hTNFα scFv antibody) displayed, and even more important, the survival rate. We found that the cell survival rate was reversely proportional to the expression level, and INPNC-fused antibody had much lower antigen-binding ability than Ag43β-fused antibody. In conclusion, the combination of Ag43β-fusion and lac promoter is the best choice for scFv antibody display on *E. coli*.

1 Materials and methods

1.1 Construction of vectors

The gene encoding the *INPNC*, *Ag43β* and *ATscFv* were synthesized by polymerase chain reaction (PCR) using the primer set in Table 1. Primers were designed using Primer Premier 5.0 and a His tag

Table 1 List of primers used in this study

Primer name	Sequence	Comment
P1	GTACATGAATTCGACTCTCGACAAGGCGTTG	P1 and P2 were used to obtain PCR fragment coding for INPNC to insert to pET22b plasmid. *Eco*R Ⅰ and *Hin*dⅢ sites were introduced.
P2	GTACATAAGCTTCTTTACCTCTATCCAGTC	
P3	GTACATAAGCTTGATATCGGAATTAATTCGGATC	P3 and P4 were used to obtain PCR fragment coding for ATscFv to insert to pET22b plasmid. *Hin*dⅢ and *Xho* Ⅰ sites were introduced.
P4	GTACATCTCGAGCCGTTTTATTTCC	
P5	GTACATGGTACCTTAAAGAGGAGAAAGGTCATGA AATACCTGCTGCCGAC	P5 and P6 were used to obtain PCR fragment coding for PelB signal peptide and INPNC-ATscFv to insert to pBAD30 plasmid. *Kpn* Ⅰ and *Sal* Ⅰ sites were introduced. A His tag was introduced in P6.
P6	GTACATGTCGACTTAGTGATGATGATGATGATGCT CGAGCCGTTTTATTTCCAAC	
P7	GTACATGAATTCACTCTCGACAAGGCGTTGG	P7 and P8 were used to obtain PCR fragment coding for INPNC to insert to pAK201 plasmid. *Eco*R Ⅰ and *Sal* Ⅰ sites were introduced.
P8	GTACATGTCGACCTTTACCTCTATCCAGTCATC	
P9	GTACATGTCGACGATATCGGAATTAATTCGGATC	P9 and P10 were used to obtain PCR fragment coding for ATscFv to insert to pAK201 plasmid. *Sal* Ⅰ and *Xho* Ⅰ sites were introduced. A His tag was introduced in P10.
P10	GTACATCTCGAGTTAGTGATGATGATGATGATGC CGTTTTATTTCCAACTTTGTC	
P11	GTACATGAATTCGCGCAGTGAAAATGCTTATCGTG	P11 and P12 were used to obtain PCR fragment coding for Ag43β to insert to pET22b plasmid. *Eco*R Ⅰ and *Sal* Ⅰ sites were introduced.
P12	GTACATGTCGACTCAGAAGGTCACATTCAGTGTG	
P13	GTACATCCATGGATCACCACCACCACCACCACGA TATCGGAATTAATTCGGATC	P13 and P14 were used to obtain PCR fragment coding for ATscFv to insert to pET22b plasmid. *Nco* Ⅰ and *Eco*R Ⅰ sites were introduced. A His tag was introduced in P13.
P14	GTACATGAATTCGGCTCGAGCCGTTTTATTTCC	
P15	GTACATGGTACCTTAAAGAGGAGAAAGGTCATGA AATACCTGCTGCCGAC	P15 and P12 were used to obtain PCR fragment coding for ATscFv-Ag43β to insert to pBAD30 plasmid. *Kpn* Ⅰ and *Sal* Ⅰ sites were introduced.
P16	GTACATGAATTCCACCACCACCACCACCACGATA TCGGAATTAATTCGGATC	P16 and P17 were used to obtain PCR fragment coding for ATscFv to insert to pAK201 plasmid. *Eco*R Ⅰ and *Sal* Ⅰ sites were introduced. A His tag was introduced in P16.
P17	GTACATGTCGACCCGTTTTATTTCCAACTTTGTC	
P18	GTACATGTCGACCGCAGTGAAAATGCTTATCGTG	P18 and P19 were used to obtain PCR fragment coding for Ag43β to insert to pAK201 plasmid. *Sal* Ⅰ and *Xho* Ⅰ sites were introduced.
P19	GTACATCTCGAGTTATCAGAAGGTCACATTCAGTGTG	

was introduced by primer design (Figure 1a). The template for PCR to obtain gene fragments coding for *INPNC* was a kind gift from the laboratory of FENG Yan (Shanghai Jiao Tong University, Shanghai). Genomic DNA of *E. coli* K12 was used as template to obtain *Ag43β* fragments. The gene of *ATscFv* was cloned by TANG Jie's research group. The pBAD30 and pAK201 plasmids were kind gifts from the laboratory of George Georgiou (University of Texas, Austin). *INPNC* and *ATscFv* were cloned into vector pET22b, pBAD30 and pAK201 to yield pET22b-INPNC-ATscFv, pBAD30-INPNC-ATscFv and pAK201-INPNC-ATscFv. *Ag43β* and *ATscFv* were cloned into vector pET22b, pBAD30 and pAK201 to yield pET22b-ATscFv-Ag43β, pBAD30-ATscFv-Ag43β and pAK201-ATscFv-Ag43β (Figure 1a).

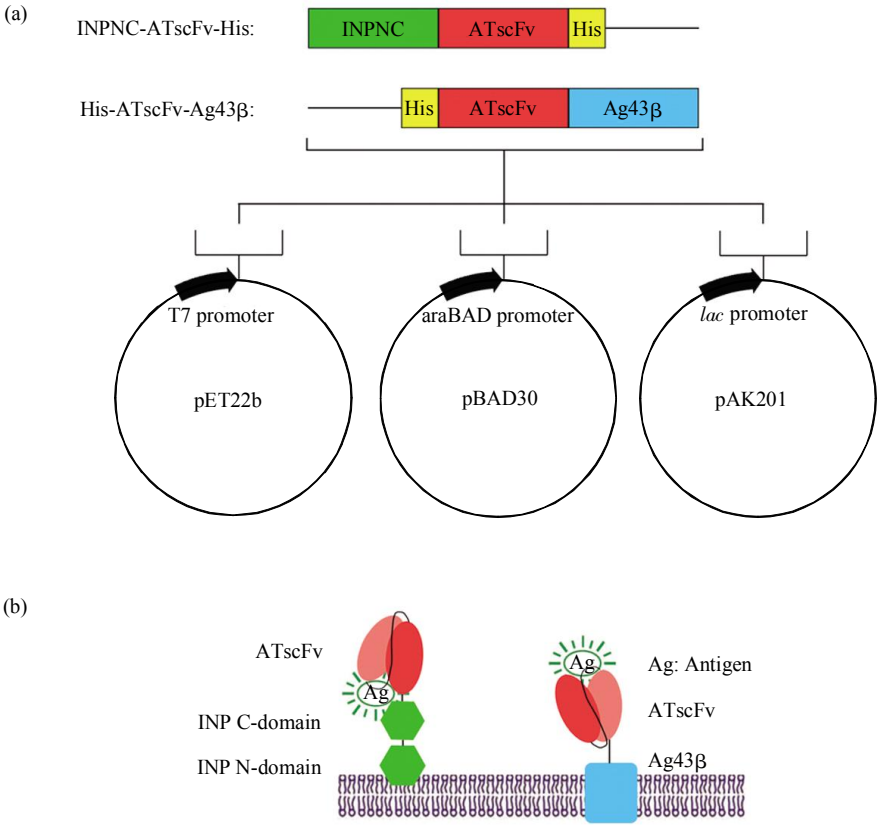

Fig. 1 ATscFv display on the surface of *E. coli* cells

(a) Schematic diagram of passenger protein ATscFv (red) fused with carrier proteins INPNC (green) and Ag43β (blue) used in this study. A His tag (yellow) was introduced. (b) Schematic illustration of INPNC and Ag43β surface display systems and interactions of antibody with antigen (GFP-hTNFα). The left and right panels shows that the ATscFv (red) is displayed by INPNC (green) and Ag43β(blue) respectively.

1.2 Bacteria cultures

All studies were carried out using *E. coli* BL21 (DE3) cells (Novagen). BL21 (DE3) cells carrying pET22b-INPNC-ATscFv, pET22b-ATscFv-Ag43β, pBAD30-INPNC-ATscFv and pBAD30-ATscFv-Ag43β were grown at 37℃ in Lysogeny Broth (LB) medium supplemented with Ampicillin (50 mg/L). BL21 (DE3) cells carrying pAK201-INPNC-ATscFv and pAK201-ATscFv-Ag43β were grown at 37℃ in LB medium supplemented with chloramphenicol (40 mg/L). pET22b-INPNC-ATscFv, pBAD30-INPNC-ATscFv and pAK201-INPNC-ATscFv were used to express and display C-terminally fused ATscFv by INPNC with different promoters, and pET22b-ATscFv-Ag43β, pBAD30-ATscFv-Ag43β and pAK201-ATscFv-Ag43β were used to express and display N-terminally fused ATscFv by Ag43β with different promoters. After overnight growth, the cells were diluted 1∶100 in fresh LB medium with ampicillin (50 mg/L) or chloramphenicol (40 mg/L)

and incubated at 37℃ to about 0.5 A_{600}. Then, the cells were transferred to 25 ℃ , and 0.2 mmol/L of IPTG (isopropyl-1-thio-b-D galactopyranoside) or 0.6% (w/v) arabinose were added to induce the expression.

1.3 Western blotting

After induction for 12 h, the amount of 1 A_{600} cells of all samples were collected by centrifugation, washed twice in 1 ml of phosphate buffer solution (PBS), and resuspended in 100 μl of 2×SDS-PAGE loading buffer(100 mmol/L Tris-HCl pH 6.8, 4% SDS, 0.2% PBP, 20% glycerol, 2% β-mercaptoethanol), boiled for 6 min to lyse the cells, and taken 5 μl to analyze by Western blot. Mouse anti-his monoclonal antibody (Invitrogen) was used as 1st antibody at dilution of 1∶2 000. HRP conjunct rabbit anti-mouse antibody (Promega) was used as 2nd antibody at dilution of 1∶5 000.

1.4 Assay with flow cytometry

To detect ATscFv display level, the amount of 0.5 A_{600} cells of all samples after induction for 12 h were collected by centrifugation, washed twice in 1 ml of PBS, and resuspended in 500 μl of PBS containing 1% BSA. Then the cells were incubated with mouse anti-his monoclonal antibody (Invitrogen, 1∶500 in PBS containing 1% BSA) for 1 h at room temperature. After that, the cells were washed once in PBS containing 1% BSA, and incubated with FITC conjunct rabbit anti-mouse antibody (Jackson ImmunoResearch, 1∶500 in PBS containing 1% BSA) for 1 h at room temperature in dark.

To detect antigen-binding ability of displayed ATscFv, the amount of 0.5 A_{600} cells of all samples after induction for 12 h were collected by centrifugation, washed twice in 1 ml of PBS, and resuspended in 500 μl of PBS with 1% BSA. Then the cells were incubated with GFP-hTNFα(2 mg/L in PBS containing 1% BSA) for 1 h at room temperature in dark. The fluorescence associated with the cells was detected and sorted by FACSCalibur and FACSInflux cell sorter (BD Biosciences).

1.5 Immunofluorescence assay

The amount of 0.5 A_{600} cells of all samples after induction for 12 h were labeled anti-His antibody or GFP-hTNFα antigen as above. After labeling, cells were resuspended in 100 μl of PBS with 1% BSA. Then 7 μl of samples were moved to microscope slide and observed by confocal laser scanning microscope Olympus LSCMFV500.

1.6 Survival rate of displayed cells

The cells labeled with GFP-hTNFα were sorted with a sorting gate3. About 5×10^4 cells were collected and spread on SOB plate with Ampicillin (50 mg/L) or chloramphenicol (40 mg/L). After cultured in 37℃ for 8 h, colonies were counted and the survival rate can be calculated.

2 Results

2.1 Expression and display of single chain antibody fused to ice nucleation protein or autotransporter

Before binding to antigen, scFv antibody needs to be expressed and displayed onto the cell surface. We employed three promoters: strong (T7), medium (*lac*) and weak (araBAD) to test the expression level and display efficiency of scFv antibody. We also used two different carrier proteins: Ag43β (an autotransporter) and INPNC (ice nucleation protein). An important difference between the two carriers is the type of fusion: INPNC is fused to the passenger protein with its C-terminus, while Ag43β to the passenger protein with its N-terminal end (Figure 1b). The different fusion types lead to different directions of displayed scFv antibodies. We used His tag to monitor the display level and used GFP-hTNFα as antigen to monitor the antigen-binding ability of displayed ATscFv antibody.

The cell viability is critical for maintaining isolated *E. coli* with desired antibody genes. However, there is a paradox in protein expression in *E. coli* and cell viability: a strong promoter leads to a high level expression but to a low survival rate at the same time; on the contrary, a weak promoter confers a high viability but only induces a low antibody expression level [19-20]. To investigate the effect of different promoters on surface display, we employed pET22b, pAK201 and pBAD30 that bears T7 promoter, *lac* promoter and araBAD promoter respectively to express the single chain antibody ATscFv. As expected [21], expression level of T7 promoter (pET22b) was about 10 times that of *lac* promoter (pAK201), and about 100 times that of araBAD promoter (pBAD30). Interestingly, the carrier proteins also affected the expression level. The expression level of INPNC-ATscFv was higher than that of ATscFv-Ag43β in T7 promoter and araBAD promoter (Figure 2a).

Besides the expression level of the fusion

proteins, we also investigated the effect of carrier protein and promoters on the ATscFv display level on *E. coli* cells. *E. coli* cells bearing different plasmids respectively were incubated first with anti-His antibody and subsequently with anti-mouse-FITC antibody. As shown in Figure 2b, despite the 10 times higher in expression level, the antibody display levels on T7 and *lac* promoter-containing bacterial cells did not differ much (INPNC: 27.18% *vs* 39.29%, Ag43β: 31.09% *vs* 18.17%). It might because the sec pathway which transports proteins across the inner membrane (IM) is limited. A large proportion of proteins in T7 promoter bearing cells were not transported to the outer membrane. The display level of araBAD promoter-containing cells was obviously lower than that of the other two cells, probably because the expression level was too low that the transportation was not the limitation (Figure 2b).

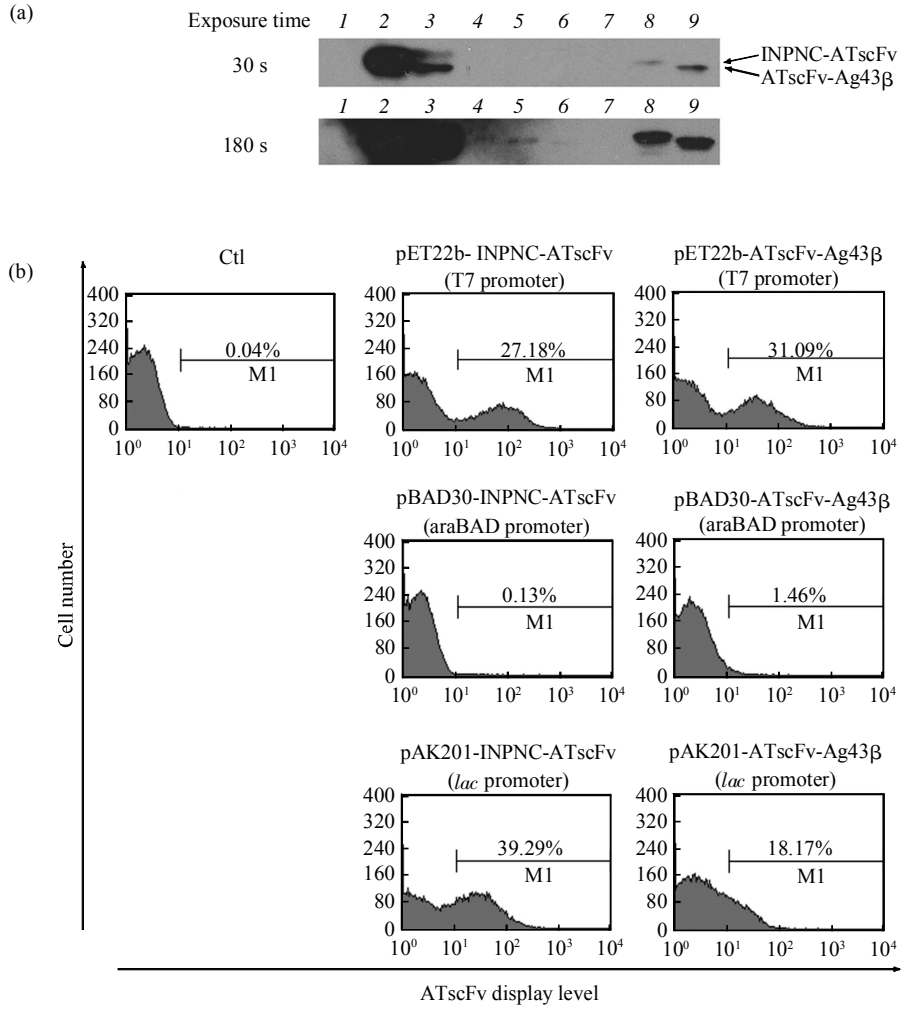

Fig. 2 ATscFv expression in and display on the *E. coli* cell surface

(a) ATscFv was expressed and analyzed by Western blotting. Cells containing different plasmids (*1*: pET22b as control; *2*: pET22b-INPNC-ATscFv; *3*: pET22b-ATscFv-Ag43β; *4*: pBAD30 as control; *5*: pBAD30-INPNC-ATscFv; *6*: pBAD30-ATscFv-Ag43β; *7*: pAK201 as control; *8*: pAK201-INPNC-ATscFv; *9*: pAK201-ATscFv-Ag43β) were introduced and analyzed. The upper panel shows the result in a shorter exposure time (30 s) and the lower panel shows the longer exposure time (180 s). Samples prepared from equal numbers of cells were loaded in each sample lane. (b) Surface display levels of ATscFv fused to INPNC and Ag43β quantified by flow cytometry. The ATscFv on the cell surface was labeled with anti-His antibody. The upper panel: display levels of ATscFv fused to INPNC and Ag43β, driven by T7 promoter. The middle panel: display levels of ATscFv fused to INPNC and Ag43β, driven by araBAD promoter. The lower panel: display levels of ATscFv fused to INPNC and Ag43β, driven by *lac* promoter. The cells containing pET22b plasmid were used as control.

2.2 Antigen-binding ability of displayed ATscFv by different carriers: INPNC and Ag43β

Antigen-binding ability of an antibody also depends on the accessibility of its complementarity-determining regions (CDR regions). CDR regions are located near N-terminal of a scFv antibody in secondary structure. As shown in Figure 1b, ATscFv is fused to Ag43β in ATscFv-Ag43β through its C-terminus and thus its CDR regions are freely accessed to the antigen GFP-hTNFα, while the CDR regions of ATscFv-INPNC is probably not freely accessed because ATscFv is linked to INPNC by its N-terminus which makes the CDR regions too close to the carrier protein and the cell surface that can not be approached easily by a macromolecule antigen. Indeed, our data showed that the ATscFv displayed by the two carriers manifested very different antigen-binding abilities despite of the close display level. In the T7 promoter containing cells, the display level of INPNC was close to that of Ag43β(27.18% *vs* 31.09%)(Figure 2b), but the antigen-binding ability of ATscFv displayed by INPNC was obviously lower than that displayed by Ag43β(15.07% *vs* 59.49%)(Figure 3). In the *lac* promoter group, the display level of INPNC

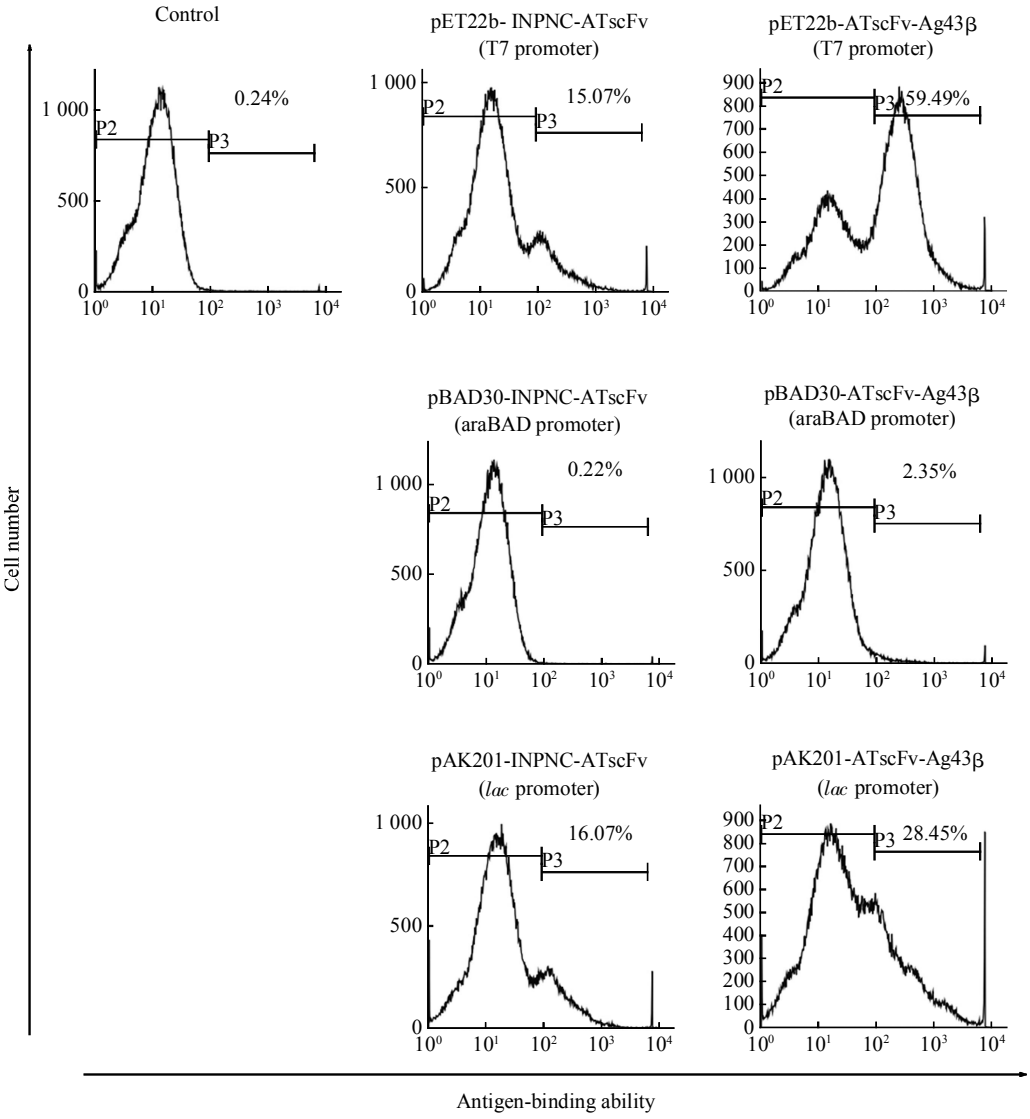

Fig. 3 Antigen-binding ability of displayed ATscFv quantified by flow cytometry

The ATscFv on the cell surface was labeled with its antigen GFP-hTNFα. The upper panel: antigen-binding ability of ATscFv fused to INPNC and Ag43β, driven by T7 promoter. The middle panel: antigen-binding ability of ATscFv fused to INPNC and Ag43β, driven by araBAD promoter. The lower panel: antigen-binding ability of ATscFv fused to INPNC and Ag43β, driven by *lac* promoter. The cells containing pET22b plasmid were used as control. The fluorescence positive cells were also sorted by flow cytometry to test survival rates.

was over two times that of Ag43β (39.29% *vs* 18.17%) (Figure 2b), but the antigen-binding ability of ATscFv displayed by INPNC was also much lower than that displayed by Ag43β (16.07% *vs* 28.45%) (Figure 3). In the araBAD promoter group, the antigen-binding ability of ATscFv displayed by INPNC was lower than that displayed by Ag43β (0.22% *vs* 2.35%) (Figure 3). Similar results were obtained by fluorescence microscope assay (Figure 4). In the cells driven by T7 or *lac* promoter, the display levels of INPNC-ATscFv were higher than that of ATscFv-Ag43β, but the antigen-binding abilities of INPNC-ATscFv were lower than ATscFv-Ag43β. Similar difference was not seen in the cells bearing araBAD promoter, probably because the fluorescence was too weak. To further confirm that the steric hindrance caused the low antigen-binding ability of the ATscFv displayed by INPNC, a $(GGGGS)_4$ linker was added between INPNC and ATscFv to distance ATscFv away from INPNC and cell surface. As expected, the linker addition enhanced the antigen-binding ability evidently (Figure 5). Therefore, antigen-binding ability of an antibody on *E. coli* display system is critically dependent on the accessibility of CDR regions, but does not or minimally depends on promoters.

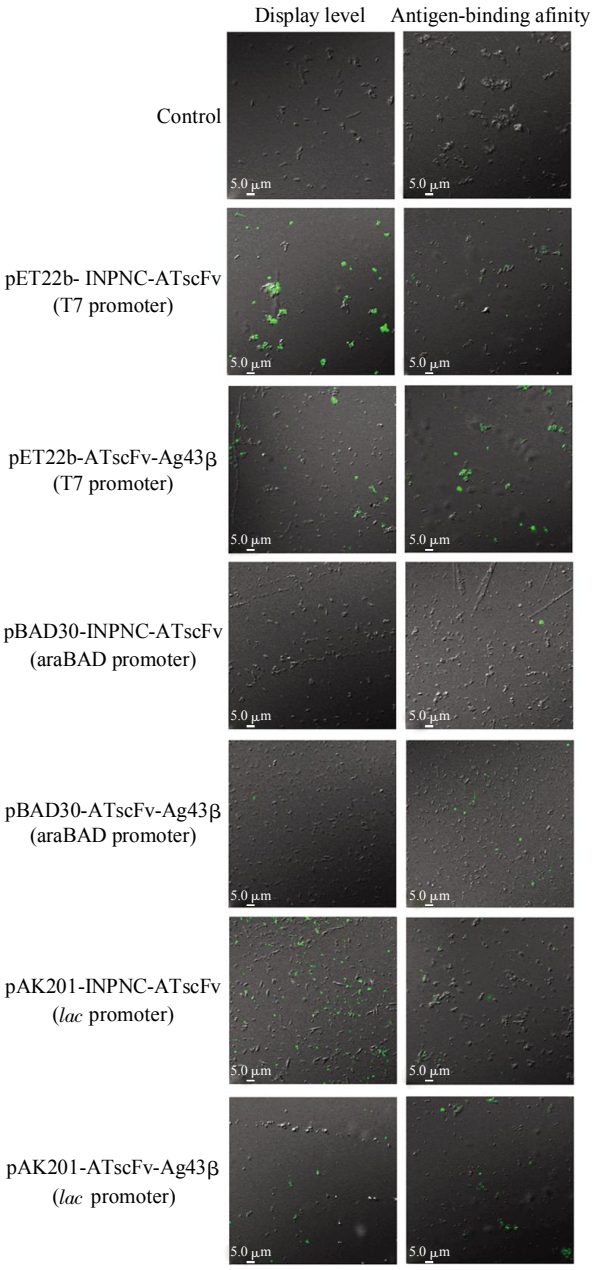

Fig. 4 Microscopic images of cells displaying ATscFv

Cells containing different plasmids (pET22b as control, pET22b-INPNC-ATscFv, pET22b-ATscFv-Ag43β, pBAD30-INPNC-ATscFv, pBAD30-ATscFv-Ag43β, pAK201-INPNC-ATscFv, pAK201-ATscFv-Ag43β) were observed using fluorescence microscope. The left panel shows the display level of ATscFv (labeled by anti-His antibody). The right panel shows the antigen-binding ability of ATscFv (labeled by its antigen GFP-hTNFα).

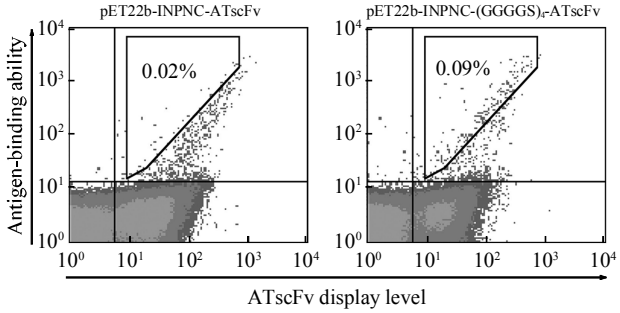

Fig. 5 The enhancement of the antigen-binding ability of displayed ATscFv by inserting a $(GGGGS)_4$ linker

Cells displaying ATscFv were labeled with anti-His antibody and GFP-hTNFα to monitor the display level and antigen-binding ability, respectively. The left panel shows the display level and antigen-binding ability of ATscFv directly fused to INPNC. The right panel shows the display level and antigen-binding ability of ATscFv when a $(GGGGS)_4$ linker was introduced between ATscFv and INPNC.

2.3 Survival rates rise significantly by changing promoters and carrier proteins

High survival rate is a key aspect of an antibody display system. In order to investigate the survival rate, cells labeled GFP-hTNFα were sorted with a sorting gate as shown in Figure 3. The collected cells were spread on SOB plate and the colonies were counted after culture to calculate the survival rate. As shown in Figure 6, promoters were dominant factors for the survival rate. When using T7 promoter, the survival rates were 0.0033% (INPNC) and 0.02% (Ag43β). However, when changed to lac promoter, the survival

rates dramatically rose to 2.04% (INPNC) and 13.27% (Ag43β). The difference between the survival rates was over 600 times. The survival rates of the araBAD promoter group were 37.80% (INPNC), (Ag43β) respectively (data not shown). The extremely low survival rate of T7 promoter-bearing cells and the very high rate of araBAD promoter-containing cells were most likely due to their extremely high and low expressions of the fusion proteins respectively. Interestingly, carrier proteins also influenced the survival rate. In all promoter-bearing cells, cells containing INPNC-fusion proteins had 3 ～7 times lower survival rate than that of cells containing Ag43β (Figure 6).

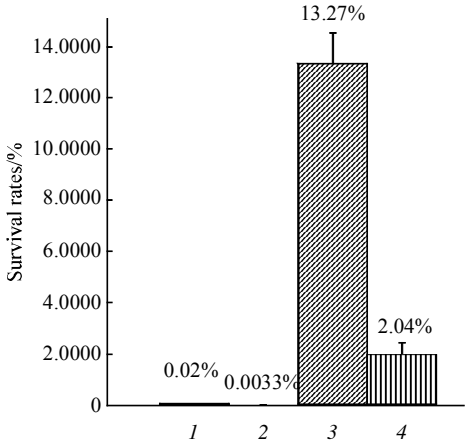

Fig. 6 Survival rates of host cells with various combinations of promoters and carrier proteins

Cells containing different plasmids (pET22b-INPNC-ATscFv, pET22b-ATscFv-Ag43β, pBAD30-INPNC-ATscFv, pBAD30-ATscFv-Ag43β, pAK201-INPNC-ATscFv, pAK201-ATscFv-Ag43β) were labeled GFP-hTNFα after induced. 5×10^4 fluorescence positive cells were sorted by flow cytometry and cultured in SOB plate. After 8 h, colonies on the plates were counted to calculate the survival rates. The survival rate of host cells with either pBAD30-INPNC-ATscFv or pBAD30-ATscFv-Ag43β was very high (close to cells not expressing protein) and not shown in this Figure. *1*: pET22b-ATscFv-Ag43β (T7 promoter); *2*: pET22b-INPNC-ATscFv (T7 promoter); *3*: pAK201-ATscFv-Ag43β (*lac* promoter); *4*: pAK201-INPNC-ATscFv(*lac* promoter).

3 Discussion

In this study, three promoters (T7, *lac* and araBAD) and two carriers (INPNC and Ag43β) were systematically studied on their effects on expression level, display level and antigen-binding affinity of displayed antibody, as well as survival rate of host cells. We found that the type of carrier protein is critical to the antigen-binding ability of displayed scFv antibody, the N-terminal fusion carrier Ag43β conferred higher antigen-binding ability to the displayed antibody than that of C-terminal fusion carrier INPNC (Figure 2b and 3). T7 promoter and araBAD promoter was not ideal to be used in the display technology because T7 promoter based expression system resulted in a very low survival rate of host cells, and araBAD based system was too weak to provide a sufficient level of antibody display. Considering both antigen-binding ability and survival rate, a combination of the *lac* promoter and the Ag43β carrier is the best choice for antibody display.

Our data showed that the display levels did not differ much between *lac* and T7 driven expressions, either using INPNC or Ag43β as carrier proteins (Figure 2a) although there was 10 fold difference in expression level between cells bearing T7 and *lac* promoters respectively (Figure 2b). It is likely that protein transportation across the membrane needs some cofactors[22], and the translocation rate is limited by the amount of these factors. Therefore, when expression rate was higher than translocation rate, the excessive proteins would not be transported onto the cell surface. The T7 promoter driven expression likely exceeded the rate limit of translocation, and the *lac* promoter driven expression was close to, but did not surpass the transportation limit, and thus the display levels in the cells bearing the two promoters respectively were close to each other. The very low display level in the araBAD driven cells was very likely due to its very low protein expression level (Figure 2). Ramesh *et al.*[6] reported a different result using several fragments of Ag43β as carrier proteins to display M18 scFv antibody driven by an araBAD promoter in which M18 scFv antibody display level reached 56% . The obvious difference between this result and ours might be due to the differences in passenger scFv antibodies and the different types of the host *E. coli* cells. It warrants a test whether increased expression and display level will not reduce survival level significantly.

There was only one report in which the displayed scFv in *E. coli* cells pretreated with EDTA had an antigen-binding level slightly higher than the background level [5]. In the present study, the antigen-binding signal of ATscFv displayed by INPNC was about 5 times higher than the background signal

(Figure 3), but the ratio of its antigen-binding ability over its expression level using either T7 or *lac* promoter was significantly lower than that of the Ag43β presented ATscFv, likely because of the steric hindrance in immunolabelling since its antigen-binding CDR regions faces toward the cell wall while the antibody CDRs of ATscFv-Ag43β stretch away from the cell wall (Figure 1b). The experimental result that lengthening the link between INPNC and ATscFv (to give more space surrounding its CDRs) significantly enhanced the antigen binding ability of INPNC-ATscFv (Figure 5) confirms the above explanation. Therefore, Ag43β is probably a better antibody carrier protein than INPNC if an antibody library using these two carrier proteins is screened for high affinity binders. However, a firmer conclusion cannot be made until more antibodies have tested. In contrast to the above result of antibody display, display systems using ice nucleation protein or its fragments had many successful applications in displaying various proteins [23-25]. It is very likely that a protein will be successfully displayed if its C-terminus involved in forming its active domain.

Compared to other inner membrane display systems such as APEx, retaining the viability of host cells is the main advantage of an outer membrane display system. However, over expression of heterogenous protein is generally harmful to host cells [19-20]. When expression was promoted by the strongest T7 promoter in this study, nearly all cells expressed fusion proteins were dead (Figure 6). We reason that the T7 RNA polymerase is five times more rapid than the *E. coli* RNA polymerase in mRNA synthesis; it rapidly decreases nutrient resources, and is therefore unbearable stress for host cells [26]. Besides promoters, different carriers also influenced the survival rate of host cells (Figure 6). The carrier Ag43β resulted in a higher survival rate than INPNC, likely due to the fact that Ag43β was an *E. coli* native protein of which *E. coli* cells could tolerate over expression better than the heterogenous protein INPNC. Taken together, the Ag43β surface display system combined with *lac* promoter-driven expression is the best choice for scFv antibody library display and sorting in our experimental conditions.

In this study, we achieved 13.27% survival rate of antibody expression *E. coli* cells (Figure 6), and this rate will significantly decrease the diversity of sorted cells. One strategy to solve the problem is to increase the number of sorting cells. For example, when the survival rate is 13.27%, the sorting number should be 8 times more than that when the survival rate is 100% in statistical calculation. Another strategy we will use is to test more carrier proteins and host *E. coli* strains to achieve higher survival rates.

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两种主流载体蛋白在大肠杆菌表面展示抗体应用中的系统分析 *

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摘要　抗体表面展示技术对于新抗体的筛选和抗体亲和力的成熟是非常重要的工具．目前，较为广泛应用的表面展示技术是噬菌体的表面展示和酵母的表面展示．大肠杆菌，以其培养简单和基因改造便捷，有望成为非常好的一种表面展示的宿主．但是，目前为止，大肠杆菌还没有被广泛地应用于抗体的表面展示技术中．主要的原因之一是在大肠杆菌外膜展示抗体的效率还不够高．作为外膜展示的载体，许多蛋白都被研究过，其中自转运蛋白(autotransporter，AT)和冰核蛋白(ice nucleation protein，INP)是人们研究最多的两种载体蛋白．还有一个原因是大肠杆菌作为宿主，在表达异源基因和展示异源蛋白过程中的存活率问题．在本研究中，系统地研究了 Ag43β(一种自转运蛋白 Antigen43 的 β 结构域)和 INPNC(去掉中间冗余序列的冰核蛋白的 N 端和 C 端)两种载体蛋白在强弱不同的三种启动子(T7、araBAD 和 *lac*)诱导表达的情况下，表达量、展示率、抗原亲和力以及宿主菌存活率的差异．我们发现，Ag43β 展示的抗体在抗原亲和力上优于 INPNC 展示的抗体．在存活率方面，T7 启动子诱导表达的存活率很低：用 INPNC 作为载体蛋白时只有 0.0033%，用 Ag43β 作为载体蛋白时只有 0.02%存活率．*lac* 启动子诱导表达的存活率：用 INPNC 作为载体蛋白时为 2.04%，用 Ag43β 作为载体蛋白时为 13.27%．araBAD 启动子诱导表达的存活率很高：用 INPNC 作为载体蛋白时为 37.80%，用 Ag43β 作为载体蛋白时高达 90.23%．但是 araBAD 诱导表达量和展示率都很低，所以其表现出的宿主高存活率意义有限．综合看来，由 *lac* 启动子驱动的、以 Ag43β 为载体蛋白的抗体表面展示系统是最好的选择．

关键词　表面展示，自转运蛋白，冰核蛋白，scFv，存活率
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