Appl Microbiol Biotechnol (2007) 73:1150–1157
DOI 10.1007/s00253-006-0571-7

pspA overexpression in *Streptomyces lividans* improves both Sec- and Tat-dependent protein secretion

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Received: 23 May 2006 / Revised: 7 July 2006 / Accepted: 9 July 2006 / Published online: 15 November 2006

Abstract *Streptomyces* is an interesting host for the secretory production of recombinant proteins because of its innate capacity to secrete proteins at high level in the culture medium. In this report, we evaluated the importance of the phage-shock protein A (PspA) homologue on the protein secretion yield in *Streptomyces lividans*. The PspA protein is supposed to play a role in the maintenance of the proton motive force (PMF). As the PMF is an energy source for both Sec- and Tat-dependent secretion, we evaluated the influence of the PspA protein on both pathways by modulating the *pspA* expression. Results indicated that *pspA* overexpression can improve the Tat-dependent protein secretion as illustrated for the Tat-dependent xylanase C and enhanced green fluorescent protein (EGFP). The effect on Sec-dependent secretion was less pronounced and appeared to be protein dependent as evidenced by the increase in subtilisin inhibitor (Sti-1) secretion but the lack of increase in human tumour necrosis factor (hTNFα) secretion in a *pspA*-overexpressing strain.

Keywords *Streptomyces* · Protein production · Phage-shock protein · pspA · Twin arginine · Secretion

Introduction

Streptomyces lividans has already been shown to be a valuable host for the secretion of heterologous proteins, of both eukaryotic and prokaryotic origin (Hong et al. 2003; Lara et al. 2004; Pozidis et al. 2001; Sianidis et al. 2006). In most cases where *Streptomyces* was used as host for the secretion of heterologous proteins, the Sec system has been used. Nevertheless, the recently detected Tat pathway (reviewed in Berks et al. 2003) can also be used for the translocation of heterologous proteins across the cytoplasmic membrane of *Streptomyces* (Schaerlaekens et al. 2004). The latter pathway has some pronounced differences compared to the Sec pathway. The Sec machinery transports proteins in an unfolded state across the cytoplasmic membrane, requiring both ATP hydrolysis and the proton motive force (PMF) for energy (Economou 1999). On the other hand, the Tat pathway transports proteins that are at least partially folded before export, and this translocation route is solely dependent on the PMF (DeLisa et al. 2003). As the PMF plays a role in both secretion pathways, an *S. lividans* strain with an improved PMF maintenance could be advantageous for the secretion of recombinant proteins. However, little is known about the way in which the PMF is maintained and regulated in Gram-positive bacteria. In *Escherichia coli*, the phage-shock-protein (psp) response is presumed to play a role in PMF maintenance (reviewed in Darwin 2005).

This psp response is activated by several forms of extracytoplasmic stress that compromise the energy status of the cell. These include, but are not limited to, filamentous phage infection, high osmolarity and elevated ethanol concentrations, alkaline shock and the presence of proton ionophores such as carbonylcyanide *m*-chlorophenylhydrazone (CCCP) (Model et al. 1997). The complete psp system in *E. coli* is comprised of the *pspABCD* operon and its transcriptional regulator, *pspF*. In this system, PspA is thought to be the main effector protein, as overexpression of *pspA* alone can alleviate the effects caused by deletion of

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both *pspB* and *pspC* (Kleerebezem et al. 1996). With respect to the production of heterologous proteins, it is important to note that *pspA* overexpression alone was sufficient to increase almost threefold Tat-dependent secretion of the SufI, CueO and ssTorA-GFP proteins in *E. coli* (DeLisa et al. 2004). So far, it has been thought that homologues of the PspA protein in bacteria that did not have the complete psp system in their chromosome, such as *S. lividans*, were unlikely to be a part of a real psp response (Darwin 2005).

In this work, we tested the impact of the *S. lividans pspA* homologue on protein secretion in this strain. To evaluate the effect of *pspA* overexpression on protein translocation, secretion of both native and heterologous proteins routed via both the Sec and the Tat pathway were quantified in *S. lividans* TK24 and the *pspA*-overexpressing strain.

Materials and methods

Strains, media and growth conditions

E. coli strain TG1 was used as host for cloning purposes while *E. coli* S17-1 (Simon et al. 1983) was used for conjugation of DNA from *E. coli* to *Streptomyces.* Cultures were grown at 37 °C (300 rpm) in Luria Bertani broth, supplemented with the appropriate antibiotics. *S. lividans* TK24 and its derivatives were precultured in 5 ml phage medium (Korn et al. 1978) supplemented with thiostrepton (10 μg/ml) or apramycin (50 μg/ml), if necessary, and grown at 27 °C with continuous shaking at 300 rpm for 48 h. After homogenization of the mycelium, the strains were inoculated in NM medium (Van Mellaert et al. 1994). For solid medium, MRYE (Anné et al. 1990) was used supplemented with thiostrepton (50 μg/ml) or apramycin (50 μg/ml), if applicable. Protoplast formation and subsequent transformation of *S. lividans* as well as conjugation from *E. coli* to *S. lividans* were carried out as described by Kieser et al. (2000). To test the distinct activities, precultures of *S. lividans* transformants grown in phage medium for 48 h were used to inoculate 50 ml NM medium and cultures were subsequently cultivated for 24–48 h.

DNA manipulations and vector constructions

For all DNA manipulations, standard techniques were followed (Sambrook et al. 1989; Kieser et al. 2000). Restriction endonucleases and DNA modifying enzymes were from Invitrogen and Roche Diagnostics. Plasmids and oligonucleotides used in this work are listed in Table 1.

To overproduce PspA in *S. lividans*, the *pspA* gene was cloned under control of the *Streptomyces venezuelae* subtilisin inhibitor (*vsi*) promoter in the *Streptomyces* vector pIJ486 (Ward et al. 1986). Therefore, the *pspA* gene was amplified by polymerase chain reaction (PCR) using the primers PspAF and PspAR. After cloning of the acquired PCR fragment in pGEM-T Easy (Promega), DNA sequences were verified, and the resulting pGEMPspA plasmid was digested with *Hin*dIII and *Eco*RI to allow insertion downstream of the *vsi* promoter into a *Hin*dIII/*Eco*RI-digested pBSVsi plasmid (Lammertyn 2000), resulting in pBSVsiPspA. This vector was digested with *Xba*I and *Hin*dIII, and the resulting *pspA*-expression cassette was ligated into the *Xba*I/*Hin*dIII-restricted pIJ486 vector, resulting in the *Streptomyces* vector pIJ486VPspA.

For the expression and secretion of hTNFα, XlnC and enhanced green fluorescent protein (EGFP), three pSSV05 (unpublished) derivatives were constructed. A cassette containing the cDNA of the mature hTNFα, fused in frame to the *vsi* signal sequence, was excised from the V-TNF plasmid (Schaerlaekens et al. 2004) as an *Xba*I/*Hin*dIII fragment and ligated into *Xba*I/*Hin*dIII-digested pSSV05 resulting in pSSV-TNF. Similarly, a cassette containing the *S. lividans xlnC* gene under control of the *vsi* promoter, was excised from pBSvsixyl (Schaerlaekens et al. 2004) as an *Xba*I/*Hin*dIII fragment and ligated into *Xba*I/*Hin*dIII-digested pSSV05 resulting in pSSV-XlnC.

For the secretory production of EGFP, the *egfp* gene was amplified by PCR using plasmid pIJ8668 (Sun et al. 1999) as template with the primers EGFPFor and EGFP-Rev. Upon cloning the PCR fragment in pGEM-T Easy, the resulting vector was first digested with *Pst*I, then treated with T4 DNA polymerase to remove the 3-protruding ends, and finally digested with *Eco*RI. Subsequently, the *egfp* gene was placed under control of the *vsi* promoter and the *xlnC* signal sequence by cloning the obtained restriction fragment in *Nsi*I/T4 DNA polymerase/*Eco*RI-treated pBSVX (Schaerlaekens et al. 2004). The resulting pBSVX-EGFP plasmid was digested with *Xba*I and *Eco*RI, and the resulting fragment was cloned into *Xba*I/*Eco*RI digested pSSV05, resulting in pSSVX-EGFP.

Transformation of *S. lividans*

To express EGFP, hTNFα and XlnC in the *S. lividans* TK24 wild-type and *pspA*-overexpressing strain, the pSSVX-EGFP, pSSV-TNF and pSSV-XlnC plasmids were, respectively, conjugated from *E. coli* S17-1 cells to spore suspensions of both *S. lividans* strains. *Sti*-1, which is encoded on the chromosome, was not cloned on a multicopy plasmid, as satisfactory amounts of protein could be detected when encoded from the chromosome.

Activity assays

Xylanase activity was assayed using the dinitrosalicylic acid assay as described previously (De Keersmaeker et al.

Table 1 Oligonucleotides and plasmids used in this work

Oligonucleotides			Plasmids		
Name	Sequence (5′–3′ direction)*	Restriction sites	Name	Relevant properties	References
PspAF	ATGAATTCTCATGAAG CGTATGGGGATG	*Eco*RI	*E. coli* plasmids		
PspAR	ATAAGCTTCCTCCTAG CCGTGGCTA	*Hin*dIII	pGEM-T Easy	Multiple cloning site, Ap^R	Promega
EGFPFor	ATCTGCAGTGAGCAA GGGCGAGGAGC	*Pst*I	pBSVsi	pBluescript KS(+) derivative containing the *S. venezuelae vsi* promoter	Lammertyn 2000
EGFPRev	ATGAATTCCTATTACTT GTACAGCTCGTCC	*Eco*RI	pBSVX	pBluescript KS(+) derivative containing the *S. venezuelae vsi* promoter and the signal sequence of *S. lividans xlnC*	Schaerlaekens et al. 2004
del1	ATGGATCCGTATCACG TTGCGCACTCCC	*Bam*HI	pBSvsixyl	pBluescript KS(+) derivative containing the *S. venezuelae vsi* promoter and *S. lividans xlnC*	Schaerlaekens et al. 2004
del2	ATCTGCAGTCTTTACCT TCTCACCGAGGCGGC	*Pst*I	pBSVX-EGFP	pBluescript KS(+) derivative containing the *S. venezuelae vsi* promoter and *S. lividans xlnC* signal sequence fused in frame to the EGFP cDNA	This work
del2′	ATGAATTCTCTTTACCTT CTCACCGAGGCGGC	*Eco*RI	*E. coli*→*Streptomyces* conjugational plasmids		
del3	ATGAATTCACGAGATGG GAACGTGGGTT	*Eco*RI	pSSV05	Conjugational derivative of the pSVH1plasmid containing the *oriT* of pSET152 and *aac(3)IV*	Unpublished
del4	ATGATATCTCACGTCGTC GTAGCCGTTG	*Eco*RV	pSSV-TNF	Derivative of pSSV05 containing *S. venezuelae vsi* promoter and signal sequence fused in frame to the hTNFα cDNA	This work
tat1′	ATGAATTCGAACCGGCT GAAACCCGCCACG	*Eco*RI	pSSV-XlnC	Derivative of pSSV05 containing the *S. lividans xlnC* gene behind the *S. venezuelae vsi* promoter	This work
tatA3′	TCTAGAAGGAGCAAGG ACTGTGAGC	*Xba*I	pSSVX-EGFP	Derivative of pSSV05 containing *S. venezuelae vsi* promoter and *S. lividans xlnC* signal sequence fused in frame to the EGFP cDNA	This work
tatA5′	GATATCCTGCGTCAGC GCTTGGTCG	*Eco*RV	*Streptomyces* plasmids		
			pIJ486	Multiple cloning site, Tsr^R	Ward et al. 1986
			pIJ486VPspA	pIJ486 derivative containing the *pspA* gene behind the *S. venezuelae vsi* promoter	This work
			V-TNF	pIJ486 derivative containing the *S. venezuelae vsi* promoter and signal sequence and the hTNFα cDNA (mature part)	Schaerlaekens et al. 2004
			pIJ8668	pIJ8600 derivative containing the *egfp* gene	Sun et al. 1999

* Restriction endonuclease cleavage sites are underlined.

2005). Briefly, after 24 h of growth, cultures of *S. lividans* were centrifuged (10 min, 4,000×*g*, 4 °C); the obtained supernatants were diluted in the assay buffer, and the amount of reducing sugar was quantified. One unit of xylanase was defined as the amount of enzyme that produces 1 mg reducing sugar in 10 min at 60 °C from a saturated xylan solution. Values were expressed as units per mg mycelial dry weight to correct for differences in growth rate between the different *S. lividans* strains.

EGFP activity was assayed by measuring fluorescence of 200 µl spent medium using a Fluoroskan Ascent FL fluorophotometer (Labsystems), with the excitation filter set to 485 nm and the emission filter to 520 nm.

Subtilisin inhibitor activity was determined in the presence of subtilisin BPN' and the substrate *N*-succinyl-L-Ala-L-Ala-L-Pro-L-Phe-*p*-nitroanilide as described by Kojima et al. (1990). Extracellular fractions of 24- or 48-h cultures were diluted in assay buffer, and the percentage of

subtilisin activity was measured. One unit was defined as the amount of enzyme that inhibited 5 μg subtilisin during 10 min incubation at 25 °C.

ELISA assays

The amount of hTNFα secreted in the extracellular medium of the different *S. lividans* cultures was determined by enzyme linked immunosorbent assay (ELISA) (DiaMed EuroGen, Turnhout, Belgium) according to the manufacturer's recommendations.

SDS-PAGE and Western blot analysis

To check accumulation of XlnC, and EGFP in the extracellular medium in *S. lividans*, Western blot analysis was performed. Proteins present in the supernatant corresponding to cultures with the same dry weight were TCA-precipitated and were subsequently separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by Western blotting and immunodetection with specific antibodies in combination with a suited alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma) and the chromogenic substrate solution NBT/BCIP (Roche Diagnostics).

Results

S. lividans pspA gene

PspA homologues are widespread in bacteria and higher organisms. In the *Streptomyces coelicolor* genome bank (http://www.sanger.ac.uk), we found a *pspA* homologue (previously SCO2168) which forms an operon with SCO2167, a gene with no homology to any known protein. With primers based on the *S. coelicolor* sequence, we were able to amplify the corresponding gene from *S. lividans* TK24. *pspA* was isolated as a 780-bp gene. The *S. lividans* and *S. coelicolor pspA* genes show 99.3% identity at the nucleotide level and 98.1% identity at the amino acid level, respectively.

The CLUSTALW identity and similarity scores with the *E. coli pspA* are 23 and 39%, respectively, but more importantly, no homologues of the *pspB*, *pspC*, *pspD* or *pspG* genes could be identified. Of these genes, *pspA*, *B* and *C* are, so far, assumed to be needed for a functional, minimal psp response (Darwin 2005).

The influence of *pspA* overexpression on the total amount of secreted protein

To overexpress *pspA*, S. *lividans* TK24 was transformed with the multicopy plasmid pIJ486VPspA, containing the *pspA* gene cloned behind the *vsi* promoter region. To compare the total amount of secreted protein, both wild-type and the *pspA*-overexpressing strain were grown in 50 ml minimal medium NMMP. After 24 h of growth, the amount of protein present in the culture supernatant was determined by the Bradford assay (Bio-Rad) using bovine serum albumin as the standard. The spent medium of the wild-type strain contained 27.6±0.9 μg of protein (mg dry wt)$^{-1}$, whereas the *pspA*-overexpressing strain contained 36.1±0.8 μg of protein (mg dry wt)$^{-1}$. This represents a significant increase in the total amount of secreted protein of 32%.

Furthermore, the *pspA*-overexpressing strain showed a similar growth curve when grown in the NM medium that was used in further secretion tests (shown in Fig. 1), indicating that the obtained results are not due to a growth difference between both strains.

Influence of *pspA* overexpression on Tat-dependent secretion

In a next step, we wanted to assess the specific effect of *pspA* overexpression on Tat-dependent secretion. To achieve this, the *pspA*-overexpressing and the wild-type strain were transformed with the plasmid pSSV-XlnC or pSSVX-EGFP containing, respectively, the native xylanase C gene and the "enhanced green fluorescent protein" gene under the control of the *vsi* promoter. Enzymatically active xylanase C can only be obtained after secretion via the Tat pathway (Faury et al. 2004), and the same applies for fluorescent, secreted EGFP (unpublished data).

Figure 2 shows the effect of the *pspA* coexpression on the xylanase activity, measured using the dinitrosalicylic acid assay, of *S. lividans* overexpressing *xlnC* over a period of 42 h of growth. When grown in the absence of xylan, as is the case here, the wild-type *S. lividans* does not produce detectable levels of the native XlnA, B and C proteins (Mondou et al. 1986; Vats-Mehta et al. 1990). Similarly, no xylanase activity could be detected in the *S. lividans* [pIJ486PspA] strain, meaning that the native *xlnA*, *B* and *C* genes were not responsible for the observed xylanase activity in the *xlnC*-overexpression strain. In contrast, in the XlnC-overproducing strain, the amount of XlnC measured in the supernatant was 4.8 U. When *pspA* was concomitantly overexpressed, values up to 18.5 units xylanase (mg dry wt)$^{-1}$ could be obtained, an almost fourfold increase in xylanase activity when compared to the XlnC-overproducing strain.

In parallel, the effect of PspA overproduction on heterologous protein secretion via the Tat pathway was evaluated. Figure 3 shows that in the PspA-overproducing strain, secretion of active EGFP was significantly enhanced after 24 h of growth. The relative medium fluorescence

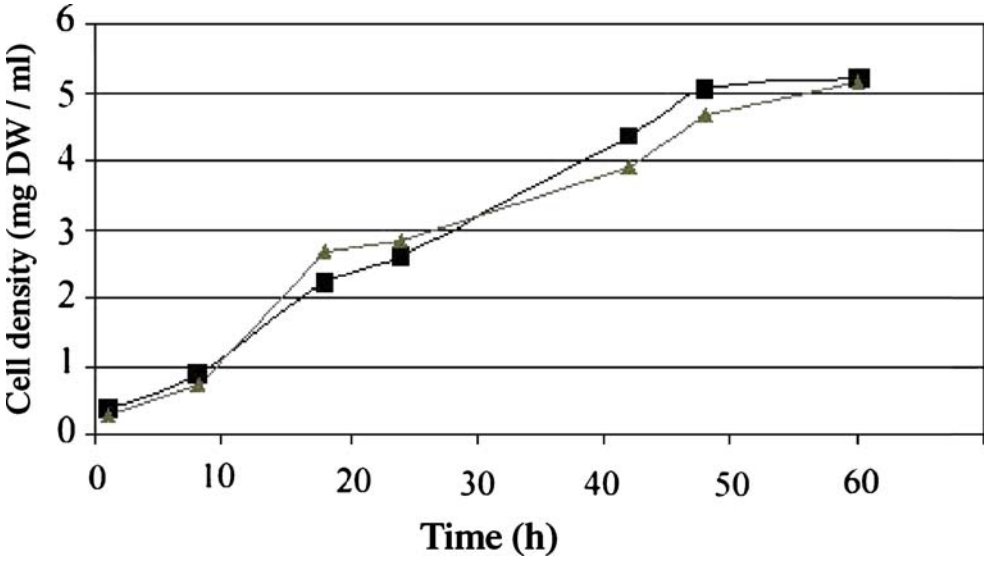

Fig. 1 Effect of *pspA* coexpression on the growth rate of *S. lividans* grown in 50 ml NM medium: *filled squares* (■), *S. lividans* [pIJ486PspA]; *filled triangles* (▲), *S. lividans* TK24

increased from 4.13 to 15.89 RFU (mg dry wt)$^{-1}$, an almost fourfold increase. This corresponds with an increase in active EGFP yield of 6.9 to 20 mg EGFP/l. From the data above, it is clear that overproduction of the *S. lividans* PspA protein has a dramatically positive effect on the secretion of the tested Tat-dependent proteins.

Influence of *pspA* overexpression on Sec-dependent secretion

To test if PspA overproduction also positively affected the Sec-dependent secretion, we evaluated the secretion of the *S. lividans* subtilisin inhibitor 1 (Sti-1) in a PspA-overproducing strain by introducing pIJ486PspA in the wild

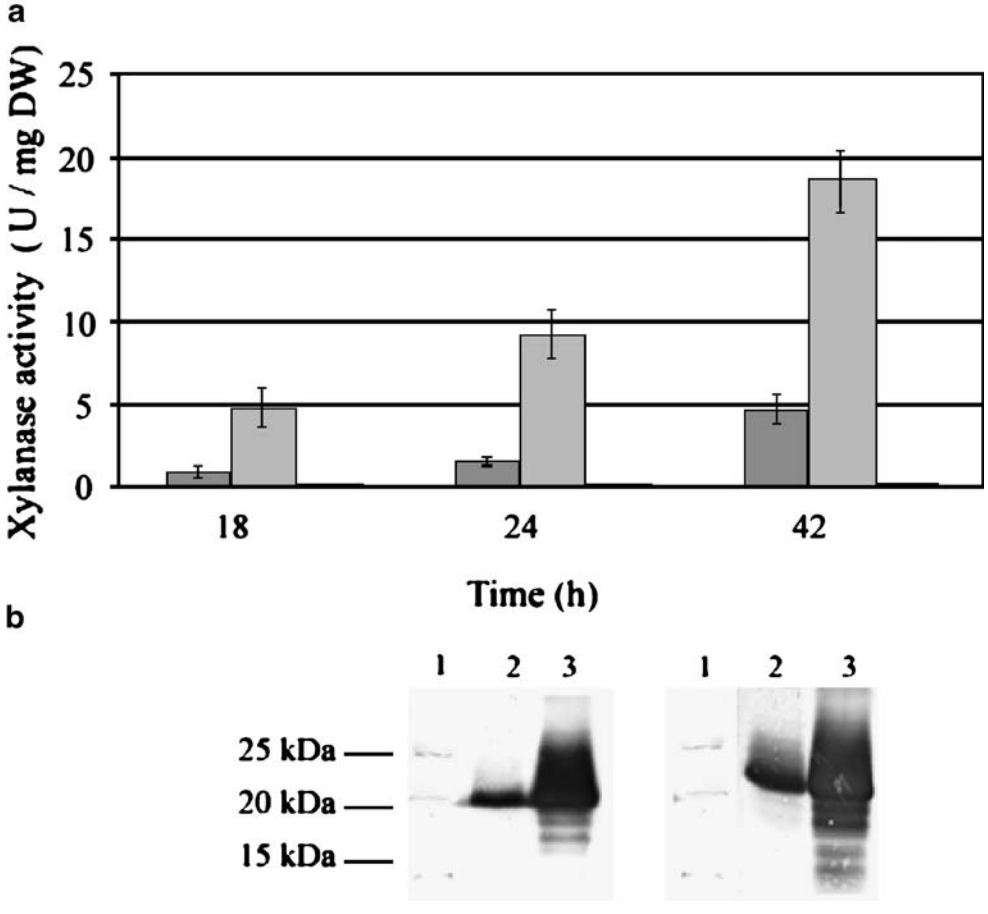

Fig. 2 **a** Effect of *pspA* coexpression on the xylanase activity found in spent medium of *S. lividans* strains expressing the *xlnC* gene and grown for 18, 24 and 42 h: *S. lividans* [pIJ486-PspA+pSSV-XlnC] (*light gray bars*), *S. lividans* [pSSV-XlnC] (*dark gray bars*), *S. lividans* [pIJ486PspA] (*dark bars*). **b** Western blotting with anti-XlnC antibodies on the extracellular fraction of *S. lividans* TK24 [pSSV-XlnC] (*lane 2*) and *S. lividans* TK24 [pIJ486PspA+pSSV-XlnC] (*lane 3*). Cultures were grown for 24 h (*left blot*) or 42 h (*right blot*) in 50 ml NM medium. In each lane, the amount of supernatant corresponding to 4 mg of dry weight was loaded. *Lane 1* shows a molecular weight marker

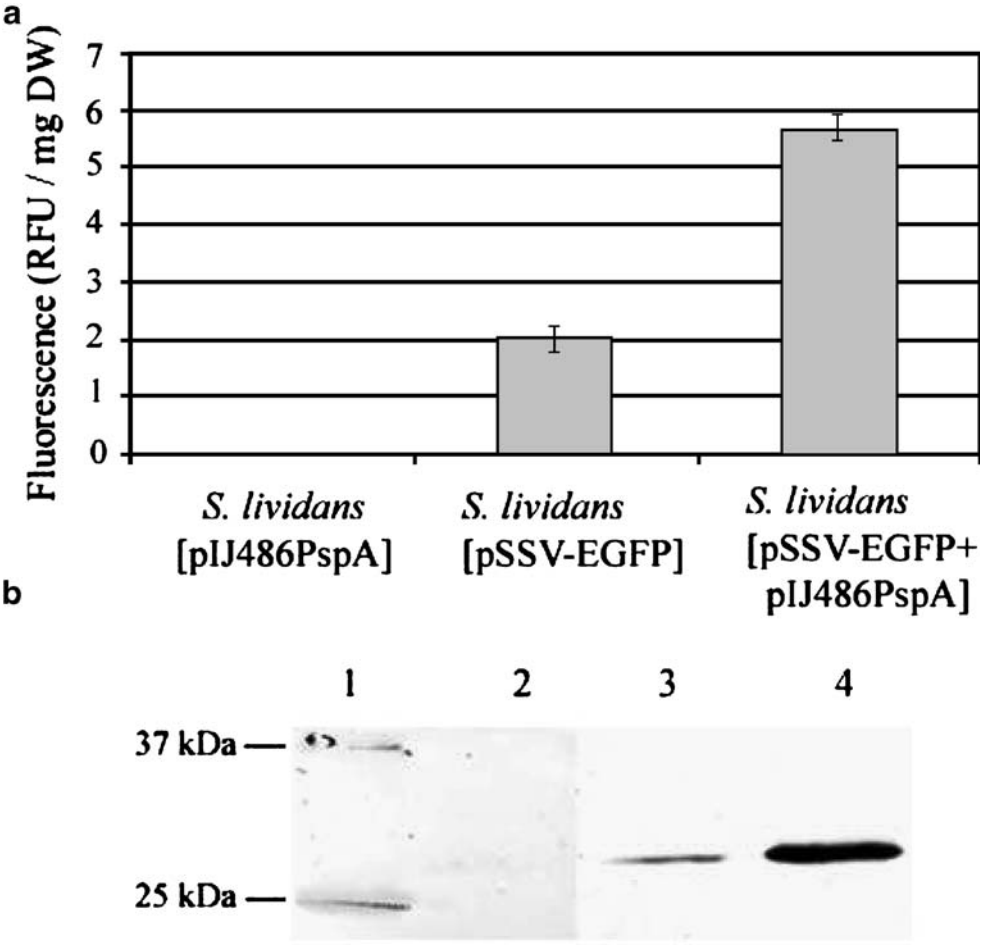

Fig. 3 **a** Effect of *pspA* coexpression on EGFP secretion in *S. lividans* strains grown for 24 h in NM medium. The amount of secreted EGFP was determined by measuring fluorescence intensities, corrected for mycelial dry weight, of spent medium from *S. lividans* [pIJ486], *S. lividans* [pSSVX-EGFP) and from *S. lividans* [pIJ486PspA+pSSVX-EGFP]. **b** Western blotting with anti-GFP antibodies on the extracellular fraction of *S. lividans* [pIJ486PspA] (*lane 2*), *S. lividans* TK24 [pSSV-EGFP] (*lane 3*) and *S. lividans TK24* [pIJ486PspA+pSSV-EGFP] (*lane 4*). Cultures were grown for 24 h in 50 ml NM medium. In each lane, the amount of supernatant corresponding to 0.1 mg of dry weight was loaded. *Lane 1* shows a molecular weight marker

type. The Sti-1 protein is already efficiently secreted from the wild-type *S. lividans*. Secretion of Sti-1 was assayed both for the wild-type and the PspA-overproduction strain grown in NM medium for 18–24 h. Subtilisin inhibitor activity in the supernatant of 18-h cultures ranged from 15.7±0.3 to 18.9±2.0 U (mg dry wt)$^{-1}$ for the wild type and the *pspA* strain, respectively (Fig. 4), indicating a small but statistically significant increase in subtilisin inhibitor activity of 20%. Similar values were obtained after 24 h of growth, confirming the positive effect of *pspA* overexpression on Sec-dependent secretion of native proteins in *S. lividans*.

Finally, to assess the effect of *pspA* overexpression on a Sec-routed heterologous protein, human tumour necrosis factor alpha (hTNFα) was tested. To this end, both the wild-type and the *pspA*-overexpressing strain were transformed with plasmid pSSV-TNF, encoding a Sec-dependent substrate consisting of the Vsi signal peptide fused in frame to the mature hTNFα. Cultures of both strains were grown for 24 h at which point the amount of secreted hTNFα was quantified by ELISA. Extracellular values of 0.72±0.15 μg hTNFα (mg dry wt)$^{-1}$ for the wild type and 1.05±0.27 μg hTNFα (mg dry wt)$^{-1}$ for the PspA-overproducing strain were obtained. Although the PspA-overproducing strain appears to secrete more hTNFα (mg dry wt)$^{-1}$, this difference is statistically not significant, and we can conclude that *pspA* overexpression does not significantly increase the secreted hTNFα yield in *S. lividans*. Taken together, *pspA* overexpression increases the secretion of all tested proteins, but the effect on Tat-dependent protein translocation is more pronounced than on the Sec-routed secretion.

Discussion

Based on the *S. coelicolor* genome, the *S. lividans pspA* gene could be isolated and identified. Remarkably, the known genomic sequence of the *S. coelicolor*, which is closely related to *S. lividans* (Bentley et al. 2002), revealed only a homologue for *pspA* but none for the *pspB* or *C* genes, thought to be needed for a functional psp response (Darwin 2005). The PspB and PspC proteins are presumed to be the sensors of extracytoplasmic stress (Adams et al. 2003), and depending on what stress condition is used, a different protein is needed to get a psp response. The response to most inducing stimuli, such as hyper-osmotic shock and 10% ethanol shock is stimulated by, but not

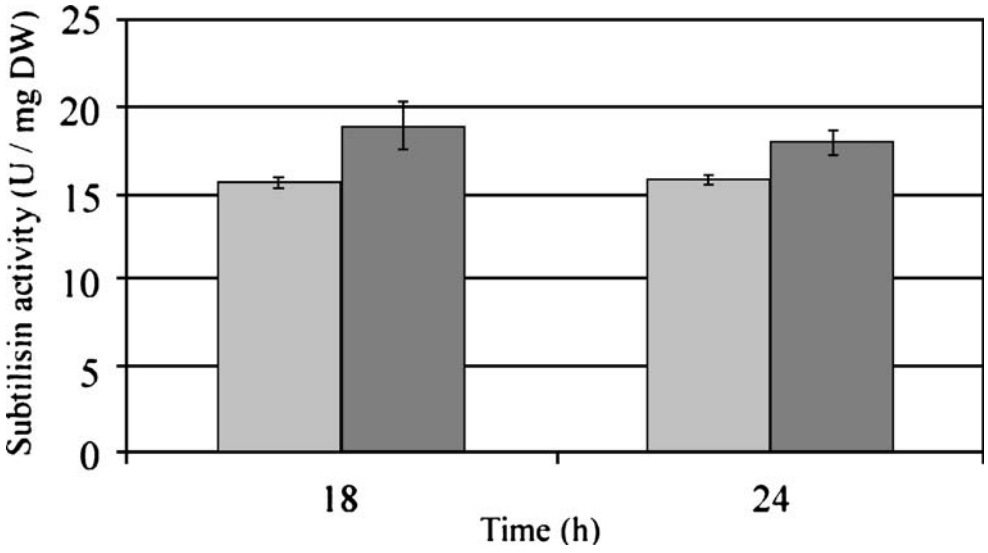

Fig. 4 Subtilisin inhibitor activity expressed as units per milligram of dry weight (DW) measured in culture supernatant of *S. lividans* TK24 [pIJ486] (*light bars*) and *S. lividans* TK24 [pIJ486PspA] (*dark bars*) upon 18 and 24 h of growth

entirely dependent on, PspB and C (Model et al. 1997). Some stress conditions, however, rely on only one of the *pspB/C* genes. In *Yersinia enterocolitica*, for instance, only the *pspB* gene is absolutely necessary to induce a psp response upon overproduction of the YsaC secretin (Maxson and Darwin 2006). Finally some responses, like the induction of PspA by heat shock in *E. coli*, do not require PspB or PspC at all (Weiner et al. 1991). The fact that *pspA* homologues are present in the vast majority of the sequenced bacterial chromosomes and that most of them are not adjacent to any other *psp* genes, indicates that PspA, as such, may play an important role in bacteria, even when PspB and C are not present.

The work presented here clearly shows that overproduction of PspA can positively influence both the Tat- and the Sec-dependent protein secretion in *S. lividans* although the effect on the Tat pathway is much more pronounced. As at least one of the roles of PspA appears to be the maintenance of the PMF (Kleerebezem et al. 1996), the more dramatic effect on the Tat secretion may be explained by the difference in energy requirement of both pathways. The Sec pathway requires ATP as its main energy source (Economou 1999), but the PMF lowers the level of ATP required for the translocation of proteins in *E. coli* (Shiozuka et al. 1990) and can increase the translocation rate of certain proteins (Yamada et al. 1989). On the other hand, the Tat pathway is solely dependent on the PMF (Berks et al. 2003).

It is clear from the obtained results that *pspA* overexpression increases the Tat-dependent protein secretion in *S. lividans* at least threefold, which is similar to the results in *E. coli*, where *pspA* overexpression resulted in a threefold increase of Tat-dependent secretion of both native and heterologous proteins (DeLisa et al. 2004). The Tat pathway has recently gained much attention, thanks to its ability to secrete proteins in a folded state, which can make it a valuable alternative for these proteins that could otherwise not be translocated in an active conformation via the Sec pathway, e.g., EGFP (Santini et al. 2001) and tPA (Kim et al. 2005). This makes the *S. lividans pspA*-overexpressing strain an interesting alternative host for recombinant protein production using the Tat pathway, as evidenced here by a yield of up to 20 mg/l EGFP, a protein that is notoriously difficult to secrete.

The effect on the Sec-dependent secretion is less pronounced and will probably vary from protein to protein. This is in correspondence with the observation made by Yamada et al. (1989) that the dissipation of the PMF has a differential effect on different secretory proteins. For some proteins, this dissipation resulted in a large decrease in translocation, whereas others were less influenced. This means that the *S. lividans pspA*-overexpressing strain might be a valuable host for the secretion of some, but not all, proteins via the Sec pathway. Whether or not overexpression of *pspA* can protect *S. lividans* against other conditions that might induce membrane-related stress is a question that we are currently investigating. Finally, this work also supports the possibility of increasing protein secretion in a wide range of hosts, all containing a *pspA* homologue but not a complete *psp* system.

Acknowledgements KV and SDK are research fellows of IWT (Vlaams Instituut voor de bevordering van het Wetenschappelijk-Technologisch onderzoek in de industrie). This research was supported by grants G.0389.05. from Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO) and OT/00/37 from K.U.Leuven and QLK3-CT-2002-02056 from the European Union.

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