JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Twin-Arginine Signal Peptide of *Bacillus subtilis* YwbN Can Direct Tat-Dependent Secretion of Methyl Parathion Hydrolase

Ruihua Liu,[†,‡] Zhenqiang Zuo,[†,§] Yingming Xu,[∥] Cunjiang Song,[§] Hong Jiang,[⊥] Chuanling Qiao,[⊥] Ping Xu,*[,#] Qixing Zhou,*[,¶] and Chao Yang*[,§]

[‡]College of Pharmacy and Tianjin Key Laboratory of Molecular Drug Research, [§]Key Laboratory of Molecular Microbiology and Technology for Ministry of Education, Department of Microbiology, College of Life Sciences, and [¶]Key Laboratory of Pollution Processes and Environmental Criteria for Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, People's Republic of China

[∥]Key Laboratory of Original Agro-environment Quality of Ministry of Agriculture and Tianjin Key Laboratory of Agro-environment and Safe-product, Tianjin 300191, People's Republic of China

[⊥]State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China

[#]State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, People's Republic of China

ABSTRACT: The twin-arginine translocation (Tat) pathway exports folded proteins across the cytoplasmic membranes of bacteria and archaea. Two parallel Tat pathways (TatAdCd and TatAyCy systems) with distinct substrate specificities have previously been discovered in *Bacillus subtilis*. In this study, to secrete methyl parathion hydrolase (MPH) into the growth medium, the twin-arginine signal peptide of *B. subtilis* YwbN was used to target MPH to the Tat pathway of *B. subtilis*. Western blot analysis and MPH assays demonstrated that active MPH was secreted into the culture supernatant of wild-type cells. No MPH secretion occurred in a total-tat_2 mutant, indicating that the observed export in wild-type cells was mediated exclusively by the Tat pathway. Export was fully blocked in a *tatAyCy* mutant. In contrast, the *tatAdCd* mutant was still capable of secreting MPH. These results indicated that the MPH secretion directed by the YwbN signal peptide was specifically mediated by the TatAyCy system. The N-terminal sequence of secreted MPH was determined as AAPQVR, demonstrating that the YwbN signal peptide had been processed correctly. This is the first report of functional secretion of a heterologous protein via the *B. subtilis* TatAyCy system. This study highlights the potential of the TatAyCy system to be used for secretion of other heterologous proteins in *B. subtilis*.

KEYWORDS: *Tat pathway, twin-arginine signal peptide, methyl parathion hydrolase, Bacillus subtilis 168*

INTRODUCTION

Synthetic organophosphates are a group of highly toxic chemicals that irreversibly inhibit acetylcholinesterase, a key enzyme in the nerve message transmission system.[1,2] Organophosphorus pesticides (OPs) are widely applied to control pests in agricultural practice in developing countries. However, the heavy use of OPs has caused problems of pesticide residues in food, water, and soil.[3−5]

Organophosphorus hydrolase (OPH) (also referred to as phosphotriesterase or parathion hydrolase), isolated from two parathion-degrading bacteria, *Pseudomonas diminuta* MG and *Flavobacterium* sp. strain ATCC 27551,[6,7] has been extensively studied for its use in the enzymatic degradation of various organophosphorus compounds. Afterward, a novel OP hydrolase gene (*mpd*) encoding methyl parathion hydrolase (MPH) was isolated from methyl parathion (MP)-degrading *Plesiomonas* sp. strain M6.[8] OPH and MPH have different substrate specificity toward OPs. We cloned the *mpd* gene from chlorpyrifos-degrading *Stenotrophomonas* sp. strain YC-1, and the gene shows 98.5% identity to that from strain M6 at the amino acid level.[9] However, the MPH in strain M6 or YC-1 is located intracellularly or secreted into the periplasm. The outer membrane acts as a permeability barrier and limits interaction between the pesticides and MPH residing within the cells.[10] Secretion of MPH to the culture medium has several advantages over intracellular production, such as simplified downstream processing, enhanced enzymatic activity, and higher protein stability and solubility.

The twin-arginine translocation (Tat) system is a protein targeting pathway with the remarkable ability to transport fully folded proteins across the cytoplasmic membranes of bacteria.[11−13] These proteins are specifically directed to the Tat pathway by N-terminal twin-arginine signal peptides bearing a consensus motif of SRRxFLK.[14] In *Escherichia coli*, the core complex for Tat-dependent protein translocation consists of the three Tat components: TatA, TatB, and TatC.[15−17] A working model has been proposed: the specific recognition for the twin-arginine motif of the Tat substrate is performed by a membrane-bound TatBC complex;[18] a separate

Received: December 19, 2013
Revised: March 11, 2014
Accepted: March 12, 2014
Published: March 12, 2014

Table 1. Strains, Plasmids, and Primers Used in This Study

strain, plasmid, or primer	description	source or reference
	Strains	
B. subtilis		
168	*trpC2*	25
total-tat_2	*trpC2*; *tatAc*::Em; *tatAy-tatCy*::Sp; *tatAd-tatCd*::Km; Em^r; Sp^r; Km^r	21
tatAyCy	*trpC2*; *tatAy-tatCy*::Sp; Sp^r	21
tatAdCd	*trpC2*; *tatAd-tatCd*::Km; Km^r	21
	Plasmids	
pUC18	pUC origin, P_{lac}, Ap^r	TaKaRa
pWH1520	*E. coli*–*B. subtilis* shuttle vector, *pBC16 ori*, *pBR ori*, P_{xylA}, *xylR*, Tc^r, Ap^r	26
pWYM20	pWH1520 derivative, secretion expression vector coding for YwbN-MPH	this study
pWTM20	pWH1520 derivative, secretion expression vector coding for TorA-MPH	23
pWM20	pWH1520 derivative, cytoplasmic expression vector coding for MPH	23
	Primers	
P1	GAATTCCATGAGCGATGAACAGAAA	this study
P2	CTGCAGCTGAGGCAACAAGCGGAGCGA	this study
P3	CTGCAGCACCGCAGGTGCGC	this study
P4	AAGCTTTCACTTGGGGTTGACGAC	this study
P5	GGTACCTCTAGAGGGTATTAATAATGAGCGATGAACAGAAA	this study
P6	GCATGCTCAGTGGTGGTGGTGGTGCTTGGGGTTGACGAC	this study

TatA complex is then recruited to form a pore through which substrates are translocated.[19] *E. coli* usually does not secrete Tat substrates into the growth medium because of the presence of the outer membrane.

The *Bacillus subtilis* Tat machinery lacks TatB and consists of three TatA (namely, TatAd, TatAy, and TatAc) and two TatC (TatCd and TatCy).[20] The TatA and TatC proteins form two active Tat translocation complexes, TatAdCd and TatAyCy, which display different substrate specificities.[21] Recently, a working model has been proposed for *B. subtilis*, in which the TatA protein has a dual function, because it can complement for the absence of both TatA and TatB in *E. coli*.[22] Moreover, Tat pathway specificity in *B. subtilis* is determined by twin-arginine signal peptide interactions with TatCd or TatCy.[21] In *B. subtilis*, Tat substrates can be secreted directly into culture media because of the absence of an outer membrane.

In a previous study, the twin-arginine signal peptide of TorA from *E. coli* directed secretion of MPH via the *B. subtilis* Tat pathway into the culture medium.[23] In this study, the MPH secretion directed by the *B. subtilis* YwbN signal peptide was achieved, leading to 1.8-fold improvement in extracellular MPH activity compared to the activity achieved with the TorA signal peptide. Furthermore, we demonstrated that the MPH secretion was specifically mediated by the TatAyCy system.

MATERIALS AND METHODS

Strains, Plasmids, Primers, and Culture Conditions. The strains, plasmids, and primers used in this study are listed in Table 1. *E. coli* DH5α was used for construction of recombinant plasmids. *B. subtilis* 168 was used for expressing recombinant MPH. All strains were grown at 37 °C in Luria–Bertani (LB) medium.[24] If necessary, media were supplemented with ampicillin (Ap; 100 μg/mL), tetracycline (Tc; 15 μg/mL), erythromycin (Em; 5 μg/mL), spectinomycin (Sp; 100 μg/mL), or kanamycin (Km; 10 μg/mL). When the *B. subtilis* culture reached an OD_{600} of 0.6, 0.5% xylose was added to induce protein expression for 24 h at 37 °C.

Construction of Recombinant *B. subtilis* 168. The nucleotide sequence encoding the twin-arginine signal peptide of *B. subtilis* YwbN was amplified from *B. subtilis* 168 chromosomal DNA[25] using primers P1 and P2, digested with *Eco*RI/*Pst*I and ligated into *Eco*RI/*Pst*I digested pUC18 to generate pUY18. The *mpd* gene was amplified from *Stenotrophomonas* sp. YC-1 chromosomal DNA[9] using primers P3 and P4, digested with *Pst*I/*Hin*dIII, and ligated into *Pst*I/*Hin*dIII-digested pUY18 to generate pUYM18. The *ywbN–mpd* fusion gene was amplified from pUYM18 using primers P5 and P6, digested with *Kpn*I/*Sph*I, and subcloned into *Kpn*I/*Sph*I-digested pWH1520,[26] an *E. coli*–*B. subtilis* shuttle vector, to create pWYM20. All plasmid constructions were verified by DNA sequence analysis. Transformation of plasmid into *B. subtilis* 168 was carried out according to the method by Sadaie and Kada.[27]

Western Blot Analysis. Cells were resuspended in lysis buffer with 5 mg/mL lysozyme, incubated at 37 °C for 30 min, and disrupted by sonication. Extracellular proteins were precipitated by adding 10% trichloroacetic acid for 12 h at 4 °C. Samples of total cell protein and extracellular protein were analyzed on 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)[24] and electrophoretically transferred onto a nitrocellulose membrane with a tank transfer system. After blocking non-specific binding sites with 3% bovine serum albumin (BSA) in TBST solution (20 mM Tris–HCl at pH 7.5, 150 mM NaCl, and 0.05% Tween-20), the membrane was incubated with rabbit anti-MPH serum at a 1:1000 dilution at 30 °C for 2 h and washed with TBST. Subsequently, the membrane was allowed to react with alkaline phosphatase-conjugated goat anti-rabbit IgG antibody (Promega) at a 1:2000 dilution at 30 °C for 1 h. The membrane was then stained with NBT/BCIP (Novagen) for visualizing antigen–antibody conjugates. The rabbit polyclonal antiserum was raised against the purified His_6-tagged MPH as described by Yang et al.[23]

MPH Assays. MPH activity was measured with MP as the substrate by monitoring an increase in absorbance at 405 nm (ε_{405} = 17 700 M^{-1} cm^{-1} for *p*-nitrophenol) at 30 °C.[28] For each assay, 100 μL of concentrated supernatant and 5 μL of 10 mg/mL MP (methanol stock solution) were added to 895 μL of 100 mM phosphate buffer (pH 7.4). One unit of activity was defined as the formation of 1 μmol of *p*-nitrophenol per minute.

Purification of His_6-Tagged MPH. For purification of the recombinant MPH from the culture supernatant of *B. subtilis* 168 carrying pWYM20, 5 mL of preculture was inoculated into 1 L of LB medium and grown to an OD_{600} of 0.6 at 37 °C. Subsequently, 0.5% xylose was added to induce protein expression at 37 °C for 24 h. The culture supernatant was concentrated 10-fold by ultrafiltration and dialyzed overnight against 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.5). Recombinant His_6-tagged MPH was purified using nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography, following the procedure described by Fu et al.[28] In brief, the concentrated fraction was loaded onto a Ni–NTA column (Novagen). His_6-tagged MPH tightly bound to the Ni–NTA

matrix and fusion proteins were eluted with elution buffer (20 mM Tris-HCl, 0.5 mM NaCl, and 300 mM imidazole at pH 7.9). The purity of purified MPH was examined by 12% SDS–PAGE. Protein concentrations were determined by the Bradford method.[24] The N-terminal sequence of purified MPH was determined by high-performance liquid chromatography–electrospray ionization mass spectrometry (HPLC–EIMS).

Pesticide Degradation Experiments. *B. subtilis* 168 cells harboring pWYM20 or pWTM20 were grown to an OD_{600} of 0.6 and then induced with 0.5% xylose for 24 h at 37 °C. Cells and supernatant were separated by centrifugation. Subsequently, 0.4 mM MP was added to the supernatant. Samples were incubated for 2 h at 30 °C and 150 rpm on a shaker. Then, residual pesticides were extracted twice with ethyl acetate, and the amounts of pesticides were determined by gas chromatography (GC). The detection conditions for GC were the same as those described by Yang et al.[23] Degradation of pesticide residues on cabbage was also performed as described by Yang et al.[23] using the supernatant of *B. subtilis* 168 cultures expressing YwbN–MPH.

RESULTS AND DISCUSSION

Tat-Dependent Secretion of MPH Directed by the YwbN Signal Peptide. Protein substrates of the Tat pathway are folded prior to translocation and are targeted to the Tat machinery by cleavable N-terminal signal peptides containing an almost invariant pair of arginine residues.[11,12] In a previous work, MPH was secreted by the *B. subtilis* Tat pathway into the growth medium when fused to the twin-arginine signal peptide of TorA from *E. coli*.[23] In this study, to enhance secretion of MPH to extracellular milieu, we used the twin-arginine signal peptide of YwbN from *B. subtilis*. A secretory expression vector pWYM20 coding for YwbN–MPH fusion protein was constructed and transformed into *B. subtilis* 168. A typical cleavage site (ASA-A) for signal peptidase I[29] was designed for producing mature MPH with an authentic N-terminal sequence of AAPQVR by cleaving the YwbN signal peptide.

To evaluate the efficiency of *B. subtilis* as the host strain for the Tat-dependent secretion of MPH, the distribution of MPH in cell lysate and culture supernatant was determined by western blotting with MPH antiserum. After 24 h of induction, the *B. subtilis* culture was fractionated into cells and culture supernatant and then these fractions were probed with anti-MPH serum. As shown in Figure 1, two specific bands corresponding to the unprocessed YwbN–MPH precursor (37 kDa) and the mature-sized MPH (32 kDa) were detected in the cell lysate of *B. subtilis* 168. As expected, mature-sized MPH was found predominantly in the culture supernatant of *B. subtilis* 168.

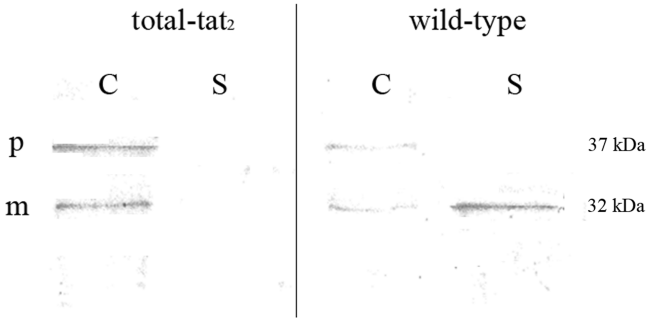

Figure 1. Secretion of MPH by the Tat pathway in *B. subtilis*. The culture of *B. subtilis* 168/pWYM20 or 168 total-tat_2/pWYM20 was fractionated into cells (C) and supernatant (S). The samples were used for SDS–PAGE and western blot using anti-MPH serum. p, YwbN–MPH precursor; m, mature-sized MPH. The protein sample loaded onto each lane in SDS–PAGE was 10 μg.

To determine whether the translocation of MPH was mediated specifically by the Tat pathway of *B. subtilis*, the secretory expression vector pWYM20 was also transformed into 168 total-tat_2 mutant strain, in which all *tat* genes encoding Tat translocases have been inactivated by insertional mutagenesis.[21] In contrast to wild-type strain, no secreted MPH was found in the culture supernatant of total-tat_2 mutant (Figure 1). Interestingly, mature-sized MPH was also found in the cellular fraction of the 168 total-tat_2 mutant, possibly resulting from proteolysis of YwbN–MPH. The degradation of TorA or YwbN chimeric proteins has previously been reported,[11,23] suggesting that the Tat signal peptides may be easily accessible to proteases. As shown in Figure 2, extracellular MPH activity

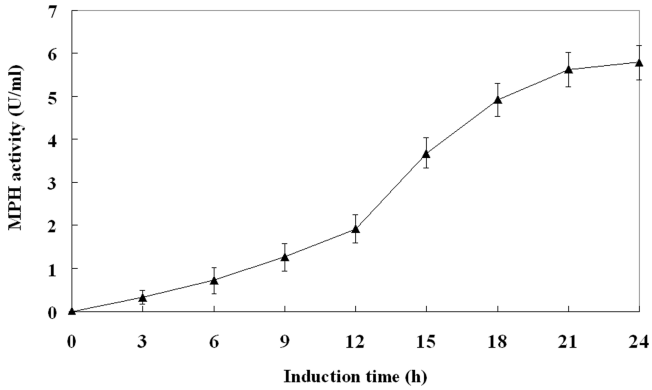

Figure 2. Extracellular MPH activity in *B. subtilis* 168 cultures capable of secreting MPH with the *B. subtilis* YwbN signal peptide. Cells were cultured in LB medium containing 15 μg/mL tetracycline at 37 °C and 250 rpm and then induced at OD_{600} = 0.6 by adding 0.5% xylose. The data are the mean ± standard deviation of three replicates.

of *B. subtilis* 168 cultures expressing YwbN–MPH increased gradually after induction and accumulated to a maximum level (5.78 units/mL) at 24 h. In contrast, no MPH activity was detected in the culture supernatant of 168 total-tat_2 mutant strain expressing YwbN–MPH fusion protein. These results clearly showed that MPH could be secreted by *B. subtilis* 168 in a strictly Tat-dependent manner.

Secretion of MPH with the YwbN Signal Peptide Is TatAyCy-Dependent. The *B. subtilis* Tat machinery comprises two active Tat translocation complexes with distinct substrate specificities: PhoD is secreted by the TatAdCd complex, whereas YwbN is secreted by the TatAyCy complex.[21] To examine whether the MPH secretion directed by the YwbN signal peptide would also be TatAyCy-dependent, we expressed the YwbN–MPH fusion protein in a *tatAyCy* or *tatAdCd* mutant strain. Secretion of MPH by these *tat* mutant strains was verified by western blot with MPH antiserum. MPH was detected in the culture medium of the *tatAdCd* mutant producing YwbN–MPH, which indicated that the *tatAdCd* mutant was still capable of secreting MPH, relying on using the other TatAyCy system. In contrast, MPH was not secreted into the culture medium of the *tatAyCy* mutant expressing YwbN–MPH (data not shown).

To further demonstrate whether the MPH secretion was specifically mediated by the TatAyCy system, extracellular MPH activity was also detected with these *tat* mutant strains. As a result, no MPH activity was detected in the culture

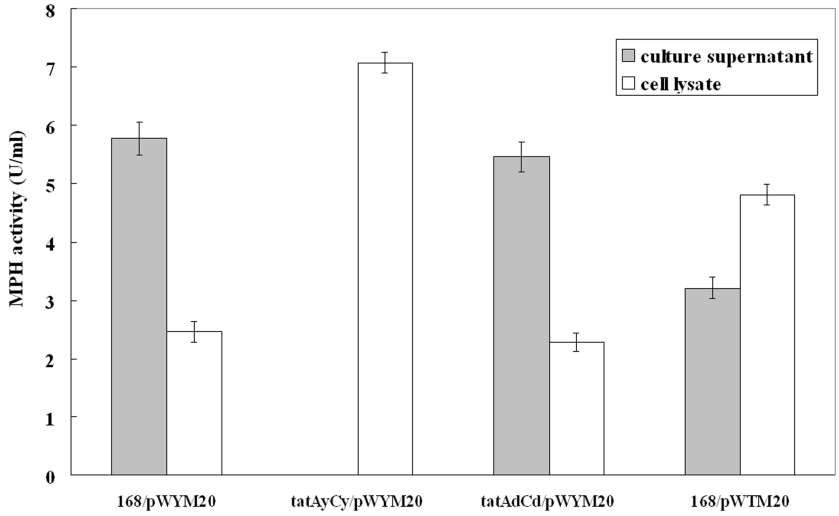

Figure 3. Specific MPH activities of the culture supernatant and total cell lysate derived from *B. subtilis* 168 and its *tat* mutant strains. 168/pWYM20, *B. subtilis* 168 expressing YwbN−MPH; *tatAyCy*/pWYM20, *tat* mutant strain of *B. subtilis* 168 lacking functional TatAyCy translocase; *tatAdCd*/pWYM20, *tat* mutant strain of *B. subtilis* 168 lacking functional TatAdCd translocase; and 168/pWTM20, *B. subtilis* 168 expressing TorA−MPH. Cells carrying pWYM20 or pWTM20 were incubated at 37 °C for 24 h after induction with 0.5% xylose. The data are the mean ± standard deviation of three replicates.

Table 2. Purification of Recombinant His_6-Tagged MPH from 1 L of Culture Supernatant by Metal Affinity Chromatography

purification step	total protein (mg)	total activity (units)	specific activity (units/mg of protein)	yield (%)	fold
culture supernatant	426	5260	12	100	1
Ni−NTA column	13	4576	352	87	29

supernatant of the *tatAyCy* mutant expressing YwbN−MPH. In contrast, MPH activity (5.46 units/mL) was found in the culture supernatant of the *tatAdCd* mutant expressing YwbN−MPH (Figure 3). These results indicated that the YwbN signal peptide directed MPH secretion in a strictly TatAyCy-dependent manner.

Efficiency of Tat-Dependent Secretion of MPH in *B. subtilis* 168. The majority of total MPH activity (70%) was found in the supernatant of *B. subtilis* 168 cultures expressing YwbN−MPH. The cell lysate contained lower activity (30% of total), and the data are consistent with the MPH immunoblots. Extracellular MPH activity of *B. subtilis* cultures expressing YwbN−MPH was 1.8-fold higher than that of *B. subtilis* cultures expressing TorA−MPH (Figure 3). Enhanced secretion of MPH activity was achieved with the YwbN signal peptide, indicating that the selection of Tat signal peptides is crucial to improve Tat-dependent protein secretion efficiency.

To determine the amount of MPH that was secreted by *B. subtilis* 168, the recombinant MPH was purified from 1 L of culture supernatant by metal affinity chromatography using a Ni−NTA column following the procedures described in the Materials and Methods. His_6-tagged MPH tightly bound to the Ni−NTA matrix, and fusion proteins were eluted with elution buffer containing 300 mM imidazole, thereby allowing for their one-step purification to electrophoretic homogeneity. The purified His_6-tagged MPH gave a single band appearing at the position of 32 kDa in SDS−PAGE (data not shown), which is in good agreement with the molecular mass estimated from the deduced amino acid sequence of the fusion protein. From 1 L of culture supernatant, we routinely obtained 13 mg of purified MPH with a purification of 29-fold, 87% final yield, and a specific activity of 352 units/mg. The data on the purification are summarized in Table 2. The N-terminal sequence of the purified MPH was determined by HPLC−EIMS as AAPQVR, demonstrating that the YwbN signal peptide had been processed correctly.

Degradation of Methyl Parathion by Recombinant *B. subtilis*. In this study, *B. subtilis* 168 cultures capable of secreting MPH (pWYM20) degraded MP 12-fold faster than *B. subtilis* 168 cultures expressing cytoplasmic MPH (pWM20). In contrast, no MP degradation was observed with *B. subtilis* 168 transformed with the empty vector pWH1520. Extracellular secretion of MPH is advantageous over intracellular production, because it allows MPH to freely bind extracellular substrates. This will greatly improve the application efficiency of the engineered strain to degrade organophosphorus pesticides in farm products and contaminated soil and water.

In this study, the supernatant can be separated from the cells by one-step centrifugation and used as the crude enzyme for the removal of pesticide residues on vegetables. The removal efficiencies for MP and chlorpyrifos on cabbage were 99 and 96%, respectively. Moreover, the supernatant containing secreted MPH was shown to effectively degrade MP (Figure 4). In contrast, the supernatant from *B. subtilis* cultures expressing YwbN−MPH (pWYM20) degraded MP 2-fold faster than the supernatant from *B. subtilis* cultures expressing TorA−MPH (pWTM20), which indicated that the efficiency of MPH secretion had been improved by use of the YwbN signal peptide.

To test whether extracellular secretion of MPH inhibits cell growth, the growth kinetics of cells carrying pWYM20 or pWH1520 were compared. No growth inhibition was observed for cells expressing YwbN−MPH. The two cultures showed the same growth profile and reached the same final cell density after 48 h of incubation. To monitor the stability of MPH activity of the concentrated supernatant, the activity was

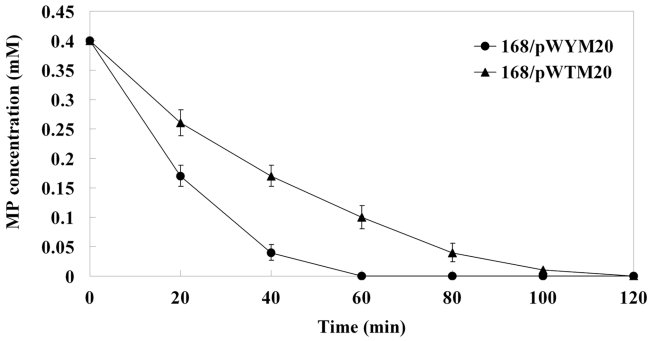

Figure 4. Degradation of MP by the culture supernatant of *B. subtilis* 168 cells capable of secreting MPH with the *B. subtilis* YwbN signal peptide (pWYM20) or *E. coli* TorA signal peptide (pWTM20). Methyl parathion was added at an initial concentration of 0.4 mM. The data are the mean ± standard deviation of three replicates.

determined each day over a 2-week period. MPH activity of the concentrated supernatant remained at essentially the original level over the duration of 2 weeks (Figure 5). The stability of

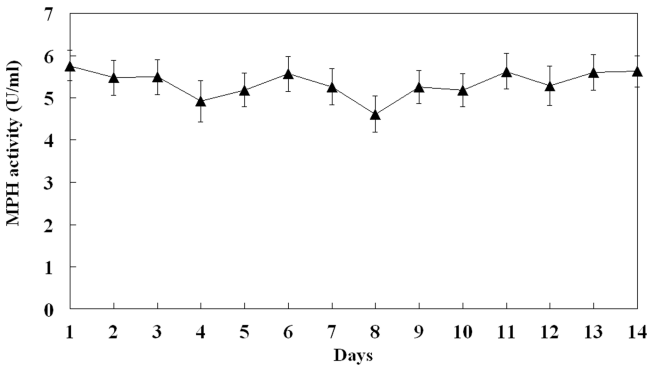

Figure 5. Stability of MPH activity of the culture supernatant of *B. subtilis* 168 cells capable of secreting MPH with the *B. subtilis* YwbN signal peptide (pWYM20) over the duration of 2 weeks. MPH activity was measured with MP as the substrate. The data are the mean ± standard deviation of three replicates.

the MPH activity observed here is in line with the results of previous studies in which MPH was secreted by *B. subtilis* 168 with the TorA signal peptide of *E. coli*.[23]

The suitability and popularity of *B. subtilis* as one of the main hosts for recombinant protein production are due to several reasons: generally recognized as safe as a result of its lack of pathogenicity and the absence of endotoxins, the absence of an outer membrane, easy for genetic manipulation and handling, and capable of large-scale fermentation.[25] These merits make recombinant *B. subtilis* a promising candidate for extracellular production of MPH for practical industrial applications, for example, enzyme-based products.

In previous studies, the YwbN signal peptide directed secretion of the *B. subtilis* subtilisin via the TatAyCy route. In contrast, the YwbN signal peptide directed secretion of the *Bacillus licheniformis* α-amylase and the *E. coli* SufI in a Tat-independent manner.[29] The *E. coli* phytase AppA was shown to export TatAdCd dependently when fused to the twin-arginine signal peptide of PhoD.[30] Tat-dependent secretion of the green fluorescent protein was reported, albeit in an inactive form.[31] Thus far, there are no reports of TatAyCy-dependent export of an active heterologous protein in *B. subtilis*. In this study, active MPH was exported to the extracellular environment via the *B. subtilis* TatAyCy system when fused to the twin-arginine signal peptide of YwbN. Here, we demonstrated for the first time TatAyCy-dependent secretion of an active heterologous protein in *B. subtilis*. In future studies, we will further explore the capability of the *B. subtilis* TatAyCy system for secretion of other heterologous proteins.

AUTHOR INFORMATION

Corresponding Authors

*Telephone/Fax: 86-21-34206723. E-mail: pingxu@sjtu.edu.cn.
*Telephone: 86-22-23507800. Fax: 86-22-66229562. E-mail: zhouqx523@yahoo.com.
*Telephone/Fax: 86-22-23503866. E-mail: yang_chao2008@hotmail.com.

Author Contributions

†Ruihua Liu and Zhenqiang Zuo contributed equally to this work.

Funding

The authors gratefully acknowledge the financial support from the National High Technology Research and Development Program of China ("863" Program, 2013AA06A210), the National Natural Science Foundation of China (31300032), the Open Project Funding of the State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University (MMLKF13-06), the Project of Tianjin, China (13JCQNJC09700), and the Open Project Funding of the Key Laboratory of Original Agro-environment Quality of Ministry of Agriculture and Tianjin Key Laboratory of Agro-environment and Safe-product (2013-KF-06).

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Singh, B. K.; Walker, A. Microbial degradation of organophosphorus compounds. *FEMS Microbiol. Rev.* **2006**, *30*, 428−471.
(2) Singh, B. K. Organophosphorus-degrading bacteria: Ecology and industrial applications. *Nat. Rev. Microbiol.* **2009**, *7*, 156−164.
(3) Bai, Y.; Zhou, L.; Wang, J. Organophosphorus pesticide residues in market foods in Shaanxi area, China. *Food Chem.* **2006**, *98*, 240−242.
(4) Salas, J.; Noa, M.; Perez, N.; Gutierrez, R.; Zazueta, H.; Osuna, I. Organophosphorus pesticide residues in Mexican commercial pasteurized milk. *J. Agric. Food Chem.* **2003**, *51*, 4468−4471.
(5) Uchimiya, M.; Wartelle, L.; Boddu, V. Sorption of triazine and organophosphorus pesticides on soil and biochar. *J. Agric. Food Chem.* **2012**, *60*, 2989−2997.
(6) Mulbry, W. W.; Karns, J. S. Parathion hydrolase specified by the *Flavobacterium opd* gene: Relationship between the gene and protein. *J. Bacteriol.* **1989**, *171*, 6740−6746.
(7) Serdar, C. M.; Gibson, D. T. Enzymatic hydrolysis of organophosphates: Cloning and expression of a parathion hydrolase gene from *Pseudomonas diminuta*. *Biotechnology* **1985**, *3*, 567−571.
(8) Cui, Z.; Li, S.; Fu, G. Isolation of methyl parathion-degrading strain M6 and cloning of the methyl parathion hydrolase gene. *Appl. Environ. Microbiol.* **2001**, *67*, 4922−4925.
(9) Yang, C.; Liu, N.; Guo, X.; Qiao, C. Cloning of *mpd* gene from a chlorpyrifos-degrading bacterium and use of this strain in bioremediation of contaminated soil. *FEMS Microbiol. Lett.* **2006**, *265*, 118−125.
(10) Richins, R. D.; Kaneva, I.; Mulchandani, A.; Chen, W. Biodegradation of organophosphorus pesticides by surface-expressed organophosphorus hydrolase. *Nat. Biotechnol.* **1997**, *15*, 984−987.
(11) Thomas, J. D.; Daniel, R. A.; Errington, J.; Robinson, C. Export of active green fluorescent protein to the periplasm by the twin-

arginine translocase (Tat) pathway in *Escherichia coli*. *Mol. Microbiol.* **2001**, *39*, 47−53.
(12) DeLisa, M. P.; Tullman, D.; Georgiou, G. Folding quality control in the export of proteins by the bacterial twin-arginine translocation pathway. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 6115−6120.
(13) Palmer, T.; Berks, B. C. The twin-arginine translocation (Tat) protein export pathway. *Nat. Rev. Microbiol.* **2012**, *10*, 483−496.
(14) Berks, B. C. A common export pathway for proteins binding complex redox cofactors? *Mol. Microbiol.* **1996**, *22*, 393−404.
(15) Bogsch, E. G.; Sargent, F.; Stanley, N. R.; Berks, B. C.; Robinson, C.; Palmer, T. An essential component of a novel bacterial protein export system with homologues in plastids and mitochondria. *J. Biol. Chem.* **1998**, *273*, 18003−18006.
(16) Sargent, F.; Bogsch, E. G.; Stanley, N. R.; Wexler, M.; Robinson, C.; Berks, B. C.; Palmer, T. Overlapping functions of components of a bacterial Sec-independent protein export pathway. *EMBO J.* **1998**, *17*, 3640−3650.
(17) Sargent, F.; Stanley, N. R.; Berks, B. C.; Palmer, T. Sec-independent protein translocation in *Escherichia coli*: A distinct and pivotal role for the TatB protein. *J. Biol. Chem.* **1999**, *274*, 36073−36082.
(18) Alami, M.; Lüke, I.; Deitermann, S.; Eisner, G.; Koch, H. G.; Brunner, J.; Müller, M. Differential interactions between a twin-arginine signal peptide and its translocase in *Escherichia coli*. *Mol. Cell* **2003**, *12*, 937−946.
(19) Gohlke, U.; Pullan, L.; McDevitt, C. A.; Porcelli, I.; de Leeuw, E.; Palmer, T.; Saibil, H. R.; Berks, B. C. The TatA component of the twin-arginine protein transport system forms channel complexes of variable diameter. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 10482−10486.
(20) Jongbloed, J. D. H.; Martin, U.; Antelmann, H.; Hecker, M.; Tjalsma, H.; Venema, G.; et al. TatC is a specificity determinant for protein secretion via the twin-arginine translocation pathway. *J. Biol. Chem.* **2000**, *275*, 41350−41357.
(21) Jongbloed, J. D.; Grieger, U.; Antelmann, H.; Hecker, M.; Nijland, R.; Bron, S.; van Dijl, J. M. Two minimal Tat translocases in *Bacillus*. *Mol. Microbiol.* **2004**, *54*, 1319−1325.
(22) Barnett, J. P.; Eijlander, R. T.; Kuipers, O. P.; Robinson, C. A minimal Tat system from a Gram-positive organism: A bifunctional TatA subunit participates in discrete TatAC and TatA complexes. *J. Biol. Chem.* **2008**, *283*, 2534−2542.
(23) Yang, C.; Song, C.; Freudl, R.; Mulchandani, A.; Qiao, C. Twin-arginine translocation of methyl parathion hydrolase in *Bacillus subtilis*. *Environ. Sci. Technol.* **2010**, *44*, 7607−7612.
(24) Sambrook, J.; Russel, D. W. *Molecular Cloning: A Laboratory Manual*, 3rd ed.; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY, 2001.
(25) Kunst, F.; Ogasawara, N.; Moszer, I.; et al. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. *Nature* **1997**, *390*, 249−256.
(26) Rygus, T.; Hillen, W. Inducible high-level expression of heterologous genes in *Bacillus megaterium* using the regulatory elements of the xylose-utilization operon. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 594−599.
(27) Sadaie, Y.; Kada, T. Formation of competent *Bacillus subtilis* cells. *J. Bacteriol.* **1983**, *153*, 813−821.
(28) Fu, G.; Cui, Z.; Huang, T.; Li, S. Expression, purification, and characterization of a novel methyl parathion hydrolase. *Protein Expression Purif.* **2004**, *36*, 170−176.
(29) Kolkman, M. A. B.; van der Ploeg, R.; Bertels, M.; van Dijk, M.; van der Laan, J.; van Dijl, J. M.; Ferrari, E. The twin-arginine signal peptide of *Bacillus subtilis* YwbN can direct either Tat- or Sec-dependent secretion of different cargo proteins: Secretion of active subtilisin via the *B. subtilis* Tat pathway. *Appl. Environ. Microbiol.* **2008**, *74*, 7507−7513.
(30) Gerlach, R.; Pop, O.; Muller, J. P. Tat dependent export of *E. coli* phytase AppA by using the PhoD-specific transport system of *Bacillus subtilis*. *J. Basic Microbiol.* **2004**, *44*, 351−359.
(31) Meissner, D.; Vollstedt, A.; van Dijl, J. M.; Freudl, R. Comparative analysis of twin-arginine (Tat)-dependent protein secretion of a heterologous model protein (GFP) in three different Gram-positive bacteria. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 633−642.