BUKTI KOREPONDENSI

DAFTAR ISIAN PENELITIAN

JURNAL ILMIAH

NASIONAL PERINGKAT 1 DAN 2

NO	JENIS ISIAN	ISIAN
1	**Judul Artikel**	Expression of Mycobacterium tuberculosis Protein Tyrosine Phosphatase B in Escherichia coli and Its Recovery from Inclusion Body
2	**Penulis**	**1. Lalu Rudyat Telly Savalas; 2.** Prapti Sedijani; 3. Saprizal Hadisaputra; 4. Jannatin Ardhuha; 5. Chomsa Asih Lestari; 6. Etty Nurul Wahidah
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No	Tanggal	Aktivitas	Keterangan
1	17 Nov 2016	Submission	Via email
2	17 Nov 2016	Submission acknowledgment	Email dari editor
3	27 Nov 2016	Hasil review	Email dari editor dengan attachment
4	16 Maret 2017	Respons terhadap editor	Email ke editor
5	27 Des 2017	Revisi terakhir/copyedit	File via email ke editor

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K1 Submission via email 17 Nov 2016

Lalu Rudyat Telly Savalas <telly@unram.ac.id>

Manuscript submission Lalu Rudyat Telly Savalas UNRAM

1 message

Lalu Rudyat Telly Savalas <telly@unram.ac.id> 17 November 2016 at 06:47
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Thank you and I look forward to hearing from you.

Sincerely yours,

Lalu Rudyat Telly Savalas
corresponding author

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Dr. rer. nat. Lalu Rudyat Telly Savalas,M.Si.
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Faculty of Teacher Training and Education
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K2 Submission acknowledgment 17 Nov 2017

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Biosaintifika Unnes <biosaintifika@gmail.com> 17 November 2016 at 15:07
To: Lalu Rudyat Telly Savalas <telly@unram.ac.id>

terima kasih pak Lalu Rudyat, artikel Bpk ada peluang untuk kami terbitkan, hanya perlu disesuikan dengan kisi kisi jurnal kami, bersama ini kami krm kisi kisi, silahkan disesuikan lalu krm kembali. terima kasih

On Thu, Nov 17, 2016 at 5:47 AM, Lalu Rudyat Telly Savalas <telly@unram.ac.id> wrote:

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> corresponding author
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> Dept of Mathematics and Natural Sciences Education
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Kisi-kisi yang dikirimkan akan kami gunakan sebagai self-assessment untuk menyempurnakan manuskrip yang kami kirim.
Kami upayakan perbaikannya pada akhir pekan ini.
Atas kerja sama yang baik dari rekan-rekan pengelola Biosaintifika kami sampaikan terima kasih.

Hormat kami
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K3 Hasil review 27 Nov 2016

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1 message

Biosaintifika Unnes <biosaintifika@gmail.com> 27 November 2016 at 08:31
To: Lalu Rudyat Telly Savalas <telly@unram.ac.id>

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Cloning and Expression of N-terminally tagged *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase B in *Escherichia* coli

LALU RUDYAT TELLY SAVALAS[1*)], PRAPTI SEDIJANI[2)], SAPRIZAL HADISAPUTRA[1)], JANNATIN 'ARDHUHA[3)], CHOMSA ASIH LESTARI[4)], AND BAIQ REPIKA NURUL FURQON[4)]

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*) telly@unram.ac.id

Abstract

Commented [U1]: Abstrak mhn diperjelas tujuan, metode hasil, simpulan dan manfaat peleitian, lihat kisi kisi

Protein tyrosine phosphatase B of *Mycobacterium tuberculosis*, along with several other proteins secreted by Mtb, plays an important role in the development of latent *Mycobacterium tuberculosis* infection (LBTI). Mtb secrete several virulence proteins to the cytoplasm of infected macrophage cells immediately after the bacteria enter the defense cells in a process known as endocytosis. Mtb reside within an organelle called phagosome. Secretion of the LBTI related proteins prevent the fusion even between bacteria-containing-phagosome and lysosome, the event that further lead to the avoidance of an otherwise harsh environment that normally kill the bacteria. In order to study the biochemistry of PtpB, we have cloned and successfully expressed PtpB of Mtb in *Escherichia coli* BL21(DE3). Overexpression of PtpB provides a way to further investigate the human protein interacting with PtpB, as well as searching for chemical compound that has potential to inhibits this virulence factor of Mtb.

Key words: *Mycobacterium tuberculosis*, latent infection, PtpB, overexpression.

Introduction

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Tuberculosis still poses major a threat in global health. The death toll caused by TB is estimated to be around 1.5 to 2 million casualties annually. The figure is worsened by HIV co-infection as well as by the development of multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains of the bacteria (Palomino & Martin, 2016). In addition to TB cases, attention has recently been given to the latent *Mycobacterium tuberculosis* infection. Increasing diabetic mellitus disease in the developing world also contributes to the elevated risk of active TB (Jeon & Murray, 2008).

Following infection, the bacteria are engulfed in macrophage and killed by a process known as phagocytosis (Wahyuningsih et al, 2016). However, the bacteria have developed survival strategy to avoid macrophage degradation by secreting several modulator proteins, such as PtpA, PtpB, PknG, PknF, etc. (Sundaramurthy & Pieters, 2007; Ahmad, 2011). One notable protein secreted by Mtb is Tyrosine phosphatase B (PtpB). It has been described that Mtb bacteria defect in PtpB are not capable of developing latent Mtb infection in Guinea pig 10 weeks post inoculation (Chauhan et al., 2013). This report underlines the important role of PtpB in the development of LBTI. Nevertheless, the detail mechanism by which PtpB interfere with macrophage degradation or which human protein interacts with PtpB is not clear. Apart from this, PtpB has been a target for drug development. By inhibiting PtpB, it is expected that macrophage maintains its ability to degrade Mtb. Hence, it is necessary to study the biochemistry of PtpB, i.e. the pathway that it interferes and possible way to inhibit the activity of PtpB.

As a prerequisite to investigate the biochemistry of PtpB, heterologous expression of PtpB is an important task to provide PtpB in sufficient amount and purity. In this study, we have cloned Mtb PtpB gene and successfully overexpressed PtpB under T7 promotor and IPTG induction in *Escherichia coli* BL21 (DE3) cells. Further investigation regarding the possibility to inhibit PtpB is yet to be tested.

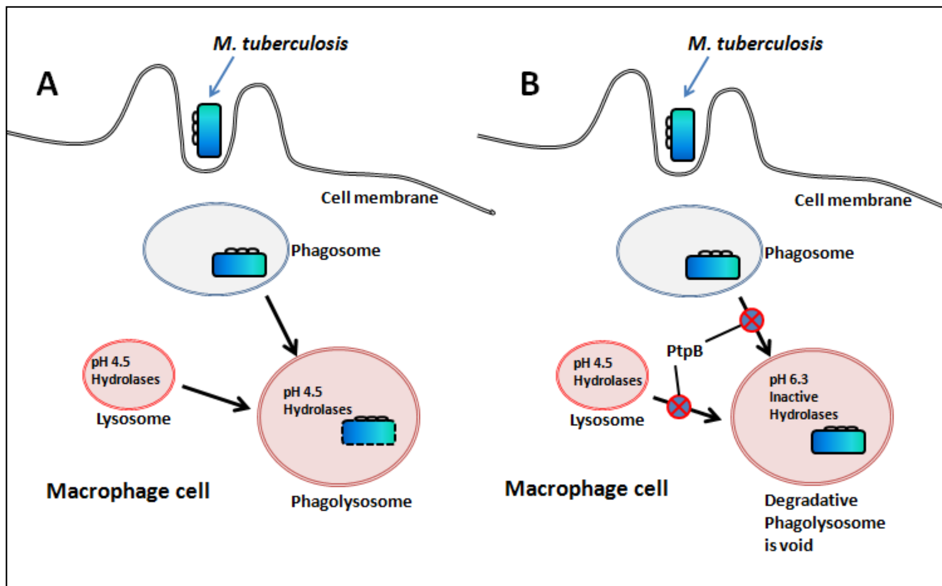

Figure 1. Development of latent *M. tuberculosis* infection. In normal pathway, upon infection, Mtb is engulfed in phagosome which later fuse with lysosome to form phagolysosome. Phagolysosome gets acidified and recruits hydrolytic enzymes from lysosome. Hydrolytics enzymes degrade Mtb (A). In latent infection, Mtb secretes several virulence factors. One such virulence factor is PtpB that prevent the fusion event between phagosome and lysosome (B).

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Pendahuluan tdk perlu gambar, lebih baik ditulis dlm kalimat mengalir diserai refernsi

Methods

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PtpB gene was amplified from pGS-21-PtpB template by using forward primer 5'-CATGGTACCGCTGTCCGTGAACTGCC-3' and reverse primer 5'-CAGCTCGAGTCCGAGCAGCACCCCG-3' which contain *Kpn*I and *Xho*I restriction site in the forward and reverse primer, respectively. The amplified fragment and pET-30a vector were digested with *Kpn*I and *Xho*I prior to ligation. Cloning strategy is depicted in Figure 1.

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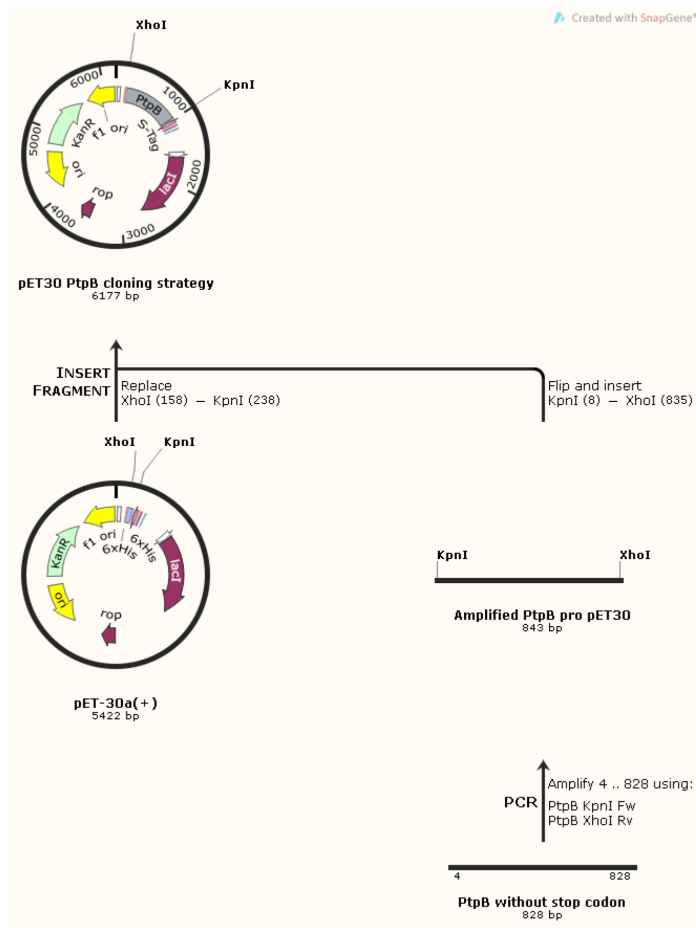

Figure 2. Cloning strategy of PtpB gene by using pET-30a as vector.

The resulted recombinant plasmid was sequenced to ensure the absent of mutation. The resulted recombinant plasmid was further used to transform competent *E. coli* BL21(DE) by using electroporator (Gene Pulser, Bio-Rad). Production of PtpB was undertaken within LB medium containing 25 ug/mL kanamycin. To induce the expression of PtpB, transformed *E. coli* BL21

(DE3) was cultured in LB medium until OD_{600} of 0.3 is reached. At this point, Isopropyl 1-Thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.5 mM and the culture was maintained at 37°C with rigorous shaking (250 rpm). Culture was harvested 4 hours after induction and cells were sedimented by centrifugation at low speed. Cells were further resuspended in lysis buffer containing protease inhibitor PMSF and the cells were disrupted by sonication. Lysate was separated from debris by high speed centrifugation. Both soluble lysate and insoluble fraction were subjected for SDS-PAGE analysis. All chemicals used in this study were of molecular biology grade and supplied by major provider. Design of cloning strategy and DNA sequence analysis were performed by SnapGene version 2.5 (GSL Biotech LLC, USA).

Results and Discussion

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Cloning of PtpB gene.

PtpB gene has successfully inserted within pET30a vector via *Kpn*I and *Xho*I restriction site. This vector allows overexpression of inserted gene since it has T7 promoter. In *E. coli* BL21 (DE3), production of RNA polymerase of phage DE3 is induced by addition of lactose analog Isopropyl 1-Thio-β-D-galactopyranoside (IPTG). RNA polymerase of phage T7 recognizes T7 promoter at the upstream position of PtpB gene. The expression system has long time been established after initiated by Studier and Moffat (Studier & Moffat, 1986). An hexa-histitine sequence is located at the up-stream on PtpB gene, a design that expected to enrich the purification of PtpB, since we previously experienced difficulty to purify related protein tagged with hexa-Histidine sequence at its C-terminus or (unpublished work).

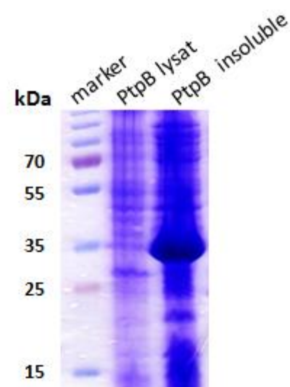

Figure 3. Expression of PtpB in *E. coli* BL21 (DE3) under T7 promoter and induced by 0.5 mM IPTG.

Expression of PtpB in E. coli

By using pET30a expression vector, we were able to overexpress Mtb PtpB as shown in Figure 3. As expected, PtpB appears at molecular weight of aroung 31 kD (Grundner, Ng, & Alber, 2005). However, PtpB is found in insoluble fraction. This indicates that PtpB is synthesized at both high rate and quantity that lead to inclusion body formation. Formation of inclusion body is not a rare even, especially when the synthesis of foreign protein takes place at a very high rate. Fortunately, strategies to recover proteins from inclusion bodies are now established (Singh et al., 2015) and this task will comprise our immediately future work.

Perspective

The importance of the study of virulence factors or Mtb has been widely recognized in order to combat this life-threatening disease. Since PtpB increasingly becomes an interesting target to prevent LBTI (Zhou et al., 2010), we propose to test the potencies of several chemical agents resulted from *in silico* study (Dhanjal et al., 2014) to inhibit PtpB both *in vitro* and *in vivo.*

Conclusion

We have successfully cloned *M. tuberculosis* PtpB gene within pET-30a as an N-terminally hexa-His tagged protein. Expression of PtpB has been performed in *E. coli* and it was shown that PtpB is overexpressed as inclusion body. For further biochemistry study of PtpB, the protein needs to be recovered. The overall approach chosen in this study provides a way to study the biochemistry property of PtpB.

Acknowledgement

This research is funded by the Ministry of Research, Technology and Higher Education through INSInas research grant awarded to LRTS.

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Wahyuningsih, S. P. A., Pramudya, M., & Sugiharto, S. (2016). Influence of Polysaccharide Krestin from Coriolus versicolor Extract on Nitrite and Malondialdehyde Concencentrations of Mus musculus Serum Exposed by Mycobacterium tuberculosis. *Biosaintifika: Journal of Biology & Biology Education*, *8*(1), 12. https://doi.org/10.15294/biosaintifika.v8i1.4969

Zhou, B., He, Y., Zhang, X., Xu, J., Luo, Y., Wang, Y., … Zhang, Z.-Y. (2010). Targeting mycobacterium protein tyrosine phosphatase B for antituberculosis agents. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(10), 4573–8. https://doi.org/10.1073/pnas.0909133107

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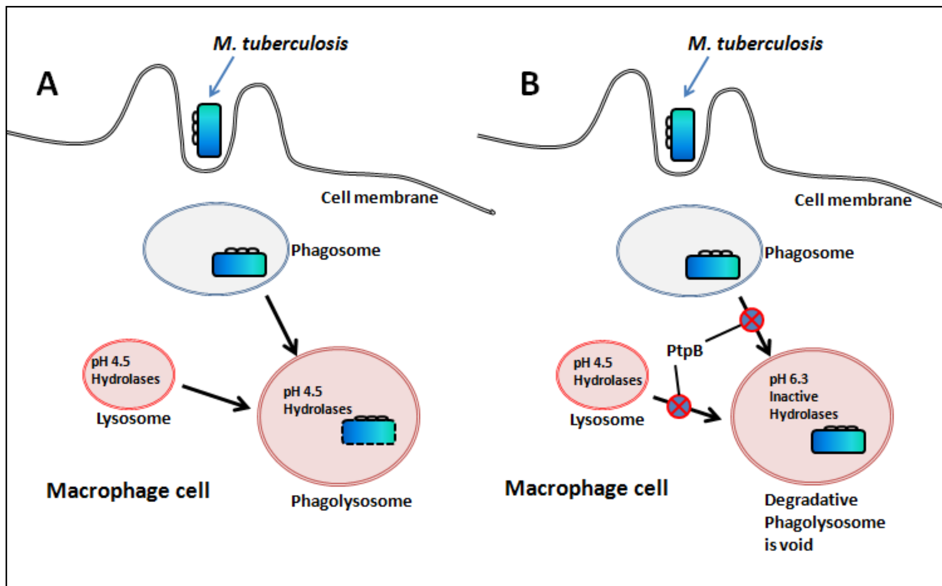

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Commented [MA7]: Jika gambar ini merupakan bagian dari metode (teknik yang diusulkan) mohon tidak diletakkan di latar belakang.

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Pendahuluan tdk perlu gambar, lebih baik ditulis dlm kalimat mengalir diserai refernsi

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Commented [MA11]: Jika gambar ini merupakan bagian dari metode (teknik yang diusulkan) mohon tidak diletakkan di latar belakang.

Commented [MA12]: Referensi?

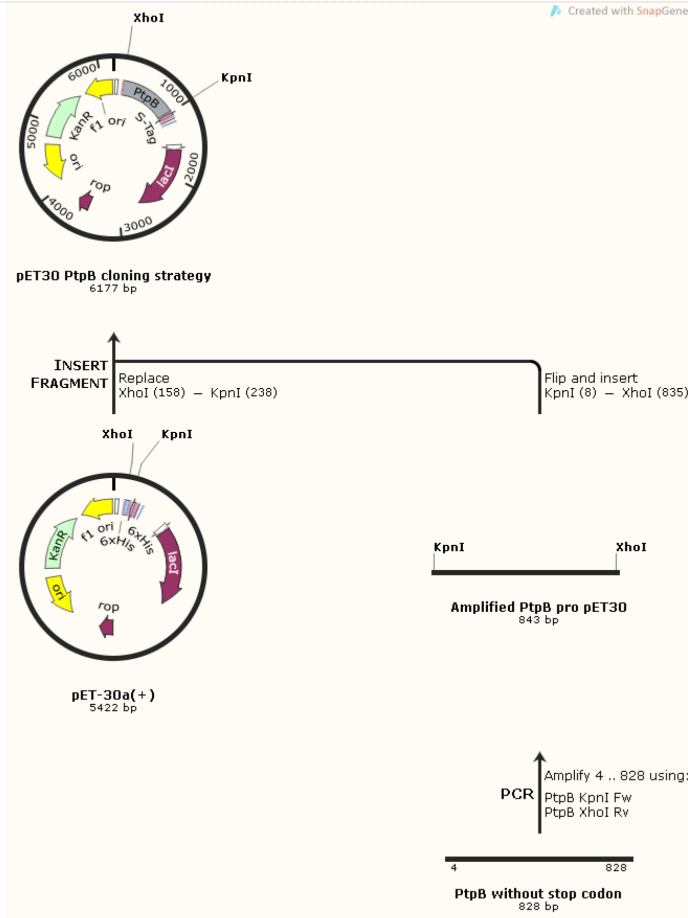

Figure 2. Cloning strategy of PtpB gene by using pET-30a as vector.

The resulted recombinant plasmid was sequenced to ensure the absent of mutation. The resulted recombinant plasmid was further used to transform competent *E. coli* BL21(DE) by using electroporator (Gene Pulser, Bio-Rad).Production of PtpB was undertaken within LB medium containing 25 ug/mL kanamycin. To induce the expression of PtpB, transformed *E. coli* BL21

(DE3) was cultured in LB medium until OD_{600} of 0.3 is reached. At this point, Isopropyl 1-Thio-β-D-galactopyranoside (IPTG) was added to a final concentration of 0.5 mM and the culture was maintained at 37°C with rigorous shaking (250 rpm). Culture was harvested 4 hours after induction and cells were sedimented by centrifugation at low speed. Cells were further resuspended in lysis buffer containing protease inhibitor PMSF and the cells were disrupted by sonication. Lysate was separated from debris by high speed centrifugation. Both soluble lysate and insoluble fraction were subjected for SDS-PAGE analysis. All chemicals used in this study were of molecular biology grade and supplied by major provider. Design of cloning strategy and DNA sequence analysis were performed by SnapGene version 2.5 (GSL Biotech LLC, USA).

Results and Discussion

Commented [U13]: Hasil terlalu sedikit, mhn ditambah data,

Commented [MA14]: Pembahasan masih terlalu sedikit. Terkesan hanya mendeskripsikan hasil. Mohon ditambah data dan informasi hasil dari penelitian lain yang mendukung.

Cloning of PtpB gene.

PtpB gene has successfully inserted within pET30a vector via *Kpn*I and *Xho*I restriction site. This vector allows overexpression of inserted gene since it has T7 promoter. In *E. coli* BL21 (DE3), production of RNA polymerase of phage DE3 is induced by addition of lactose analog Isopropyl 1-Thio-β-D-galactopyranoside(IPTG). RNA polymerase of phage T7 recognizes T7 promoter at the upstream position of PtpB gene. The expression system has long time been established after initiated by Studier and Moffat (Studier & Moffat, 1986). An hexa-histitine sequence is located at the up-stream on PtpB gene, a design that expected to enrich the purification of PtpB, since we previously experienced difficulty to purify related protein tagged with hexa-Histidine sequence at its C-terminus or (unpublished work).

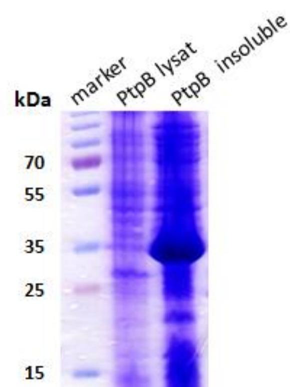

Figure 3. Expression of PtpB in *E. coli* BL21 (DE3) under T7 promoter and induced by 0.5 mM IPTG.

Expression of PtpB in E. coli

By using pET30a expression vector, we were able to overexpress Mtb PtpB as shown in Figure 3. As expected, PtpB appears at molecular weight of aroung 31kD(Grundner, Ng, & Alber, 2005).However, PtpB is found in insoluble fraction. This indicates that PtpB is synthesized at both high rate and quantity that lead to inclusion body formation.Formation of inclusion body is not a rare even, especially when the synthesis of foreign protein takes place at a very high rate. Fortunately, strategies to recover proteins from inclusion bodies are now established (Singh et al., 2015) and this task will comprise our immediately future work.

Perspective

The importance of the study of virulence factors or Mtb has been widely recognized in order to combat this life-threatening disease. Since PtpB increasingly becomes an interesting target to prevent LBTI (Zhou et al., 2010), we propose to test the potencies of several chemical agents resulted from *in silico* study(Dhanjal et al., 2014) to inhibit PtpB both *in vitro* and *in vivo.*

Conclusion

We have successfully cloned *M. tuberculosis* PtpB gene within pET-30a as an N-terminally hexa-His tagged protein. Expression of PtpB has been performed in *E. coli* and it was shown that PtpB is overexpressed as inclusion body. For further biochemistry study of PtpB, the protein needs to be recovered. The overall approach chosen in this study provides a way to study the biochemistry property of PtpB.

Acknowledgement

This research is funded by the Ministry of Research, Technology and Higher Education through INSInas research grant awarded to LRTS.

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Commented [MA15]: Mohon sitasi artikel dari Biosaintifika juga.

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K4 Respons terhadap editor 16 Maret 2017

Lalu Rudyat Telly Savalas <telly@unram.ac.id>

Re: pemberitahuan ke 2

1 message

Lalu Rudyat Telly Savalas <telly@unram.ac.id> 16 March 2017 at 21:38
To: Biosaintifika Unnes <biosaintifika@gmail.com>

Ysh Dewan Redaksi Biosaintifika,
terima kasih atas email dari Bpk/Ibu dan mohon maaf belum memberi respons. Saat ini kami baru mendapat beberapa tambahan data yang signifikan untuk memenuhi tanggapan dari mitra bestari, sehingga makalah kami semakin baik. Insyaallah dalam sepekan hasil revisi akan kami kirimkan kembali.
Atas kerja sama yang baik kami sampaikan terima kasih..

Hormat saya,

Lalu Rudyat Telly Savalas
corresponding author

On 16 March 2017 at 05:58, Biosaintifika Unnes <biosaintifika@gmail.com> wrote:

> yth bapak Lalu, bersama ini kami krm hasil review silahkan disesuaikan dengan saran dan kisi kisi, lalu krm kembali terima kasih.
>
> --
>
> **Nationally Accredited by the Directorate of Higher Education, Indonesia based on the Decree of the Minister of Research, Technology and Higher Education, Number 2/E/KPT/2015**
>
> **Biosaintifika: Journal of Biology & Biology Education [Nationally Accredited]** published scientific papers on the results of biological research and education covering the fields of biology including botany, zoology, environmental, and biotechnology. Editor accepts the article has not been published in other media with the writing format as listed on page manuscript writing guidelines. Manuscripts will be reviewed by an expert editor and managing editor. The journal is published twice a year, every April, August & December.The journal has been indexed in **Google Scholar**, **DOAJ**, **DOI Crossref**, **EBSCO**, **CABI more**

Lalu Rudyat Telly Savalas <telly@unram.ac.id>

K5 Itemize

Re: pemberitahuan ke 2

1 message

Biosaintifika Unnes <biosaintifika@gmail.com> 17 March 2017 at 12:21
To: Lalu Rudyat Telly Savalas <telly@unram.ac.id>

kami tunggu pak Lalu, agar dpt dipublikasi edisi Agustus. trims

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Nationally Accredited by the Directorate of Higher Education, Indonesia based on the Decree of the Minister of Research, Technology and Higher Education, Number 2/E/KPT/2015

Biosaintifika: Journal of Biology & Biology Education [Nationally Accredited] published scientific papers on the results of biological research and education covering the fields of biology including botany, zoology, environmental, and biotechnology. Editor accepts the article has not been published in other media with the writing format as listed on page manuscript writing guidelines. Manuscripts will be reviewed by an expert editor and managing editor. The journal is published twice a year, every April, August & December.The journal has been indexed in **Google Scholar**, **DOAJ**, **DOI Crossref**, **EBSCO**, **CABI more**

On Thu, Mar 16, 2017 at 8:38 PM, Lalu Rudyat Telly Savalas <telly@unram.ac.id> wrote:

> Ysh Dewan Redaksi Biosaintifika,
> terima kasih atas email dari Bpk/Ibu dan mohon maaf belum memberi respons. Saat ini kami baru mendapat beberapa tambahan data yang signifikan untuk memenuhi tanggapan dari mitra bestari, sehingga makalah kami semakin baik. Insyaallah dalam sepekan hasil revisi akan kami kirimkan kembali.
> Atas kerja sama yang baik kami sampaikan terima kasih..
>
> Hormat saya,
>
> Lalu Rudyat Telly Savalas
> corresponding author
>
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>
>> yth bapak Lalu, bersama ini kami krm hasil review silahkan disesuaikan dengan saran dan kisi kisi, lalu krm kembali terima kasih.
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>> **Nationally Accredited by the Directorate of Higher Education, Indonesia based on the Decree of the Minister of Research, Technology and Higher Education, Number 2/E/KPT/2015**
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>> **Biosaintifika: Journal of Biology & Biology Education [Nationally Accredited]** published scientific papers on the results of biological research and education covering the fields of biology including botany, zoology, environmental, and biotechnology. Editor accepts the article has not been published in other media with the writing format as listed on page manuscript writing guidelines. Manuscripts will be reviewed by an expert editor and managing editor. The journal is published twice a year, every April, August & December.The journal has been indexed in **Google Scholar**, **DOAJ**, **DOI Crossref**, **EBSCO**, **CABI more**

K5 Revisi naskah sesuai permintaan reviewer Des 2017

DOI: 10.15294/biosaintifika.v9i3.12384

Ysh Tim Editor Biosaintifika,

Terima kasih atas kerja sama yang baik sehingga manuskrip kami dengan nomor DOI di atas telah diterbitkan.

Selanjutnya kami mohon maaf atas keterlambatan memberikan tanggapan, karena saya berpikir besok (Rabu 27 Desember adalah kesempatan terakhir kami mengirimkan email tanggapan, sementara pdf final manuskrip kami sudah muncul pada laman jurnal. Beberapa hal yang perlu diperbaiki diikhtisarkan pada tabel di bawah ini:

No	Hal (lama)	Hal (baru)	Tertulis	Perbaikan dan Komentar
1	345	530	Abstract	Abstrak ini telah diringkas dan saya setuju.
2	346	531	lity in dormant phase in a different environment (Boshoff & Barry, 2005)whereas others restrict bacterial growth without necessarily sterilizing the infecting microorganisms. The physical and biochemical milieu in these lesions is poorly de-fined. None of the existing animal models for tu-berculosis (except perhaps non-human primates. Reactivation of the so-called LTBI (latent tuber-	Mohon ditambahkan koma dan spasi setelah (Boschoff & Barry, 2005) dan tutup kurung setelah ….non-human primates.
3	346	531	Biosaintifika 9 (3) (2017) XXX-XXX ven to the latent *Mycobacterium tuberculosis* infec-tion and it belongs to an essential part of WHO	Sebaiknya disingkat *M. tuberculosis* agar lebih konsisten
4	346	531	The present study focuses on the Tyrosi-ne phosphatase B (PtpB). It has been described that Mtb bacteria defect in PtpB are not capable of developing latent Mtb infection in Guinea pig ten weeks post inoculation (Chauhan *et al.*, 2013) we have generated a mutant of M.tuberculosis (Mtb\u2206mms. This report underlines the im-portant role of PtpB in the development of LBTI. Nevertheless, the detailed mechanism by which	Mohon yang di-*highlight* dihilangkan. Selain karena kemunculannya tiba-tiba (tidak ada dalam manuskrip kami), sisipan ini juga mengganggu. Jadi, setelah sitasi *(Chauhan *et al.*, 2013) langsung diikuti titik.
5	346 ph. 5	531	vector. We have also successfully overexpressed PtpB under T7 promotor and IPTG induction in *Escherichia coli*BL21(DE3) cells first in the form of inclusion body and further re-solubilize it into its	Promoter diganti promoter. Mohon ditambahkan spasi antara ….*coli* dan BL21.
	346 ph. 5	531	inclusion body and further re-solubilize it into its active form. This result thus provides materials in to investigate the biochemical property of PtpB, as well as assaying inhibitory potential of several PtpB inhibitor candidates resulted from compu-tational analysis recently reported (Dhanjal *et al.*, 2014) protein tyrosine phosphatase B (mPTPB.	…materials in to investigate… menjadi ….materials in order to investigate… Setelah sitasi (Dhanjal et al., 2014) diikuti titik. Tambahan protein tyrosine phosphtase B (mPTPB) mohon dihilangkan karena tidak ada dlm manuskrip awal dan tidak ada konteksnya.

6	346	531	re- growth media was standard LB which consists of yeast extract (BD), back to tryptone (Bio- gi- Basic), NaCl (Merck) and bacto agar (Difco). 346	…bacto tryptone
7	347	532	the so-called washed inclusion body (washed IB) was prepared by resuspending insoluble fraction in refolding buffer (50 mM Tris pH 8; 0,1 mM NaCl; 0,1 mM EDTA, 5% glycerol; 0,1 mM DTT). Following sonication, Triton X-100 was added to the lysate to a final concentration of 5% and let	Mohon tanda koma diganti titik untuk konsentrasi larutan. Setelah DTT tidak ada spasi, langsung tutup kurung.
8	347	532	**Determination of optimum PtpBactivity assay** The activity of PtpB recovered from inclusion body was assayed on a 96-well plate by measuring its ability to hydrolyze *para*-Nitrophenyl	Mohon ditambah spasi di antara PtpB dan activity
9	348	533	Figure 2	Penting: Mohon gambar elektroforegram dirotasi 90° searah jarum jam
10	349	533	that the recovered protein presents in a sufficient purity (Li et al., 2015)Tuna AI (PTHIKWGD on SDS-PAGE electroforegram in Figure 3 (right).	Mohon yang di-*highlight* dihilangkan. Selain bukan berasal dari manuskrip kami, sisipan ini tidak jelas konteksnya dan mengganggu
11	349	534	phosphate, pNPP, a colorless substance in its solution form which is commonly used to measure phosphatase activity (Lorenz, 2011)from early development to fully differentiated effector function. Since the opposing actions of protein tyrosine kinases (PTKs. Upon hydrolysis by PtpB, the resulted *para*-Nitrophenol (Figure 4) ap-	Mohon yang di-*highlight* dihilangkan. Selain bukan berasal dari manuskrip kami, sisipan ini tidak jelas konteksnya dan mengganggu
12	350	535	potential TB development. Since PtpB increasingly becomes an interesting target to prevent LBTI (Zhou et al., 2010)where it mediates mycobacterial survival in the host. Consequently, there is considerable interest in understanding the mechanism by which mPTPB evades the host immune responses, and in developing potent and selective mPTPB inhibitors as unique antituberculosis (antiTB, by using this result to produce functional PtpB, it is tempting in the future to test the potencies of several chemical agents resulted from *in silico* study (Dhanjal et al., 2014)protein tyrosine phosphatase B (mPTPB to inhibit PtpB both *in vitro* and *in vivo*. Of equally important, screening of inhibitory effect of new compounds	…(Zhoue et al., 2010), where… (koma dan spasi). … in developing potent and selective mPtpB inhibitors a unique antituberculosis (anti-TB). By using this result…. (mPtpB agar lebih konsisten). 'anti-TB' di dalam dua kurung dan diikuti titik kemudian dianjutkan kalimat baru "By using …. Setelah kutipan (Dhanjal et al., 2014) diikuti titik. Kalimat protein tyrosine phosphatase B (mPTPB dihilangkan

Response to reviewer notes:

U1: Abstrak mhn diperjelas tujuan, metode hasil, simpulan dan manfaat peleitian, lihat kisi kisi:

Beberapa hal yang ditanyakan reviewer telah dicantumkan secara implisit di dalam abstrak. Dalam revisi ini, abstrak telah diperbaiki untuk memperjelas bagian-bagian yang ditanyakan sbb:

Tujuan penelitian: ….., expression of active PtpB in a simple expressing system…..

Metode: …… Mtb PtpB gene has been cloned in pET30a vector and overexpressed in E. coli BL 21(DE3) under IPTG induction as inclusion body. Following resolubilization by urea ……

Hasil: ……the resulted PtpB has also been been shown to be active against para-Nitrophenyl phosphate…….

Simpulan dan manfaat: It is concluded that the resulted PtpB has had been recovered from inclusion body to give the active form of the enzyme, and thus the success in overexpressing PtpB provides the required material to further investigate the biochemical properties of the pathogen virulence factor

U2: Tiap paragraph baru menjork ke dalam, lihat cth artikel yg sdh terbit

Tiap paragraph telah diperbaiki cara penulisannya sebagaimana disarankan reviewer

U3: Gambar ini dari hasil penelitian penulisa taua refernsi?? Pendahuluan tdk perlu gambar, lebih baik ditulis dlm kalimat mengalir diserai refernsi

Gambar ini adalah hasil reka cipta dari pengaran berdasarkan beberapa referensi. Sesuai saran reviewer, gambar 1 telah dihilangkan dan diganti dengan penjelasan di dalam pendahuluan.

U4: Langkah langkah perlu dijelaskan lebih detail

Langkah-langkah penelitian telah dijelaskan lebih detail.

U5: Gambar ini dari referensi atau menggambar sendiri ? jika dari refernsi mhn diberi refernsi nya

Gambar ini adalah disain sendiri menggunakan perangkat lunak analisis DNA SnapGene dengan lisensi atas nama pengarang LRTS. Nomor gambar telah diubah menjadi Gambar 1, karena gambar sebelumnya pada pendahuluan telah dihilangkan.

U6: Hasil terlalu sedikit, mhn ditambah data,

Sesuai dengan tambahan pada bagian metode, data telah ditambah dengan gambar elektroforegram SDS-PAGE setelah PtpB didenaturasi dengan urea dan di-recover dengan cara dialysis. Aktivitas PtpB ditunjukkan pada Gambar 3 yang merupakan hasil hidrolisis para Nitrophenyl phosphate.