Research Article

Journal of Molecular Microbiology and Biotechnology

J Mol Microbiol Biotechnol 2010;19:169–179
DOI: 10.1159/000322157

Published online: October 29, 2010

Cloning and Targeted Disruption of Two Lipopolysaccharide Biosynthesis Genes, *kdsA* and *waaG*, of *Pseudomonas aeruginosa* PAO1 by Site-Directed Mutagenesis

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Key Words
Essential genes · Plasmid insertion mutagenesis · Lipopolysaccharide biosynthesis · Antibacterial drugs

Abstract
The emergence of antibiotic resistance in bacterial pathogens poses a great challenge to public health and emphasizes the need for new antimicrobial targets. The recent development of microbial genomics and the availability of genome sequences allows for the identification of essential genes which could be novel and potential targets for antibacterial drugs. However, these predicted targets need experimental validation to confirm essentiality. Here, we report on experimental validation of a two potential targets in the lipopolysaccharide (LPS) biosynthesis pathway of the pathogen *Pseudomonas aeruginosa* PAO1 using insertion duplication. Two genes, *kdsA* and *waaG*, from LPS encoding proteins 2-dehydro-3-deoxyphosphooctonate aldolase and UDP-glucose (heptosyl) LPS α-1,3-glucosyltransferase were selected as putative target candidates for the gene disruption experiments using plasmid insertion mutagenesis to determine essentiality. The introduction of a selectable ampicillin and kanamycin resistance marker into the chromosome resulted in lack of recovery of antibiotic-resistant colonies suggesting the essentiality of these genes for the survival of *P. aeruginosa*. Several molecular analyses were carried out in order to confirm the essentiality of these genes. We propose that the above two validated drug targets are essential and can be screened for functional inhibitors for the discovery of novel therapeutic compounds against antibiotic-resistant opportunistic pathogen *P. aeruginosa*.

Introduction

Antibiotics target genes that are required for bacterial growth and survival. Hence, knowledge on essential genes is of importance both for understanding of the

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minimal requirements for cellular life and also for drug discovery [Chalker and Lunsford, 2002; Zhang and Lin, 2009]. Identifying genes that encode functions for the bacterial growth provides possible antibiotic targets and can help facilitate drug discovery efforts [Jordan et al., 2002]. The availability of complete genome sequences and the use of comparative genomics have enabled access to a large number of candidate (putative) drug targets [Barker, 2006; Muzzi et al., 2007; Overbeek et al., 2003]. However, in order to demonstrate that the gene is essential for growth, molecular validation of the selected targets needs to be performed.

The prediction and discovery of essential genes based on genome-driven, target-based approaches has provided a repertoire of essential genes that can be used to select and validate antimicrobial targets by random transposon mutagenesis, RNA interference and systematic gene inactivation [Akerley et al., 2002; Baba et al., 2006; Chalker et al., 2001; Fire et al., 1998; Forsyth et al., 2002; Giaever et al., 2002; Glass et al., 2006; Jacobs et al., 2003; Joyce et al., 2006; Kobayashi et al., 2003; Sassetti et al., 2001; Song et al., 2005]. To experimentally validate the essentiality of the prioritized bacterial genes, site-directed gene disruption studies are performed. Disruption of single gene helps us in understanding its role in the bacterial genome and analyzing the corresponding biochemical pathways and functional activities [Falconer and Brown, 2009]. In this direction, suicide vectors carrying the internal portion of a gene are generally used in a wide range of bacteria to obtain mutants by gene disruption. Such vectors lead to inactivation of the target gene in a single crossover recombination event. Hence, for non-essential genes, recombinants can be obtained, whereas for essential genes, no recombinants can be obtained, thus determining the essentiality of genes in a targeted manner [Thanassi et al., 2002].

Most bacterial pathogens develop antibiotic resistance either by mutation in chromosomally encoded genes or by horizontal gene transfer [Mazel and Davies, 1999]. *Pseudomonas aeruginosa* is one of the best examples of the global emergence of resistance in a pathogen. The opportunistic pathogen *P. aeruginosa* causes persistent infections in the lungs of cystic fibrosis patients that are frequently associated with the emergence of antibiotic-resistant subpopulations of bacteria. It also causes urinary tract, respiratory tract and skin infections [Stover et al., 2000]. Although this bacterium is well studied, roughly one-fourth of its open reading frames are uncharacterized [Lewenza et al., 2005].

The use of broad-spectrum antibiotics to treat chronic infection may promote resistance in other pathogenic bacteria, thereby compromising their utility in the treatment of more serious infections [Freiberg and Brotz-Oesterhelt, 2005]. Hence, there is considerable interest in developing novel therapies which are both more effective and specific for *P. aeruginosa*. The search for new ways to combat *P. aeruginosa* infections has been greatly facilitated by availability of the complete genome sequence of the pathogen [Stover et al., 2000]. Previously, we carried out a differential genome analysis to identify essential genes that could be potential targets in *P. aeruginosa* [Perumal et al., 2007]. It was reported that genes involved in the lipopolysaccharide (LPS) biosynthesis are prioritized potential drug targets. LPS is the major surface component of Gram-negative bacteria, and a component of LPS, lipid A, is recognized by the innate immune system. *P. aeruginosa* can synthesize various structures of lipid A, a pathogenic factor that consists of three distinct regions: O antigen, core, and lipid A [Ernst et al., 2003]. LPS has a critical function in Gram-negative bacterial membrane integrity and resistance to host defenses, and therefore, the conserved LPS enzymes are attractive targets for novel therapeutic compounds [Goldberg and Pier, 1996].

Two genes, *kdsA* and *waaG*, encoding 2-dehydro-3-deoxyphosphooctonate aldolase and UDP-glucose (heptosyl) LPS α-1,3-glucosyltransferase determined by differential genome analyses are responsible for lipid A biosynthesis and hence have been assessed as essential genes responsible for the growth and survival of the pathogen *P. aeruginosa*. In this investigation, we confirm the essentiality of *kdsA* and *waaG* genes by targeted gene disruption experiments. We have used an insertion duplication approach to demonstrate that both *kdsA* and *waaG* genes required for peptidoglycan synthesis are essential for growth and survival in *P. aeruginosa*. To construct the mutant, the essential genes were cloned into a suicide vector and the recombinant plasmid was integrated to recombine with the wild-type *P. aeruginosa* by site-directed mutagenesis. Selectable antibiotic resistance markers introduced through suicide vector confirmed whether the genes have survived the disruption suggesting non-essentiality with colony formation in the selective media or disruption resulting in lack of recovery of antibiotic-resistant colonies, thereby suggesting the essentiality of these genes for survival of *P. aeruginosa*. We report both the *kdsA* and *waaG* genes form no recombinants, thus confirming their essentiality for growth and survival of *P. aeruginosa*.

Experimental Procedures

The experimental procedures are presented in detail in online supplementary file 2 (for all online suppl. material, see www.karger.com/doi/10.1159/000322157) under the following subheadings: Bacterial strains, Plasmids and media, Chemicals and reagents, Isolation of plasmid and genomic DNA, Polymerase chain reaction (PCR), Cloning of PCR product, Restriction endonuclease digestion of DNA, Dephosphorylation of vector DNA, Ligation of restriction endonuclease digested DNA to vector, Preparation of competent cells and chemical transformation, Bacterial conjugation, Preparation of DNA probes, Dot-blot hybridization, Southern blot hybridization, and DNA sequencing analysis.

Results

The results of analyses are presented in online supplementary file 3 under the following subheadings: Construction of mutants using pJET1.2/blunt cloning vector, Subcloning of $kdsA_{306}$ and $waaG_{507}$ gene fragments into suicide vector pFS100, Generation of the mutants by insertion duplication mutagenesis in the chromosome of *P. aeruginosa* PAO1, PCR amplification of cell lysate to check for the loss of *kdsA* and *waaG* genes in cetrimide media.

Discussion

LPS of Gram-negative bacteria represents a large class of exopolysaccharides that vary considerably among different species. However, they all share common features, such as the lipid A core, embedding the polymer into the outer membrane of the microbial genomes. Lipid A core is considered an essential component of the LPS molecule by providing structure to the outer membrane [Kipnis et al., 2006; Pier, 2007].

P. aeruginosa is a Gram-negative bacterium producing LPS and has the ability to express a variety of virulence determinants and as a result causes infections in immunocompromised patients [Mesaros et al., 2007; Pier et al., 1996]. Moreover, the ability to cause a wide range of chronic human infections suggests an evolutionary mechanism existing in this bacterium which facilitates its survival in any particular environment. It is also likely that the relatively large genome of *P. aeruginosa* strains (6–7 Mb) could include a substantial set of conserved genes, coding for functions necessary for survival in most environments.

LPS has an important role in the structural integrity of this bacterium and its defense against the host, and hence the pathways of these enzymes are attractive drug targets. Interestingly, viable deletion mutants, which fail to generate lipid A, have been created in *Neisseria meningitidis* [Steeghs et al., 1998]. The enzymes of the LPS pathway have no human homologues and hence they serve as potential targets. A total of 13 enzymes formed this pathway of which 7 matched with the list of candidate essential genes obtained by transposon mutagenesis [Jacobs et al., 2003]. A differential genome analysis was carried out in *P. aeruginosa* and four novel drug targets *(lpxC, kdsA, kdsB* and *waaG)* were predicted in the LPS biosynthesis [Perumal et al., 2007]. The position and role of the KdsA and WaaG proteins in the LPS pathway are shown in online supplementary S1 – figure 8. Molecular validation of lpxC as a potential drug target has been reported in *P. aeruginosa* [Mdluli et al., 2006].

Here, we performed targeted gene disruption for two genes, *kdsA* and *waaG*, by site-directed mutagenesis. We confirm that both genes are essential for growth and survival of *P. aeruginosa*.

Generating knockout mutants is usually the first step in determining the function of a gene. Targeted gene disruption can be performed using plasmids that are non-replicative. Additionally, the simple method of homologous recombination can be used to determine gene essentiality. In plasmid insertion mutagenesis, homologous recombination is used to insert a suicide vector sequence into the appropriate chromosomal location by a single cross-over event. Our rationale was to use an insertion duplication method which would allow us to determine in a single step whether a gene was essential or not, based on the number of transformants (recombinants) obtained after bacterial conjugation. The method followed with homologous recombination between an introduced plasmid carrying only the internal fragment of the gene and the chromosomal copy of *P. aeruginosa*.

A single recombination event will lead to a single cross-over strain in which the entire plasmid with selectable marker is integrated and flanked by two partial copies of the gene (online suppl. S1 – fig. 4). Neither of these copies represents a functional gene and so the strain generated will be a mutant. If the gene is found to be essential, no transformants will be obtained. The target gene will be disrupted in the chromosome using the selectable marker such as antibiotic resistance genes. If the recombination event can recover the antibiotic resistance in a disrupted gene, then it implies the non-essentiality for that target. In order to check that the loss of the genes

Table 1. Bacterial strains used in this study

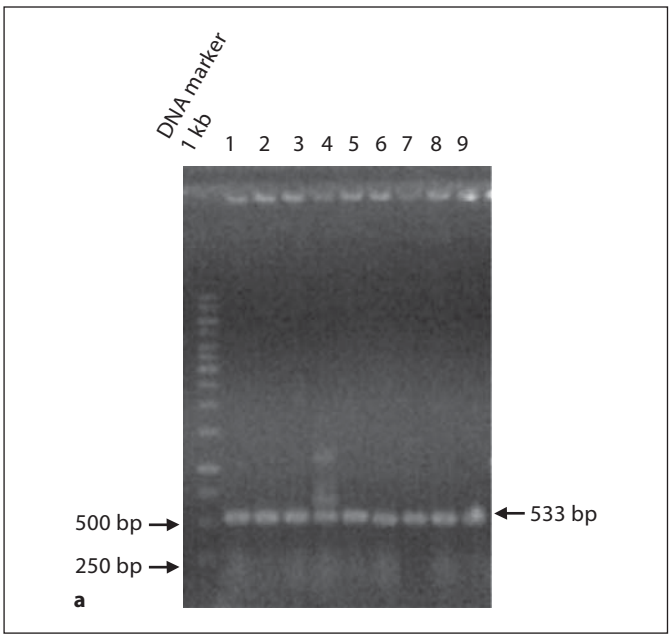
Strain	Description	References
P. aeruginosa PAO1	Wild-type, ATCC 15692	Cox, 1982
E. coli MC1061(λ *pir*)	*(λ pir), thi thr-1 leu6 proA2 his-4 argE2 lacY1 galK2 ara14 xyl5 supE44* λ *pir*	Rubires et al., 1997
E. coli SM10 λ *pir*	λ *pir (thi thr leu tonA lacY supE recA::RP4-2-Tc::Mu Km)* used for transformation of recombinant plasmid	Donnenberg and Kaper, 1991

Table 2. Plasmids used in this study

Vector	Description	References
pFS100	pGP704 suicide vector, λ *pir* dependent, Km^R Amp^R low copy number	Rubires et al., 1997
pJET1.2/blunt cloning vector	Cloning vector for PCR product, ampicillin resistance	Fermentas
pJET::$kdsA_{421}$	pJET1.2/blunt cloning vector containing 421-bp fragment of *kdsA* gene from *P. aeruginosa* PAO1 strain, ampicillin resistance	This study
pFS100::$kdsA_{306}$	pFS100 suicide vector containing 306-bp fragment of BglII digested *kdsA* gene from pJET::$kdsA_{421}$, ampicillin and kanamycin resistance	This study
pJET::$waaG_{459}$	pJET1.2/blunt cloning vector containing 459-bp fragment of *waaG* gene from *P. aeruginosa* PAO1 strain, ampicillin resistance	This study
pFS100::$waaG_{507}$	pFS100 suicide vector containing 507-bp fragment of BglII digested *waaG* gene from pJET::$waaG_{459}$, ampicillin and kanamycin resistance	This study

Table 3. Oligonucleotides used in this study

Oligonucleotides	Sequence	Location/description
pJET1.2F	5′-CGA CTC ACT ATA GGG AGA GCG GC-3′	Priming site is at bases 310–332 of pJET1.2/blunt cloning vector
pJET1.2R	5′-AAG AAC ATC GAT TTT CCA TGG CAG-3′	Priming site is at bases 405–428 of pJET1.2/blunt cloning vector
kdsAF	5′-CAG GTC TGC GAA GAA TAC G-3′	*P. aeruginosa* nt 106–124
kdsAR	5′-TGT TGT AAC CGA AGG AGG A-3′	*P. aeruginosa* nt 508–526
waaGF	5′-TCT TCG TCA AGC ACT ACG G-3′	*P. aeruginosa* nt 449–467
waaGR	5′-AGA CAT CGG TCA CCA ACA C-3′	*P. aeruginosa* nt 889–907
orKdsAF	5′-ATA TCC AGA TCG CAA ACG AC-3′	*P. aeruginosa* nt 376–395
orKdsAR	5′-TGA GCA TCG TGA TCT TCA GG-3′	*P. aeruginosa* nt 1345–1364
orWaaGF	5′-GCG GAA AAA CTC TAC GAA CG-3′	*P. aeruginosa* nt 170–189
orWaaGR	5′-CAG ACA TCG GTC ACC AAC AC-3′	*P. aeruginosa* nt 1385–1404



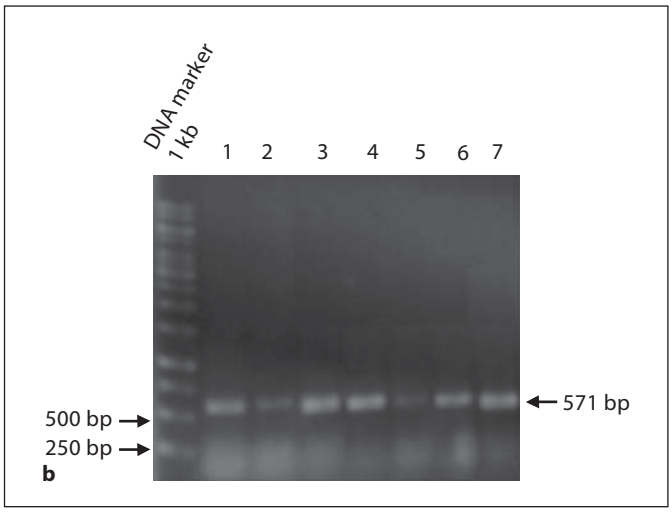
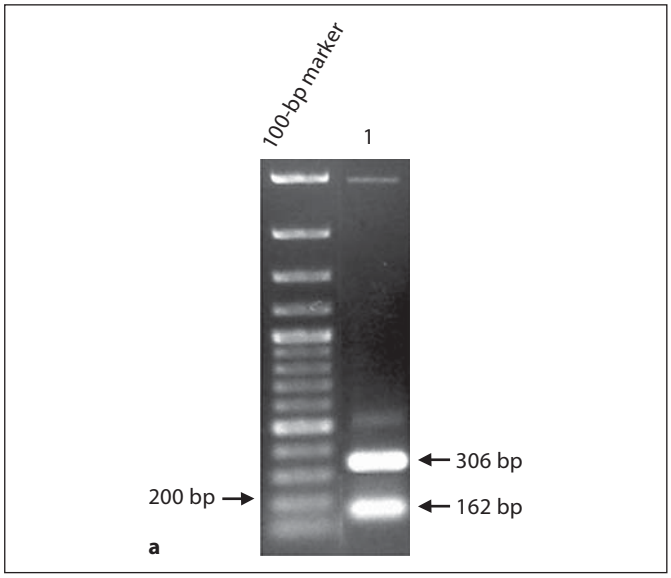


Fig. 1. PCR analysis of the pJET::$kdsA_{421}$ and pJET::$waaG_{459}$ clones. **a** PCR analysis of $kdsA_{421}$ fragment cloned to pJET vector using pJET1.2 forward and pJET1.2 reverse sequencing primers. The expected 533-bp PCR products containing $kdsA_{421}$ fragment and multiple cloning sites of pJET vector are indicated by an arrow. Lanes 1–9 represent the colonies. The 1-kb DNA marker used is indicated on the left. **b** PCR analysis of $waaG_{459}$ fragment cloned to pJET vector using pJET1.2 forward and pJET 1.2 reverse primers. The expected 571-bp PCR products containing $waaG_{459}$ fragment are indicated by an arrow. Lanes 1–7 represent the positive colonies. The 1-kb DNA marker used is shown in the figure.



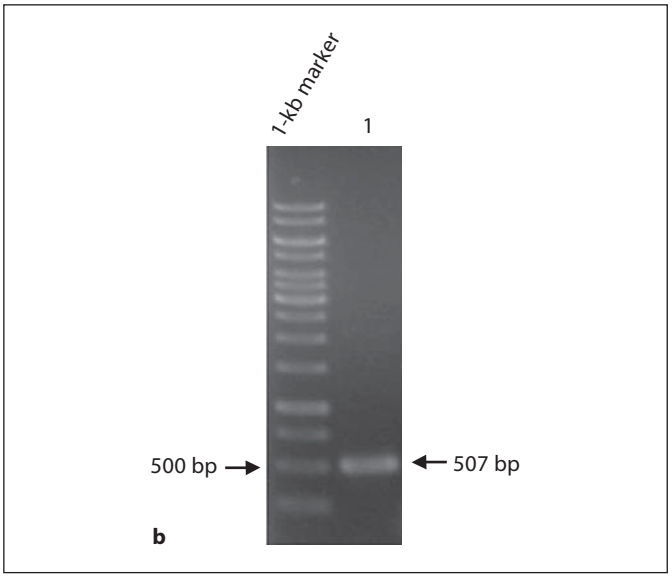


Fig. 2. Restriction digestion analysis of pJET::*kdsA* and pJET::*waaG* PCR products. PCR product containing $kdsA_{421}$ fragment from pJET::*kdsA* and $waaG_{459}$ fragment from pJET::*waaG* were digested with BglII enzyme and were run on 0.8% agarose gel. **a** The 533-bp PCR product using pJET primers when digested with BglII cuts at three sites resulting in four different sizes (30, 162, 306, 35 bp). The smaller-size fragments (30, 35 bp) were not visible, whereas two bands of size 162 and 306 bp are shown in the figure. The 100-bp DNA marker used is shown on the left of lane 1. **b** The 571-bp PCR product using pJET primers when digested with BglII cuts at two sites resulting in three different sizes (30, 507, 34 bp). The smaller-size fragments (30, 34 bp) were not visible, whereas the larger fragment of size 507 bp is shown in the figure.

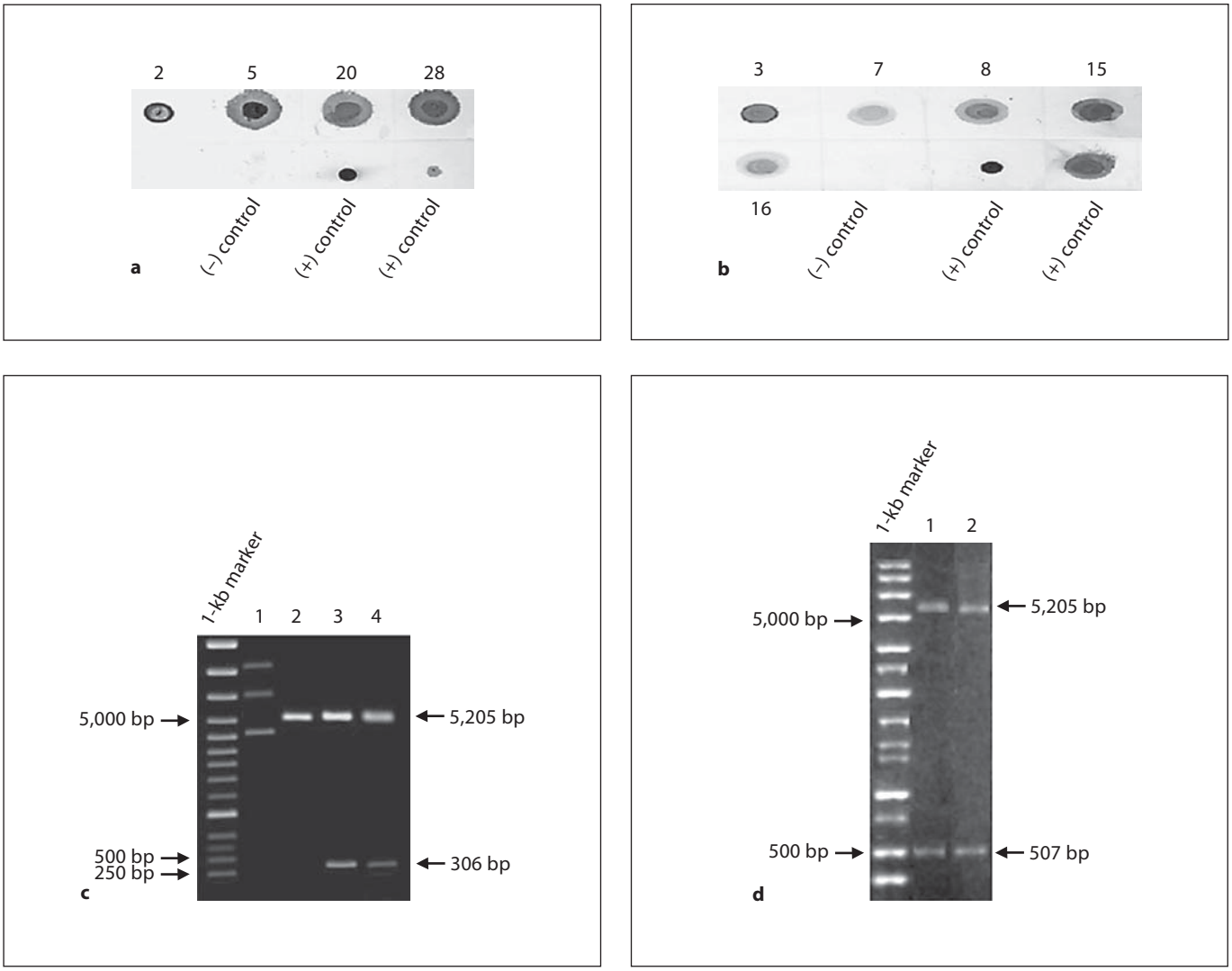


Fig. 3. Dot-blot hybridization (**a**, **b**) and restriction digestion analyses (**c**, **d**) to confirm the presence of the *kdsA* and *waaG* gene fragments. **a** Presence of $kdsA_{306}$ gene fragment in pFS100. Crude DNA extracted from pFS100::$kdsA_{306}$ clones were applied onto Nylon membrane and hybridized with DIG-labeled 421-bp *kdsA* PCR product. The $kdsA_{421}$ PCR product from *P. aeruginosa* genomic DNA was positive control, whereas pFS100 plasmid DNA was used as negative control. The numerical number indicates pFS100::$kdsA_{306}$ clones obtained after selecting on LB agar supplemented with ampicillin (100 μg/ml) and kanamycin (50 μg/ml). The strong hybridization reactions were seen in clone numbers 2, 5, 20 and 28. **b** Presence of $waaG_{507}$ gene fragment in pFS100. Crude DNA extracted from pFS100::$waaG_{507}$ clones were applied onto Nylon membrane and hybridized with DIG-labeled 459-bp *waaG* PCR product. The $waaG_{459}$ PCR product from *P. aeruginosa* genomic DNA was positive control, whereas pFS100 plasmid DNA was used as negative control. The numbers indicate pFS100::$waaG_{507}$ clones. The strong hybridization reactions were seen in clone numbers 3, 7, 8, 15 and 16. **c** Restriction analysis of the pFS100::$kdsA_{306}$ plasmid. Plasmid DNA preparations from pFS100::$kdsA_{306}$ bacterial clones were digested with BglII run on 0.8% agarose gel. Lane 1: extracted pFS100 plasmid DNA, lane 2: pFS100 digested with BglII enzyme resulting in a linear band of size 5,205 bp, lanes 3 and 4: recombinant plasmid pFS100::$kdsA_{306}$ (clone numbers 2 and 5) digested with BglII resulting in two bands of size 5,205 and 306 bp. The larger size 5,205 bp corresponds to plasmid pFS100 and the smaller size 306 bp corresponds to the *kdsA* gene fragment. The 1-kb marker used is indicated on the left side of the lanes. **d** Restriction analysis of the pFS100::$waaG_{507}$ plasmid. Plasmid DNA preparations from pFS100::$waaG_{507}$ bacterial clones were digested with BglII enzyme and then run on 0.8% agarose gel. Lanes 1 and 2: recombinant plasmid pFS100::$waaG_{507}$ (colony numbers 8 and 16) digested with BglII resulting in two bands of size 5,205 and 507 bp. The larger size 5,205 bp corresponds to plasmid pFS100 and the smaller size 507 bp corresponds to the *waaG* gene fragment.

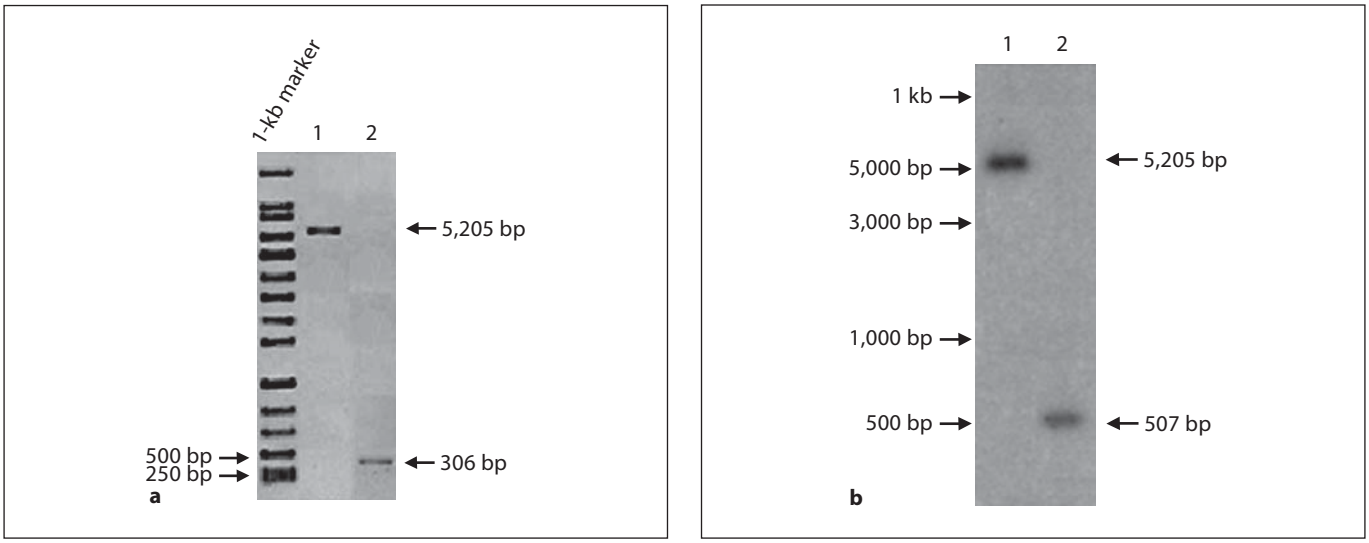
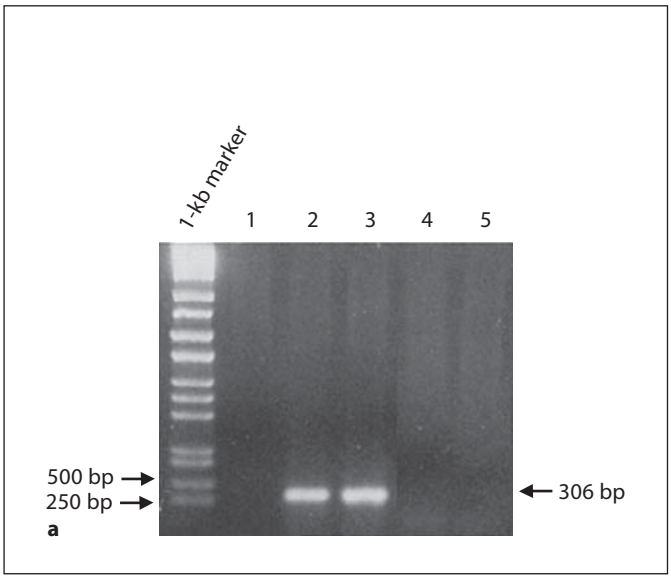


Fig. 4. Southern blot hybridization analyses of (**a**) pFS100::$kdsA_{306}$ and (**b**) pFS100::$waaG_{507}$ recombinant plasmids. **a** Southern blot analysis of the pFS100::$kdsA_{306}$ recombinant plasmid clone. The pFS100::$kdsA_{306}$ DNA bacterial clones were digested with BglII and run on 0.8% agarose gel. The denatured DNA from the gel is transferred to a nylon membrane and hybridized with DIG-labeled 421 bp *kdsA* PCR product as gene probe. Lane 1: pFS100::$kdsA_{306}$ digested with BglII and hybridized with the pFS100 probe, lane 2: pFS100::$kdsA_{306}$ digested with BglII and hybridized with the *kdsA* gene probe. The digested product size of 306 bp forms base pairs with its complementary DNA sequence from the gene probe to form double-stranded DNA. The 1-kb probe marker is shown on the left side of the lanes. **b** Southern blot analysis of the pFS100::$waaG_{507}$ recombinant plasmid clone. The pFS100::$waaG_{507}$ DNA bacterial clones were digested with BglII and run on 1% agarose gel. The denatured DNA from the gel is transferred to a nylon membrane and hybridized with DIG-labeled 459 bp *waaG* PCR product as gene probe. Lane 1: pFS100::$waaG_{507}$ digested with BglII and hybridized with the pFS100 probe, lane 2: pFS100::$waaG_{507}$ digested with BglII and hybridized with the *waaG* gene probe. The digested product size of 507 bp forms base pairs with its complementary DNA sequence from the gene probe to form double-stranded DNA.

affects growth, oligonucleotide primers were chosen so that flanking genes and intergenic regions including potential promoters would remain intact in the deletion mutant. Correct incorporation of the fused construct gives rise to a larger or smaller PCR product in a mutant than in the wild type, depending on the target gene. Our approach for the genes *kdsA* and *waaG* resulted in lack of recovery of antibiotic-resistant colonies suggesting essentiality of these genes in *P. aeruginosa*.

To further verify our result on gene essentiality of these genes, we performed an in silico analysis. We have followed the constraint-based flux balance analysis (FBA) approach [Price et al., 2004; Varma and Palsson, 1994] to simulate a previously published genome-scale metabolic model iMO1056 [Oberhardt et al., 2008] for *P. aeruginosa* to obtain in silico gene essentiality predictions under three different growth media. The three different growth media simulated were: Luria-Bertani (LB), cetrimide and rich media. In our simulations of the metabolic model iMO1056, the subset of external nutrient metabolites that are available for uptake in LB and cetrimide media are indicated in online supplementary table S2, and for the case of rich media, all external nutrient metabolites are assumed to be available for uptake. It must be mentioned that the genes predicted in silico to be essential under rich medium are likely to be essential in silico under any simulated medium. The list of in silico predicted essential gene products in *P. aeruginosa* metabolism for the three different media are given in online supplementary table S3. These in silico simulations additionally validate and strengthen our experimental results for both *kdsA* and *waaG*.

Presently, drugs which target gene products that are essential in vitro have proven to be less than optimal for treating *Pseudomonas* infections. A recent report on the antibiotic sensitivity patterns of *P. aeruginosa* has highlighted the problem of antibiotic resistance in cystic fibrosis strains in comparison with other hospital isolates [Henwood et al., 2001]. Resistance to aminoglycosides and colistin has been observed in laboratory strains of



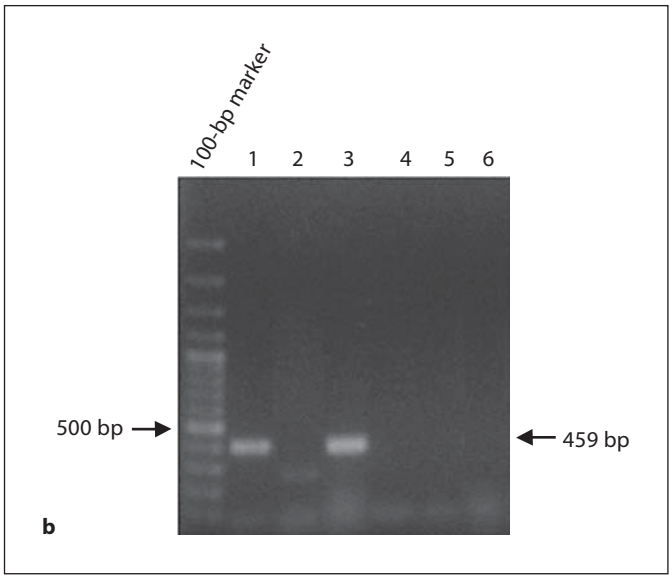


Fig. 5. Colony PCR analyses for (**a**) pFS100::$kdsA_{306}$ and (**b**) pFS100::$waaG_{507}$ recombinant plasmids. **a** The recombinant plasmid pFS100::$kdsA_{306}$ clones from the colonies 2, 5, 20 and 28 were used for colony PCR using kdsA forward and kdsA reverse primers, respectively. A 0.8% agarose gel was run and the resultant 306-bp product size of kdsA gene fragment was observed in colony numbers 2 and 5 (lanes 2, 3). No bands were observed in other colonies 20 and 28 (lanes 4, 5). Lane 1: pFS100 plasmid with no insert was used as negative control for colony PCR experiment. The 1-kb DNA marker is shown on the left. **b** The recombinant plasmid pFS100::$waaG_{507}$ clones from the colonies 3, 7, 8, 15 and 16 were used for colony PCR using waaG forward and waaG reverse primers, respectively. A 0.8% agarose gel was run and the resultant 459-bp product size of *waaG* gene fragment was observed in colony numbers 8 and 16 (lanes 1, 3). No bands were observed in other colonies 3, 7 and 15 (lanes 4–6). Lane 2: pFS100 plasmid with no insert was used as negative control for colony PCR experiment. The 100-bp DNA marker is shown on the left.

P. aeruginosa due to overexpression of an outer membrane protein, oprH, which protects the LPS from binding the antibiotics [Lambert, 2002]. The risk of emergence of antibiotic resistance varies with different antibiotic treatments. Four antipseudomonal agents, ciprofloxacin, ceftazidime, imipenem, and piperacillin, were used in a comparative analysis to check the risks of emergence of resistance [Carmeli et al., 1999]. Ceftazidime was found to be associated with the lowest risk, and imipenem had the highest risk.

P. aeruginosa exhibits the highest rates of resistance for the fluoroquinolones, with resistance to ciprofloxacin and levofloxacin ranging from 20 to 35% [Jalal et al., 2000]. Among the aminoglycosides, gentamicin with resistance rates ranging from 12 to 22% was observed. Gentamicin was the least active of the aminoglycosides, with lower rates of resistance being reported for tobramycin and amikacin [Lister et al., 2009]. *P. aeruginosa* possesses the ampC gene for the inducible chromosomal β-lactamase. Patients treated with an appropriate β-lactam based on susceptibility data failed the therapy due to the emergence of AmpC-mediated resistance [Tam et al., 2009]. The emergence of resistance/clinical failure is observed most frequently with infections in patients with cystic fibrosis and neutropenia [Aloush et al., 2006].

Complementing in silico predictions with in vitro wet-lab methods facilitates antibacterial drug discovery. This further provides an efficient method to enrich for potential prioritized target genes and identify those which are most critical for normal cell function [Zhang and Zhang, 2006]. Genes that are essential for bacterial survival are considered to be ideal targets for inhibitor screening. Recent developments in structural biology have made it possible to dock small drug-like ligand molecules with proteins to identify their potential binding sites. Computational methods in conjunction with structural information have been used to guide the design of novel antibacterial agents [Dhaliwal and Chen, 2009; Waszkowycz, 2008].Methods such as docking and virtual screening are becoming widely used in drug development. Computational ligand-protein docking can be explored through virtual screening that involves databases with relevant molecular structures that are docked into protein targets [Shoichet, 2004]. Screening drug-like

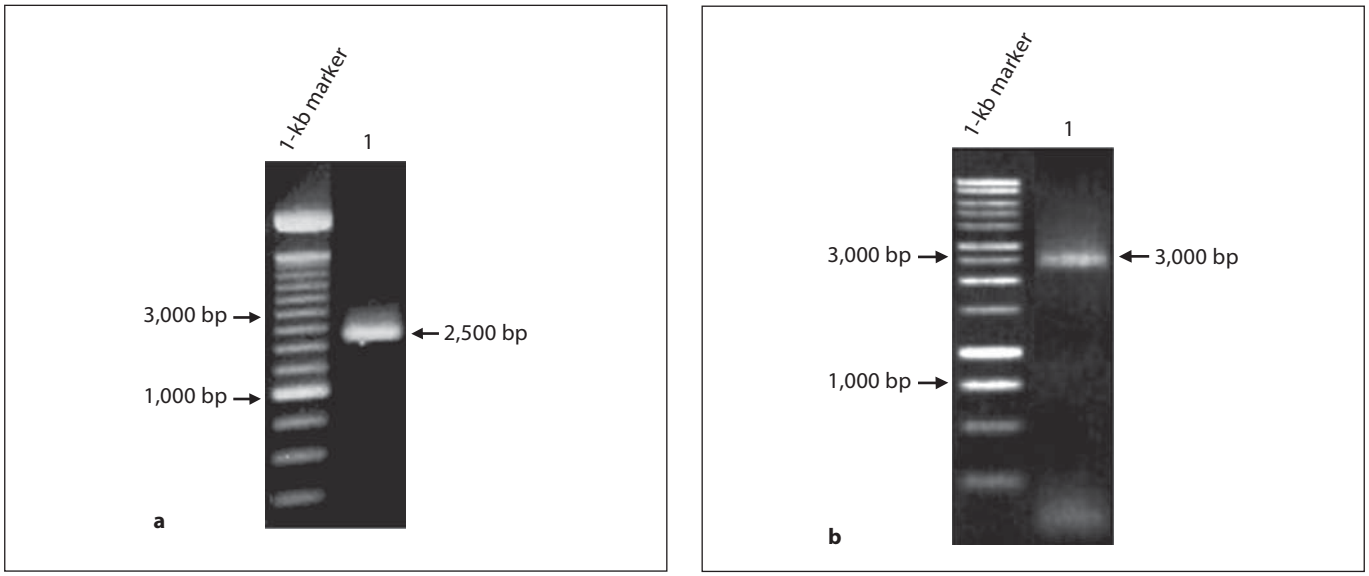


Fig. 6. PCR analysis from cell lysate to check the loss of the (**a**) *kdsA* and (**b**) *waaG* genes. **a** The cell lysate obtained after filter mating of *E. coli* SM10 λ *pir* harboring pFS100::$kdsA_{306}$ plasmid with the recipient *P. aeruginosa* (wild type) was used as DNA sample for PCR. Two primers outside region of *kdsA* gene orKdsAF and orKdsAR were used which amplified a product of size 2,500 bp. Lane 1 shows the PCR product with size 2,500 bp corresponding to the 1-kb DNA marker in the left. **b** The cell lysate obtained after filter mating of *E. coli* SM10 λ *pir* harboring pFS100::$waaG_{507}$ plasmid with the recipient *P. aeruginosa* (wild type) was used as DNA sample for PCR. Two primers outside region of *waaG* gene orWaaGF and orWaaGR were used which amplified a product of size 3,000 bp. Lane 1 shows the PCR product with size 3,000 bp corresponding to the 1-kb DNA marker in the left.

compounds by computational docking against the structure of a known target has become a promising avenue in lead discovery. This approach is arguably much cheaper than experimental high-throughput screening and has been validated in other lead discovery projects [McInnes, 2007; Villoutreix et al., 2009].

The molecular validation of the above studied two essential genes will lead to design of putative inhibitory compounds in silico which will involve computing a target structure with the small molecules. We therefore suggest that the products of these essential genes merit further investigation as potential targets for novel, highly specific antibacterial agents.

Conclusion

Several important technology advances have helped us to identify genes that are essential for bacterial growth and that could serve as potential antibiotic targets. These have been very useful for studying the roles of pathogenic genes during in vitro growth. Our experimental results confirm the fact that combined computational and molecular biology methods will provide researchers with a markedly improved repertoire of research tools that can help us to better understand the complexities of infectious pathogens like *P. aeruginosa*. Extensive laboratory procedures and time are required to validate genes that when inhibited result in rapid and complete understanding of bacterial infections. Nonetheless, the in silico and in vitro data for the genes involved in the LPS biosynthesis highlights the importance of gene essentiality for the purpose of target identification for novel antimicrobial drug discovery.

Acknowledgements

We would like to thank Dr. Juan M. Tomas and Dr. Leung Ka Yin for providing the pFS100 suicide plasmid, *E. coli* MC1061 and *E. coli* SM10 bacterial strains for this work. We also would like to thank Mya Myintzu Hlaing for critical comments which helped improve the manuscript.

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