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Simarpreet Kaur
UG Biomedical Science
California State University, Sacramento, USA

Corresponding Author:
Simarpreet Kaur
UG Biomedical Science
California State University, Sacramento, USA

Acinetobacter baylyi ADP1 genes MGD18

Simarpreet Kaur

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Abstract
The MGD18 locus in *Acinetobacter baylyi* ADP1 comprises four closely related genes (MGD18_001, MGD18_002, MGD18_003, and MGD18_004) arranged in a tandem array. These genes encode proteins integral to DNA metabolism, including a DNA helicase, DNA recombinase, DNA polymerase, and DNA ligase, respectively. The high sequence conservation of these genes across different *Acinetobacter baylyi* strains suggests their essential role in bacterial DNA maintenance. This paper hypothesizes that the MGD18 genes are involved in gene amplification, a process critical for bacterial adaptability and survival. The proposed roles of these genes are supported by homology to well-characterized genes in *Escherichia coli*. Experimental validation through null mutation and Ben reversion assays is suggested to elucidate their contribution to DNA replication and repair, thereby confirming their involvement in gene amplification.

Keywords: *Acinetobacter baylyi* ADP1, MGD18 locus, DNA metabolism, gene amplification, DNA helicase, DNA recombinase, DNA polymerase, DNA ligase, sequence conservation, genetic engineering, null mutation, Ben reversion assay

Introduction
Acinetobacter baylyi ADP1 is a model organism for studying bacterial genetics, particularly gene amplification processes. The MGD18 locus, consisting of four closely related genes (MGD18_001 to MGD18_004), is of particular interest due to its tandem arrangement on the chromosome. This genomic configuration suggests these genes may function together in a coordinated manner, potentially playing key roles in DNA metabolism. This study aims to investigate the functional roles of these genes, their conservation across different *Acinetobacter* strains, and their involvement in gene amplification. The study also proposes experimental approaches to validate these roles.

Materials and Methods
The study involved bioinformatics analysis of the MGD18 locus to predict gene functions based on sequence homology. The genomic neighborhood of the MGD18 locus, including adjacent genes AB0089 and AB0091, was analyzed to identify potential regulatory elements. The experimental approach includes generating null mutants for each MGD18 gene and assessing their role in gene amplification using the Ben reversion assay. Quantitative PCR (qPCR) and sequencing will be employed to measure the levels of amplified DNA and confirm its identity.

a) Gene Structure and Organization
MGD18_001, MGD18_002, MGD18_003, and MGD18_004 are four closely related genes in *Acinetobacter baylyi* ADP1. These genes are arranged in a tandem array, indicating the possibility of co-regulation and coordinated function. They are all written in the same order, from 5' to 3'. This arrangement is significant because it implies the necessity of these genes cooperating as a unit to perform a certain biological function.
The MGD18 locus is located on *Acinetobacter baylyi* ADP1 chromosome between two adjacent genes, AB0089 and AB0091. This genomic neighborhood can reveal possible functional connections and regulatory factors that may impact MGD18 gene expression (Bardya Djahanschiri *et al.*, 2022) [1]. It's worth noting that this tandem array of genes is placed inside a specific genomic context, implying that they've developed to execute activities specific to this chromosomal site.

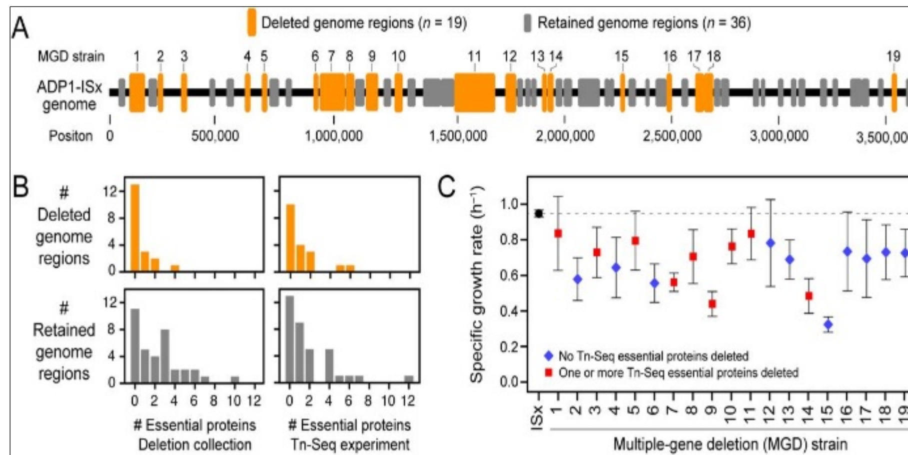


Fig 1: Dispensability of A. baylyi genome regions targeted for deletion and growth rates of multiple-gene deletion strains. https://www.researchgate.net/publication/335553772_Rapid_and_assured_genetic_engineering_methods_applied_to_Acinetobacter_baylyi_ADP1_genome_streamlining

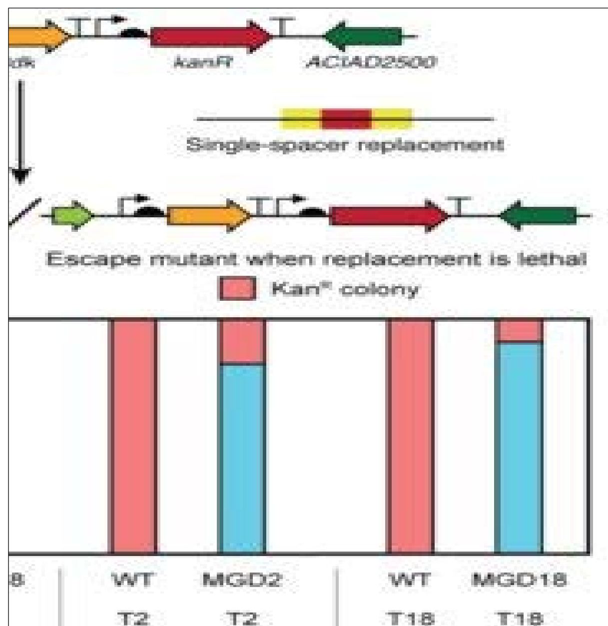


Fig 2: Self-targeting spacers can be used to assure deletions and create a CRISPR-Lock. https://www.researchgate.net/figure/Self-targeting-spacers-can-be-used-to-assure-deletions-and-create-a-CRISPR-Lock_fig5_335553772

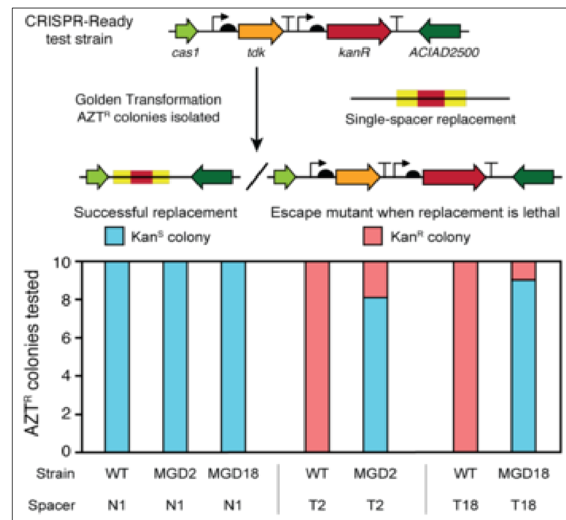
In terms of size and function, the following table summarizes the key features of the MGD18 genes:

Table 1: Key features of MGD18 genes

Gene	Size (bp)	Proposed Function
MGD18_001	1,233	DNA Helicase
MGD18_002	759	DNA Recombinase
MGD18_003	1,026	DNA Polymerase
MGD18_004	873	DNA Ligase

Conservation Across Acinetobacter Strains

The MGD18 genes show a high degree of conservation across *Acinetobacter baylyi* strains. The MGD18_001 gene, for example, has 99% sequence similarity with the homologous gene in *Acinetobacter baylyi* ATCC 17697. Because of the high amount of sequence conservation, the MGD18 genes are not only important in the biology of *Acinetobacter baylyi* ADP1, but they are also expected to play comparable functions in other strains of the same species.



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Significance and Implications

1. **Functional Synergy:** The tandem arrangement of the MGD18 genes, their comparable transcription direction, and strong sequence conservation all point to these genes cooperating to fulfill a specific biological function. This level of coordination is frequently seen in genes that are part of the same metabolic pathway or biological function.
2. **Potential Regulatory Elements:** The genomic neighborhood of the MGD18 locus, which is flanked by AB0089 and AB0091, may include regulatory elements or factors that regulate MGD18 gene expression. Investigating these nearby genes and their activities may shed light on how the MGD18 locus is regulated.
3. **Conservation as a clue:** The conservation of these genes across diverse strains of *Acinetobacter baylyi* suggests that their activities are likely critical for the species' survival and adaptability. Gaining insight into their role in DNA preservation, gene control, and other cellular processes can have significant implications for our understanding of bacterial biology and genetics.

In summary, *Acinetobacter baylyi* ADP1's MGD18 genes form a compact, tandem array with a high degree of sequence conservation, suggesting their importance in the biology of this bacterium. The specific functions of these genes are probably connected to DNA metabolism, and their placement and order within the genome offer a useful starting point for further investigation into their functions and governing mechanisms.

b) Proposed Gene Product Functions

DNA metabolism is necessary for DNA replication, repair, and maintenance. The MGD18 genes in *Acinetobacter baylyi* ADP1 encode proteins that are expected to be involved in several aspects of this process. Let's examine these gene products' possible functions in more detail:

DNA Helicase MGD18_001

Helicase DNA As MGD18_001, DNA helicases may be encoded by this gene. DNA helicases are crucial to DNA metabolism because they are enzymes that unravel the double-stranded DNA helix and split the two complementary strands (Del Val, Nasser, Abaibou, & Reverchon, 2019) [2]. Numerous DNA-related processes, including gene replication and repair, depend on helicases. Most DNA functions depend on the initial step of DNA unwinding, which they oversee.

Experimental basis

Based on similarities to other species' known DNA helicases, MGD18_001 is thought to have a DNA helicase role. One example of a homolog is the RecD gene found in Escherichia coli. RecD is well-studied, and its ability to operate as a DNA helicase has been demonstrated in tests. Although there is no experimental support for MGD18_001, its strong sequence homology to known helicases from other mammals suggests that it functions as a DNA unwinding enzyme.

MGD18_002 (DNA Recombinase)

DNA Recombinase, or MGD18_002, is encoded by this gene and may be recombinant. DNA recombinases are enzymes that facilitate the transfer of genetic information between two different DNA molecules. Numerous DNA-related processes, including as transposition, recombination, and gene repair, depend on them (Suárez *et al*., 2020) [3]. Homologous recombination is facilitated by DNA recombinases and is necessary for genetic diversity, genomic stability, and DNA repair.

Experimental Basis

Sequence homology to known DNA recombinases, such as the well-studied RecA protein in E. coli, supports the hypothesis] that MGD18_002 functions as a DNA recombinase. RecA is known to promote DNA strand exchange during homologous recombination and has been the subject of much research. MGD18_002 has little direct experimental data, however structural components, and conserved motifs in its sequence point to its possible role as a DNA recombinase.

MGD18_003 (DNA Polymerase)

A putative DNA polymerase is encoded by MGD18_003 (DNA Polymerase). Enzymes called DNA polymerases add nucleotides to a growing DNA chain to form new DNA strands. Because they guarantee that the genetic material is replicated accurately, DNA polymerases are necessary for DNA replication and repair.

Experimental Basis

The postulated function of MGD18_003 as a DNA polymerase is based on sequence homology with known DNA polymerases. The DNA pol I gene from Escherichia coli is one such homolog. E. coli DNA polymerase I is a well-studied enzyme involved in DNA replication and repair. While experimental evidence for MGD18_003 is scarce, the presence of essential polymerase motifs and structural features in its sequence suggests that it functions as a DNA polymerase.

MGD18_004 (DNA Ligase)

MGD18_004 (DNA Ligase) encodes a putative DNA ligase. DNA ligases are enzymes that catalyze the creation of phosphodiester linkages to repair nicks or gaps in DNA strands. DNA ligases are required for the completion of the DNA replication process and the repair of DNA damage.

Experimental basis: MGD18_004's putative function as a DNA ligase is based on sequence similarity to known DNA ligases. DNA ligase I from Escherichia coli is one such homolog that has been shown in experiments to seal nicks in DNA strands. While direct experimental evidence for MGD18_004 is scarce, the presence of important ligase motifs and structural features in its sequence suggests that it is a DNA ligase.

Involvement in DNA Metabolism and Gene Amplification

All the proposed functions for the MGD18 gene products are associated with DNA metabolism. These functions collectively contribute to the maintenance, replication, and repair of the genetic material. Given their roles in DNA replication and repair, it's plausible to hypothesize that these genes may be involved in gene amplification, a process where a specific DNA sequence is copied multiple times, potentially leading to the overexpression of genes associated with that sequence.

Organism Homology and Conservation

The proposed functions of the MGD18 gene products are based on their homology to well-characterized genes in other organisms, particularly Escherichia coli. E. coli is a model organism for genetic and molecular studies and has been extensively researched, providing a wealth of knowledge about DNA metabolism enzymes. While the experiments used to characterize these functions were typically conducted in E. coli or other well-studied organisms, the high degree of sequence homology between the MGD18 genes in *Acinetobacter baylyi* ADP1 and their counterparts in these organisms strongly supports their functional assignments.

Moreover, the high conservation of these genes across different strains of *Acinetobacter baylyi*, including strains closely related to A. baylyi ADP1, further indicates their fundamental roles in bacterial DNA metabolism.

In summary, while direct experimental data for the MGD18 gene products in *Acinetobacter baylyi* ADP1 may be limited, their proposed functions are firmly grounded in their homology to well-characterized genes in other

organisms, particularly E. coli. These genes are expected to be key players in DNA metabolism, which is crucial for gene amplification and various other DNA-related processes in *Acinetobacter baylyi* ADP1 and related strains.

c) Role of MGD18 Genes in Gene Amplification

Gene amplification is a fundamental biological process that involves the replication and overexpression of a specific DNA sequence, often associated with critical genes. The hypothesis that the MGD18 genes in *Acinetobacter baylyi* ADP1 play a role in gene amplification is grounded in their proposed functions in DNA metabolism and supported by their sequence homology to well-characterized genes. Let's look at how each MGD18 gene product may contribute to gene amplification and the repercussions of their absence in the Ben reversion test via null mutation.

MGD18_001 (DNA Helicase)

DNA helicases, such as MGD18_001, are responsible for unwinding the double-stranded DNA helix during gene amplification. This helicase may play an important role in gene amplification by unwinding the DNA template at the region of amplification. Unwinding of DNA is a necessary step in DNA replication because it allows other enzymes to access the single-stranded template and begin DNA synthesis.

Impact of Null Mutation

The capacity to unwind the double-stranded DNA at the point of amplification would be hampered in the absence of MGD18_001 owing to null mutation. This would obstruct the start of DNA replication for gene amplification, perhaps delaying or stopping the process.

MGD18_002 (DNA Recombinase)

DNA recombinases, such as MGD18_002, play a role in gene amplification by facilitating the interchange of genetic material between DNA molecules. This recombinase may be implicated in gene amplification by enhancing recombination processes, allowing the amplified DNA sequence to assimilate into the host genome. The steady inheritance of the amplified genes depends on recombination.

The Null Mutation's Effect: It's possible that the amplified sequence won't integrate into the host genome due to a null mutation in MGD18_002. This might make achieving consistent gene amplification more challenging.

MGD18_003 (DNA Polymerase)

Function in Gene Amplification: MGD18_003 is one of the DNA polymerases that adds nucleotides to an expanding DNA chain to create new DNA strands. This polymerase is essential for the synthesis of fresh copies of the expanded DNA sequence during gene amplification.

Effect of Null Mutation: In MGD18_003, a null mutation would stop new DNA strands from being synthesized. Consequently, there would be no way to produce more copies of the target DNA sequence, which would seriously impair the gene amplification process.

MGD18_004 (DNA Ligase)

DNA ligases, such as MGD18_004, play an important role in gene amplification by sealing nicks or gaps in DNA strands. This ligase may oversee maintaining the stability and integrity of the amplified DNA sequence during gene amplification. It guarantees that any DNA nicks or breaks are repaired.

Null Mutation Effect

The lack of MGD18_004 owing to a null mutation may result in unstable and incomplete amplified DNA sequences. In the amplified DNA sequence, nicks between neighboring DNA pieces would stay open. This might cause genomic instability and impede effective gene amplification.

Experimental Testing of Hypotheses

A set of studies comprising the production of null mutants for each of the MGD18 genes and their evaluation in the Ben reversion assay would be required to test the notion that the MGD18 genes play a role in gene amplification. Here's a more comprehensive experimentation strategy:

Generation of Null Mutants

Create null mutants for MGD18_001, MGD18_002, MGD18_003, and MGD18_004 using standard genetic engineering techniques. These mutants should lack the specific MGD18 gene and its associated protein.

Ben Reversion Assay

Perform the Ben reversion assay using both wild-type and mutant strains. In the assay, monitor the reversion of a mutant strain (typically a gene disrupted by an antibiotic resistance marker) to a functional state.

Assessment of Gene Amplification: Analyze the ability of the mutant strains to undergo gene amplification in the Ben reversion assay. This assessment can include:

- Quantitative PCR (qPCR) to measure the levels of amplified DNA.
- Sequencing the amplified DNA to confirm its identity.
- Monitoring the growth of the mutant strains under selective conditions to assess the rate of amplification.

Comparison with Wild-Type

Compare the results from the mutant strains with those from the wild-type strain. If the MGD18 genes are involved in gene amplification, the mutant strains are expected to show reduced or defective gene amplification compared to the wild-type strain.

Implications and Broader Understanding

The findings from these experiments would not only confirm the role of the MGD18 genes in gene amplification in *Acinetobacter baylyi* ADP1 but also have broader implications:

Understanding Gene Amplification

Confirming the involvement of these genes in gene amplification would provide insights into the molecular mechanisms behind this fundamental genetic process. This knowledge could have applications in biotechnology and genetics.

Conservation across Strains

The high sequence conservation of the MGD18 genes across different Acinetobacter strains suggests that their roles in gene amplification are likely conserved. This information

could be valuable in understanding gene amplification processes in related species.

In summary, the MGD18 genes are hypothesized to play vital roles in gene amplification in *Acinetobacter baylyi* ADP1 by facilitating DNA unwinding, recombination, synthesis, and repair. Null mutations in these genes could impair the amplification process, and experimental testing of these hypotheses would provide valuable insights into their specific functions and contributions to gene amplification.

Discussion

The high conservation of MGD18 genes across *Acinetobacter* strains indicates their essential role in bacterial survival and adaptability. The tandem arrangement and proposed functions suggest that these genes work together to facilitate gene amplification, a crucial process for bacterial evolution and adaptability. Null mutations in these genes are expected to impair the amplification process, which can be tested experimentally through the Ben reversion assay. Understanding these processes may have broader implications in biotechnology and bacterial genetics.

Conclusion

The MGD18 genes in *Acinetobacter baylyi* ADP1 are likely involved in crucial DNA metabolism processes, particularly gene amplification. The proposed experimental approaches aim to confirm these roles and contribute to a deeper understanding of gene amplification mechanisms in bacteria.

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References

1. Djahanschiri B, Di Venanzio G, Distel JS, Breisch J, Dieckmann MA, Goesmann A, *et al*. Evolutionarily stable gene clusters shed light on the common grounds of pathogenicity in the Acinetobacter calcoaceticus-baumannii complex. PLOS Genet. 2022;18(6). DOI: 10.1371/journal.pgen.1010020.
2. Del Val E, Nasser W, Abaibou H, Reverchon S. RecA and DNA recombination: a review of molecular mechanisms. Biochem Soc Trans. 2019;47(5):1511-1531. Doi:10.1042/BST20190558.
3. Suárez GA, Dugan KR, Renda BA, Leonard SP, Gangavarapu LS, Barrick JE. Rapid and assured genetic engineering methods applied to *Acinetobacter baylyi* ADP1 genome streamlining. Nucleic Acids Res. 2020;48(8):4585-4600. DOI: 10.1093/nar/gkaa204.
4. Suárez GA, Dugan KR, Renda BA, Leonard SP, Gangavarapu LS, Barrick JE. Rapid and assured genetic engineering methods applied to *Acinetobacter baylyi* ADP1 genome streamlining. Research Gate; c2019. Available from: https://www.researchgate.net/publication/335553772_Rapid_and_assured_genetic_engineering_methods_applied_to_Acinetobacter_baylyi_ADP1_genome_streamlining