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Transcriptional coupling of DNA repair in sporulating *Bacillus subtilis* cells

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Summary

In conditions of halted or limited genome replication, like those experienced in sporulating cells of *Bacillus subtilis*, a more immediate detriment caused by DNA damage is altering the transcriptional programme that drives this developmental process. Here, we report that *mfd*, which encodes a conserved bacterial protein that mediates transcription-coupled DNA repair (TCR), is expressed together with *uvrA* in both compartments of *B. subtilis* sporangia. The function of Mfd was found to be important for processing the genetic damage during *B. subtilis* sporulation. Disruption of *mfd* sensitized developing spores to mitomycin-C (M-C) treatment and UV-C irradiation. Interestingly, in non-growing sporulating cells, Mfd played an anti-mutagenic role as its absence promoted UV-induced mutagenesis through a pathway involving YqjH/YqjW-mediated translesion synthesis (TLS). Two observations supported the participation of Mfd-dependent TCR in spore morphogenesis: (i) disruption of *mfd* notoriously affected the efficiency of *B. subtilis* sporulation and (ii) in comparison with the wild-type strain, a significant proportion of Mfd-deficient sporangia that survived UV-C treatment developed an asporogenous phenotype. We propose that the Mfd-dependent repair pathway operates during *B. subtilis* sporulation and that its function is required to eliminate genetic damage from transcriptionally active genes.

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Introduction

Endospore formation in *Bacillus subtilis* involves a complex cellular differentiation programme that is exquisitely regulated in great part by a cascade of sigma (σ) factors that bind the core of the RNA polymerase (RNAP) and regulate temporally and spatially the expression of genes required for the synthesis of the spore (Errington, 2003; Hilbert and Piggot, 2004). This developmental process, triggered by nutrient-limiting conditions and high cell density, allows *B. subtilis* spores to survive under conditions that are detrimental for its vegetative life. The genesis of the spore begins with an asymmetric cell division that results into two unequal-sized compartments, the mother cell (larger compartment) and the forespore (smaller compartment), each with its own chromosome and pattern of gene expression (Stragier and Losick, 1996). *B. subtilis* spores show no detectable metabolism, exhibit a high degree of resistance to heat, radiation and chemical agents (Nicholson *et al.*, 2000; Setlow, 2006), and possess the ability to survive for very long periods of dormancy (Kennedy *et al.*, 1994; Pedraza-Reyes *et al.*, 2012) and germinate upon sensing growth-favourable conditions (Setlow, 2003).

During spore formation, the two cell types of the sporangium no longer replicate their chromosomes (Veening *et al.*, 2009) but process DNA damage under unfavourable metabolic conditions. Indeed, recent reports have revealed that processing of genetic damage in sporangia is necessary for an efficient spore development (Rivas-Castillo *et al.*, 2010; Ramírez-Guadiana *et al.*, 2012). Interestingly, translesion synthesis (TLS) performed by the Y-DNA polymerases YqjH and YqjW was found to be important for processing spontaneous and induced genetic damage during spore synthesis (Rivas-Castillo *et al.*, 2010). Furthermore, an alternative excision repair pathway (AER) involved in eliminating UV-induced DNA damage operates in sporangia and spores of *B. subtilis* (Ramírez-Guadiana *et al.*, 2012). So while metabolic activities directly involved in cell growth and division are decreasing, DNA repair transactions appear very active in the sporulating cell.

Premutagenic DNA lesions, if left unrepaired, lead to genotoxic events and may also affect patterns of gene expression during sporulation which has led to speculate that DNA repair could be directed to actively transcribed

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Error-Prone Processing of Apurinic/Apyrimidinic (AP) Sites by PolX Underlies a Novel Mechanism That Promotes Adaptive Mutagenesis in *Bacillus subtilis*

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In growing cells, apurinic/apyrimidinic (AP) sites generated spontaneously or resulting from the enzymatic elimination of oxidized bases must be processed by AP endonucleases before they compromise cell integrity. Here, we investigated how AP sites and the processing of these noncoding lesions by the AP endonucleases Nfo, ExoA, and Nth contribute to the production of mutations (*hisC952*, *metB5*, and *leuC427*) in starved cells of the *Bacillus subtilis* YB955 strain. Interestingly, cells from this strain that were deficient for Nfo, ExoA, and Nth accumulated a greater amount of AP sites in the stationary phase than during exponential growth. Moreover, under growth-limiting conditions, the triple *nfo exoA nth* knockout strain significantly increased the amounts of adaptive *his*, *met*, and *leu* revertants produced by the *B. subtilis* YB955 parental strain. Of note, the number of stationary-phase-associated reversions in the *his*, *met*, and *leu* alleles produced by the *nfo exoA nth* strain was significantly decreased following disruption of *polX*. In contrast, during growth, the reversion rates in the three alleles tested were significantly increased in cells of the *nfo exoA nth* knockout strain deficient for polymerase X (PolX). Therefore, we postulate that adaptive mutations in *B. subtilis* can be generated through a novel mechanism mediated by error-prone processing of AP sites accumulated in the stationary phase by the PolX DNA polymerase.

The genetic alterations that allow organisms to escape from growth-limiting conditions in response to natural or artificial selection during prolonged nonlethal selective pressure are referred to as adaptive or stationary-phase mutagenesis (1). This biological process, originally discovered in *Escherichia coli* (2, 3), was later found to occur in other prokaryotes (1, 4) as well as in some eukaryotes (5). The existence of adaptive mutagenesis was demonstrated in *Bacillus subtilis* by employing strain YB955, which allows measuring the reversion frequencies to chromosomal auxotrophies of *hisC952* (TAG nonsense mutation), *metB5* (TAA nonsense mutation), and *leuC427* (missense mutation) (1).

It has been proposed that during periods of environmental stress, such as those occurring in the stationary phase of growth, a group of cells in a *B. subtilis* culture can be differentiated into a subpopulation with suppressed DNA repair systems in which adaptive mutations may be generated (1, 6). In agreement with this idea, it has been shown that the genetic inactivation of the mismatch (MMR) and guanine-oxidized (GO) systems potentiates the mutagenic events that occur in nongrowing *B. subtilis* cells (6, 7). Thus, it appears that the accumulation of mismatched and oxidized DNA bases in nongrowing *B. subtilis* cells is a key factor that promotes mutations under conditions of nutritional or metabolic stress (6, 7).

Reactive oxygen species (ROS) generated in cells either as byproducts of normal cellular metabolism or by exogenous agents have the potential to react with lipids, proteins, and DNA (8, 9). Accordingly, it has been shown that attack of DNA by ROS results in the formation of a myriad of oxidized bases, including uracil glycol and thymine glycol, 5-hydroxy-uracyl and 5-hydroxy-cytocine, and 8-oxo-adenine and 8-oxo-guanine (8-oxo-G), among others (10, 11). However, in addition to inducing the formation of oxidized bases, ROS may generate other types of genetic injuries, including formation of apurinic/apyrimidinic (AP) sites, damage to the deoxyribose sugar, and fragmentation of the DNA backbone, producing single-strand and/or double-strand DNA breaks (12). AP sites are among of the most frequently formed lesions in DNA, and they may arise spontaneously or following the catalytic action of specific DNA glycosylases that hydrolyze damaged bases from DNA: if these enzymes possess a lyase activity, a second catalytic event causes the rupture of the deoxyribose sugar, generating a single-strand break (13–15). AP sites and strand breaks are potentially mutagenic and toxic for cells: therefore, if left unrepaired, they affect replication, transcription, and cell survival (16, 17). The first step in the processing of AP sites is carried out by AP endonucleases, a group of enzymes which excise the DNA backbone at the 5′ end of the AP site. This cut generates 5′-phosphate deoxyribose and 3′-hydroxyl deoxyribose ends that are recognized by a DNA polymerase that is responsible for incorporating the appropriate nucleotide(s); finally, the DNA ligase seals the DNA patch (8). *B. subtilis* possesses Nfo and ExoA, two AP endonucleases that are frequently found in organisms of the three domains of life (17). In addition to processing AP sites, these proteins may process 3′-OH blocking lesions, including those that result from elimination of modified bases by glycosylases with

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Aag Hypoxanthine-DNA Glycosylase Is Synthesized in the Forespore Compartment and Involved in Counteracting the Genotoxic and Mutagenic Effects of Hypoxanthine and Alkylated Bases in DNA during *Bacillus subtilis* Sporulation

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ABSTRACT

Aag from *Bacillus subtilis* has been implicated in *in vitro* removal of hypoxanthine and alkylated bases from DNA. The regulation of expression of *aag* in *B. subtilis* and the resistance to genotoxic agents and mutagenic properties of an Aag-deficient strain were studied here. A strain with a transcriptional *aag-lacZ* fusion expressed low levels of β-galactosidase during growth and early sporulation but exhibited increased transcription during late stages of this developmental process. Notably, *aag-lacZ* expression was higher inside the forespore than in the mother cell compartment, and this expression was abolished in a *sigG*-deficient background, suggesting a forespore-specific mechanism of *aag* transcription. Two additional findings supported this suggestion: (i) expression of an *aag-yfp* fusion was observed in the forespore, and (ii) *in vivo* mapping of the *aag* transcription start site revealed the existence of upstream regulatory sequences possessing homology to σ^G-dependent promoters. In comparison with the wild-type strain, disruption of *aag* significantly reduced survival of sporulating *B. subtilis* cells following nitrous acid or methyl methanesulfonate treatments, and the Rif^r mutation frequency was significantly increased in an *aag* strain. These results suggest that Aag protects the genome of developing *B. subtilis* sporangia from the cytotoxic and genotoxic effects of base deamination and alkylation.

IMPORTANCE

In this study, evidence is presented revealing that *aag*, encoding a DNA glycosylase implicated in processing of hypoxanthine and alkylated DNA bases, exhibits a forespore-specific pattern of gene expression during *B. subtilis* sporulation. Consistent with this spatiotemporal mode of expression, Aag was found to protect the sporulating cells of this microorganism from the noxious and mutagenic effects of base deamination and alkylation.

The integrity of genomes of organisms is constantly compromised by intracellular and extracellular factors that have the potential to generate different base modifications, including, oxidations, alkylations, and deaminations (1). These types of nonbulky genetic insults are detected primarily by specific DNA glycosylases and eliminated through the base excision repair (BER) pathway (2). DNA deamination is a major type of spontaneous genetic damage with which cells must contend (3), and the spontaneous loss of the exocyclic amino groups in cytosine, guanine, and adenine yields the bases uracil, xanthine, and hypoxanthine (HX), respectively (4, 5). HX in DNA is potentially mutagenic, since it can pair not only with thymine but also with cytosine and therefore would result in AT-to-GC transitions after DNA replication (6). Organisms such as *Escherichia coli* and *Saccharomyces cerevisiae* employ the 3-methyladenine DNA glycosylases AlkA and MAG, respectively, to process HX and the modified bases 3-methyladenine, 7-methylguanine, and 7-methyladenine (7, 8). Other enzymes of mammalian origin, which are structurally unrelated to *E. coli* AlkA, include alkyl-adenine-DNA glycosylase (AAG), alkyl-*N*-purine-DNA glycosylase (ANPG), and *N*-methylpurine-DNA glycosylase (MPG) from human, mouse, and rat, respectively, and these also can excise alkylated and deaminated bases from DNA (7, 9–16). The physiological relevance of eliminating the base analog HX from DNA is evidenced by the mutator phenotype exhibited by bacteria and mammals deficient in these glycosylases (6, 17).

Deaminated bases can also be excised from DNA by endonuclease V (EndoV), an endonuclease that hydrolyzes the second phosphodiester bond located at the 3′ end of the modified base, and homologs of such enzymes have been described in bacteria, archaea, and eukaryotes (18–21).

A recent report revealed that *Bacillus subtilis* employs uracil DNA glycosylase (Ung) as well as YwqL, an EndoV homolog, to contend with the mutagenic effects of base deamination (22). However, HX can be processed by another repair protein, termed Aag; this alkyl adenine glycosylase is encoded in the genome of *B. subtilis* by *aag* (formerly *ydlJ*), and its product possesses functional

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Plant organellar DNA polymerases paralogs exhibit dissimilar nucleotide incorporation fidelity

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The coding sequences of plant mitochondrial and chloroplast genomes present a lower mutation rate than the coding sequences of animal mitochondria. However, plant mitochondrial genomes frequently rearrange and present high mutation rates in their noncoding sequences. DNA replication in plant organelles is carried out by two DNA polymerases (DNAP) paralogs. In *Arabidopsis thaliana* at least one DNAP paralog (AtPolIA or AtPolIB) is necessary for plant viability, suggesting that both genes are partially redundant. To understand how AtPolIs replicate genomes that present low and high mutation rates, we measured their nucleotide incorporation for all 16-base pair combinations *in vitro*. AtPolIA presents an error rate of 7.26×10^{-5}, whereas AtPolIB has an error rate of 5.45×10^{-4}. Thus, AtPolIA and AtPolIB are 3.5 and 26-times less accurate than human mitochondrial DNAP γ. The 8-fold difference in fidelity between both AtPolIs results from a higher catalytic efficiency in AtPolIA. Both AtPolIs extend from mismatches and the fidelity of AtPolIs ranks between high fidelity and lesion bypass DNAPs. The different nucleotide incorporation fidelity between AtPolIs predicts a prevalent role of AtPolIA in DNA replication and AtPolIB in DNA repair. We hypothesize that in plant organelles, DNA mismatches generated during DNA replication are repaired via recombination-mediated or DNA mismatch repair mechanisms that selectively target the coding region and that the mismatches generated by AtPolIs may result in the frequent expansion and rearrangements present in plant mitochondrial genomes.

Introduction

The mechanisms that mediate DNA replication in plant organelles are largely unknown. Several mechanisms like D-loop, theta-like, rolling circle, and recombination-dependent DNA replication are proposed to account for DNA replication in mitochondrial and chloroplast genomes [1–5]. Animal and plant mitochondrial genomes code for approximately the same number of essential genes. However, animal mitochondrial genomes are circular DNA molecules of approximately 15 kb and have few noncoding regions, whereas plant mitochondrial genomes are predominantly large linear DNA molecules (11 Mb in some angiosperms from the genus *Silene*), present a complex structure, are abundant in noncoding regions, and frequently rearrange [6]. Chloroplast genomes are also considered to be predominantly linear and harbor inverted repeats that may function as origins of replication [7,8].

Animal and plant mitochondria differ in their content of noncoding DNA. Mutation rates in the coding region of plant organellar genomes are 50–100-fold lower than in animal mitochondria [9,10].

Abbreviations
DNAP, DNA polymerases; f_{no}, misincorporation frequencies; MDA, multiple displacement amplification; MMR, DNA mismatch repair; POPs, plant/protist organellar DNAPs; TLS, translesion DNA synthesis.

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ORIGINAL RESEARCH

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Transcriptional coupling (Mfd) and DNA damage scanning (DisA) coordinate excision repair events for efficient *Bacillus subtilis* spore outgrowth

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Abstract

The absence of base excision repair (BER) proteins involved in processing ROS-promoted genetic insults activates a DNA damage scanning (DisA)-dependent checkpoint event in outgrowing *Bacillus subtilis* spores. Here, we report that genetic disabling of transcription-coupled repair (TCR) or nucleotide excision repair (NER) pathways severely affected outgrowth of Δ*disA* spores, and much more so than the effects of these mutations on log phase growth. This defect delayed the first division of spore's nucleoid suggesting that unrepaired lesions affected transcription and/or replication during outgrowth. Accordingly, return to life of spores deficient in DisA/Mfd or DisA/UvrA was severely affected by a ROS-inducer or a replication blocking agent, hydrogen peroxide and 4-nitroquinoline-oxide, respectively. Mutation frequencies to rifampin resistance (Rif^r) revealed that DisA allowed faithful NER-dependent DNA repair but activated error-prone repair in TCR-deficient outgrowing spores. Sequencing analysis of *rpoB* from spontaneous Rif^r colonies revealed that mutations resulting from base deamination predominated in outgrowing wild-type spores. Interestingly, a wide range of base substitutions promoted by oxidized DNA bases were detected in Δ*disA* and Δ*mfd* outgrown spores. Overall, our results suggest that Mfd and DisA coordinate excision repair events in spore outgrowth to eliminate DNA lesions that interfere with replication and transcription during this developmental period.

KEYWORDS
Bacillus subtilis, DisA, germination/outgrowth, NER, TCR

1 | INTRODUCTION

Bacillus subtilis spores are metabolically dormant as well as resistant to a number of DNA-damaging agents, including, heat, radiation, desiccation, extreme pH, and oxidizing agents (Setlow, 2007). This DNA resistance is due in large part to a group of DNA-binding, acid-soluble spore proteins (α/β-SASPs), synthesized during the last stages of sporulation (Setlow, 1988). After detecting appropriate conditions, spores can return to vegetative growth through a two-step process termed germination and then outgrowth (Setlow, 2003; Setlow, Wang, & Li, 2017). This process is triggered by specific germinants, generally amino acids or sugars that are specifically sensed by receptors in the spore's inner membrane (Paidhungat & Setlow, 2002; Setlow, 2003). This receptor-germinant interaction activates several events, including

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Identification of a unique insertion in plant organellar DNA polymerases responsible for 5′-dRP lyase and strand-displacement activities: Implications for Base Excision Repair

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ABSTRACT

Plant mitochondrial and chloroplast genomes encode essential proteins for oxidative phosphorylation and photosynthesis. For proper cellular function, plant organelles must ensure genome integrity. Although plant organelles repair damaged DNA using the multi-enzyme Base Excision Repair (BER) pathway, the details of this pathway in plant organelles are largely unknown. The initial enzymatic steps in BER produce a 5′-deoxyribose phosphate (5′-dRP) moiety that must be removed to allow DNA ligation and in plant organelles, the enzymes responsible for the removal of a 5′-dRP group are unknown. In metazoans, DNA polymerases (DNAPs) remove the 5′-dRP moiety using their intrinsic lyase and/or strand-displacement activities during short or long-patch BER sub-pathways, respectively. The plant model *Arabidopsis thaliana* encodes two family-A DNAPs paralogs, AtPolIA and AtPolIB, which are the sole DNAPs in plant organelles identified to date. Herein we demonstrate that both AtPolIs present 5′-dRP lyase activities. AtPolIB performs efficient strand-displacement on a BER-associated 1-nt gap DNA substrate, whereas AtPolIA exhibits only moderate strand-displacement activity. Both lyase and strand-displacement activities are dependent on an amino acid insertion that is exclusively present in plant organellar DNAPs. Within this insertion, we identified that residue AtPolIB-Lys593 acts as nucleophile for lyase activity. Our results demonstrate that AtPolIs are functionally equipped to play a role in short-patch BER and suggest a major role of AtPolIB in a predicted long-patch BER sub-pathway. We propose that the acquisition of insertion 1 in the polymerization domain of AtPolIs was a key component in their evolution as BER associated and replicative DNAPs.

1. Introduction

Plants are sessile organisms exposed to DNA damaging agents like Reactive Oxidative Species (ROS), alkylating moieties, heavy metals or UV-radiation. Those agents create DNA lesions that alter the coding potential of DNA or potentially block the replication fork [1,2]. Besides a nuclear genome, plants harbor mitochondrial and chloroplast genomes and the integrity of those genomes is critical for plant survival. An important pathway to maintain genome integrity is Base Excision Repair (BER). BER starts the repairing process with a DNA glycosylase that hydrolyzes a damaged base leaving an apurinic/apyrimidinic (AP) site. Afterwards, an AP endonuclease cleaves immediately 5′ to the AP site generating a 3′-hydroxyl (3′-OH) and a 5′-deoxyribosephosphate (5′-dRP) moiety. Bifunctional glycosylases harbor an associated lyase activity [3–5]. From this point forward, the BER pathway is divided into two sub-pathways. In the first sub-pathway, dubbed short-patch, the 5′-dRP is removed by a DNA polymerase (DNAP) with an associated lyase activity and only a single nucleotide is incorporated. In the second sub-pathway, dubbed long-patch, a DNAP synthesizes several nucleotides after the incision site using an associated strand-displacement activity and creating a single-stranded DNA flap that is cleaved by a flap-specific nuclease (reviewed in [1–4]). In animals, the short and long-patch sub-pathways of BER are functional in the nucleus and mitochondria. As mitochondrial DNA is prone to ROS, it is suggested that BER plays a predominant role in maintaining organellar DNA integrity [1,5,6]. Functional and biochemical studies indicate that land plants perform nuclear BER via the long and short-patch sub-pathways (for recent reviews [7–12]). In contrast to the wealth of information of BER in yeast

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Non-canonical processing of DNA photodimers with *Bacillus subtilis* UV-endonuclease YwjD, 5′→3′ exonuclease YpcP and low-fidelity DNA polymerases YqjH and YqjW

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ABSTRACT

It has been shown that mutation frequency decline (MFD) and nucleotide excision repair (NER) deficiencies promote UV-induced mutagenesis in *B. subtilis* sporangia. As replication is halted in sporulating *B. subtilis* cells, in this report, we investigated if this response may result from an error-prone repair event involving the UV-endonuclease YwjD and low fidelity (LF) DNA synthesis. Accordingly, disruption of YwjD generated *B. subtilis* sporangia that were more susceptible to UV-C radiation than sporangia of the WT strain and such susceptibility increased even more after the single or simultaneous inactivation of the LF DNA polymerases YqjH and YqjW. To further explore this concept, functional His_6-tagged YwjD and Y-DNA polymerases YqjH and YqjW were produced and purified to homogeneity. *In vitro* repair assays showed that YwjD hydrolyzed the phosphodiester bond immediately located 5′-end of a *ds*-DNA substrate bearing either, cyclobutane pyrimidine dimers (CPD), 6-4 photoproducts (6-4 PD) or Dewar isomers (DWI). Notably, the 6-4 PD and DWI but not the CPDs repair intermediaries of YwjD were efficiently processed by the LF polymerase YqjH suggesting that an additional 5′→3′ exonuclease event was necessary to process PD. Accordingly, the LF polymerase YqjW efficiently processed the incision-excision repair products derived from YwjD and exonuclease YpcP attack over CPD-containing DNA. In summary, our results unveiled a novel non-canonical repair pathway that employs YwjD to incise PD-containing DNA and low fidelity synthesis contributing thus to mutagenesis, survival and spore morphogenesis in *B. subtilis*.

1. Introduction

The synthesis of spores in *B. subtilis* is characterized by the establishment of a sporangium composed of two unequally cell sized compartments, the mother cell (larger compartment) and the forespore (smaller compartment) [1,2]. After segregating into each compartment, the two identical chromosomal copies are no longer replicated in further stages of the sporulation process. Instead, completion of this developmental program is largely dependent on a transcriptional program controlled by different sigma factors that upon binding the core RNA polymerase determine in a hierarchical manner the time and the space in which sporulation-specific genes are expressed [1,3,4].

It has been shown that treatment of *B. subtilis* sporangia with Mitomycin-C (M-C) and ultraviolet light (UV) affects sporulation, suggesting that DNA crosslinks and pyrimidine dimers (PDs) may interfere with the transcriptional program that drives sporulation in this microorganism [5]. In support of this notion, sporulating cells deficient for nucleotide excision repair (NER) are severely sensitized by these genotoxic agents [5]. In addition to NER, sporulating cells rely on additional repair factors to contend with the noxious effects of UV light including the UV-endonuclease YwjD and the translesion synthesis (TLS) DNA polymerases YqjH and YqjW [6]. In agreement with this report, the yeast fission *Schizosaccharomyces pombe* employs the NER system as well as an Alternative Excision Repair (AER) pathway dependent on a homolog of YwjD to repair DNA lesions induced by UV light [7]. A recent report [8] revealed that *ywjD*, *i*) is expressed during sporulation under the control of the forespore-specific RNA polymerase factor σ^G; *ii*) its function is necessary to counteract the DNA-damaging

Abbreviations: NER, nucleotide excision repair pathway; UV, ultraviolet light; LF, low fidelity; *ds*-DNA, double stranded-DNA; PD, pyrimidine dimers; CPD, cyclobutane pyrimidine dimer; 6-4 PD, 6-4 pyrimidine photoproduct; DWI, Dewar isomer; M-C, Mitomycin-C; TLS, translesion synthesis; TCR, transcriptional coupling repair pathway

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JÓVENES EN LA CIENCIA
REVISTA DE DIVULGACIÓN CIENTÍFICA

CARACTERIZACIÓN GENÉTICA Y FISIOLÓGICA DE MUTANTES DE *BACILLUS SUBTILIS* DEFICIENTES EN LOS SISTEMAS DE REPARACIÓN DE DNA UNG, YWQL Y MMR

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Resumen

Se ha reportado que el ácido nitroso (HNO_2) desamina las bases nitrogenadas citosina, adenina y guanina dando lugar a las bases análogas uracilo, hipoxantina y xantina respectivamente, las cuales son altamente mutagénicas y deben ser reparadas eficientemente. Tal reparación se da por diferentes vías; el sistema de reparación por escisión de bases (BER), la vía de reparación por escisión alternativa (AER) y el sistema de reparación de bases erróneamente apareadas (MMR). Aquí investigamos la posible interconexión entre YwqL y el sistema MMR así como la participación de Ung, usando como modelo de estudio a *Bacillus subtilis*. Células deficientes de los sistemas antes mencionados fueron tratadas con HNO_2 y calculada su supervivencia. Nuestros resultados sugieren que MMR es altamente eficiente reconociendo malos apareamientos promovidos por HNO_2 pues una mutante carente de *mutSL* fue dramáticamente afectada por HNO_2. Interesantemente, la interrupción de YwqL (una endonucleasa del sistema AER) en el fondo *mutSL* afectó positivamente la viabilidad celular al tratamiento con HNO_2. Estos resultados sugieren que la actividad fosfohidrolítica de YwqL dependiente de bases desaminadas sobre el DNA podría propiciar la entrada del sistema MMR para corregir las lesiones promovidas por la pérdida de grupos amino del material genético.

Abstract

It has been reported that nitrous acid (HNO_2) deaminates the nucleobases cytosine, adenine and guanine generating the analogous bases uracil, hypoxanthine and xanthine respectively, which are highly mutagenic and must be promptly repaired before replication. Such repair may operate through distinct repair mechanisms; including, the base excision repair system (BER), the alternative excision repair pathway (AER) and presumably through the mismatch repair system (MMR). Here we investigated the possible interconnection between YwqL (an endonuclease of AER) and MMR system (MutS-L) as well as the participation of the uracil DNA glycosylase (Ung), using *Bacillus subtilis* as study model. *B. subtilis* cells deficient for YwqL, Ung and/or MutSL were treated with HNO_2 and the fraction of survivors was calculated by viable counts. Overall, our results suggest that MMR is highly efficient recognizing mismatches promoted by HNO_2 because a mutant lacking *mutSL* was dramatically affected by HNO_2. Interestingly, disruption of *ywqL* (AER) in the *mutSL* background increased cell viability to HNO_2 treatment. These results suggest that base-deamidated-dependent phosphohydrolytic activity of YwqL could promote the entry of MMR system to process DNA lesions promoted by the loss of amino groups in the genetic material of *B. subtilis*.

Palabras Clave | *Bacillus subtilis*; Reparación de DNA; Desaminación; Ácido Nitroso.

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African Journal of Microbiology Research

Full Length Research Paper

Prevalence of *Helicobacter pylori cagA* and *vacA* genotypes in a population from Northeastern Mexico with chronic gastritis and intestinal metaplasia

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Helicobacter pylori **is associated with the development of a variety of gastroduodenal diseases. In this study, we evaluated the prevalence of *H. pylori cagA* and *vacA* genotypes from a Northeastern Mexico population. DNA was extracted from 135 gastric biopsies from patients with gastric disease: 110 with chronic gastritis (CG) and 25 with intestinal metaplasia (IM). Polymerase Chain Reaction (PCR) was used to detect *cagA* and *vacA* (*s1, s2, m1, m2*) genes of *H. pylori*. The study showed that the dominant genotypes were *cagA vacA s1/m1* 43 (31.8%), followed by *cagA vacA s2/m2* 18 (13.3%) and *cagA vacA s1/m2* 3 (2.2%). This study did not find any presence of the genotype *vacA s2/m1*. For the genotype *cagA vacA s1/m1*, a significant association was found between its presence in patients with IM compared with patient with CG ($P < 0.05$). In conclusion, the results show that the predominant genotype in the Northeastern Mexico population was *cagA vacA s1/m1*, which was found to be significantly associated with patients with IM.**

Key words: Gastric biopsies; *Helicobacter pylori*; *cagA*; *vacA*; Chronic Gastritis; Intestinal Metaplasia.

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International Journal of Medicine and Medical Sciences

Full Length Research Paper

Standardization of the method to obtain therapeutic-quality platelet-rich plasma

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Platelet-rich plasma (PRP) is a reliable source for obtaining cells to regenerate tissues, with ease of availability inorder to implement and standardize the ideal methodology in centrifugation strength and time for obtaining therapeutic-quality PRP, allowing its application to provide better and rapid recovery of muscular injuries, tendinitis, bone and ligament lesions. To evaluate PRP therapy, 150 patients with muscular lesions, tendinitis, shoulder, knee, ankle, hand and elbow injuries were treated. On application of PRP, we obtained 100% clinically significant symptomatic improvement in all 150 patients treated, who had musculoskeletal and ligament injuries, with a marked reduction of pain and inflammation. We concluded that the ideal concentration for obtaining PRP is at 1000 rpm with a time of 5 min; in addition, under these conditions the plasma lacks leukocytes and erythrocytes. The results were reproducible because the experiment was repeated at two institutions under the same conditions and similar results were obtained. The regeneration obtained in the affected patients is due to the fact that growth factors were released from the activated platelets; these initiate and modulate cicatrization in the tissues, which is a recent innovation to promote cicatrization, accelerating the power of tissue regeneration, with a platelet concentrate suspended in plasma.

Key words: Growth factors, platelet activation, application, tissue regeneration, therapeutic quality.

INTRODUCTION

Platelet-rich plasma (PRP) is a reliable source for obtaining cells to regenerate tissues, with ease of availability. In short term clinical practice, it is utilized to concentrate growth factor-rich plasma (GFRP) by up to 388% above values found in normal plasma, for later application in tissues, in a search to enhance the osteo-induction biological cascade. The pharmaceutical way in which PRP is utilized clinically is obtained by means of its gelling on adding thrombin and $CaCl_2$ to it. PRP gel is a compound of fibrinogen and activated platelets (by the addition of

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African Journal of Microbiology Research

Review

Immunity towards tuberculosis infection: A review

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Immune response represents the initial arm of host defense against Koch's bacillus. This review describes and discusses current knowledge of the host's immune response to *Mycobacterium tuberculosis* infection. To improve the diagnosis of tuberculosis, more rapid diagnostic techniques have been investigated in recent years, such as mediators, receptors and activators of immunity, gamma-interferon, tumor necrosis factor-alpha, reactive nitrogen intermediates, T cells, and natural killer. We consider it a first priority to implement programs of education for the development of a strategy to prevent tuberculosis. It is recommended to implement an immunotherapy treatment following chemotherapy to prevent reactivation of the bacillus due to the presence of latent bacilli in tissues.

Key words: Immune response, prevention, control, reactivation, nitric oxide, interferon, latent infection, granuloma, *Mycobacterium*.

INTRODUCTION

Tuberculosis (TB) has been and remains a major global health problem. TB is a pandemic and is amongst the top 10 killer infectious diseases, second only to human immunodeficiency virus (HIV) (Jain et al., 2012). It has extensively affected millions of people world-wide. It causes bad health among millions of people each year (WHO, 2013). TB is primarily a pulmonary infectious disease (Wang et al., 2013). It affects especially young adults and therefore has a high impact on the socio-economic status of a country (Zakham et al., 2012).

In Africa, the study of TB is complicated by the parallel epidemic of HIV because co-infection is common. This makes it necessary to consider HIV infection, especially in high HIV prevalent areas (Morris et al., 2011). HIV is a prerequisite condition for the acquisition of TB. The latest estimates included in this report are that there were 8.6 million new TB cases in 2012 and 1.3 million TB deaths worldwide. The number of TB deaths is unacceptably high given that most are preventable if people can access health care for diagnosis and right treatment is provided. Short-course regimens of first-line drugs (isoniazid, pyrazinamide, ethambutol, and rifampin) that can cure

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Bacterial Diversity in Two Aerated Lagoons of a Pulp and Paper Effluent and their Interaction with a Commercial Inoculum using PCR-DGGE

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Aerated lagoons are a main unit operation for wastewater treatment in the paper industry. Many such operations involve inoculation with bacterial formulations in which *in situ* effectiveness has not been proven; this can be translated into low efficiency in treatment and unnecessary investments. Lack of knowledge of bacterial biodiversity present in a lagoon limits the capacity to exploit the maximum degradation. To overcome such problems, various methods to identify and study these microorganisms have been developed. In this study, a PCR-DGGE analysis was performed to estimate the bacterial diversity and to verify the presence of bacteria present in a commercial inoculum in two aerated lagoons of a pulp and paper effluent. Phylogenetic affiliation of predominant member's correspondent to γ- and β-proteobacteria and Firmicutes were found. The dominant bacteria present in lagoon 2 belonged to the following genus *Microbacterium* sp. *Rhodocyclaceae* sp., *Eubacterium* sp. and *B. subtilis*. In lagoon 1 the dominant genus included *Microbacterium* sp., *Rhodocyclaceae* sp. *Tepidimonas* sp., *Acetanaerobacterium* sp., and *Flavobacteria* sp. The two characterized lagoons were not similar to the commercial inoculum. In addition, non-dominant bacteria (less relative intensity) were composed mostly of bacteria of the commercial inoculum.

Keywords: Pulp and paper effluent; Bacterial diversity; PCR-DGGE; 16S rDNA; Commercial inoculum

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INTRODUCTION

Wastewaters of pulp-and-paper mills can be potentially polluting and very dangerous, so they should be treated before being discharged (Ashrafi *et al.* 2015). The most common aerobic biological methods used in the treatment of pulp mill effluents are aerated lagoons (Bajpai 2012). Of the microorganisms involved in the depuration process, bacteria stand out; they are able to convert organic matter to carbon dioxide, water, and biomass, which can be removed by physical methods (Welander *et al.* 1997; Forster *et al.* 2003). The stability and permanence of bacteria in the system ensures an efficient process. However, lagoons are subjected to various perturbations such as variations in pH, high organic loads, presence of toxic compounds, and seasonal changes (Mueller *et al.* 1977). To counteract these effects, a biomass support material (Welander *et al.* 1997) or lagoons that are inoculated with commercial inoculum are introduced.

Review of Molecular Techniques for the Identification of Bacterial Communities in Biological Effluent Treatment Facilities at Pulp and Paper Mills

Ana M. Bailón-Salas,[a,δ] Hiram Medrano-Roldán,[a] Sergio Valle-Cervantes,[a] Luis A. Ordaz-Díaz,[b] Norma Urtiz-Estrada,[c] and Juan A. Rojas-Contreras [a,*]

One of the processes most used in biotechnology today for handling industrial liquid wastes is biological wastewater treatment. The efficiency and quality of its operation depends on the composition and activity of the microbial community that is present. The application of traditional and molecular techniques has provided a glimpse into the "black box" and has given information to improve the wastewater treatment process. However, bleach pulp and paper mill effluents require a better understanding of the active bacterial population. For the study of these microorganisms, molecular techniques have been used for more than 15 years. However, there has been a lack of knowledge of the physiological requirements and relations with the environment, which seems to be very difficult to obtain involving profile on the diversity. Nowadays, high-throughput sequencing technology is a promising method that makes it possible to identify the entire profile of microbial communities. In combination with fingerprint methods, this approach allows the identification and analysis of the whole biodiversity of microbial communities. In this review, several identification techniques will be discussed.

Keywords: Microbial characterization; Pulp and paper; Biological treatment; Molecular techniques; Bacterial communities

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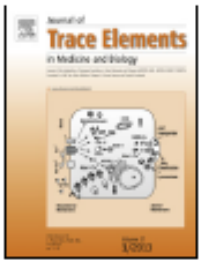

Title: Apoptosis in pancreatic β-cells is induced by arsenic and atorvastatin in Wistar rats with diabetes mellitus type 2

Authors: Tania Guadalupe Delgado-León, José Manuel Sálas-Pacheco, Fernando Vazquez-Alaniz, Ángel Antonio Vértiz-Hernández, Olga Dania López-Guzmán, Eduardo Lozano-Guzmán, Aurora Martínez-Romero, Norma Úrtiz-Estrada, Maribel Cervantes-Flores

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ORIGINAL ARTICLE

Discovery of *Entamoeba histolytica* hexokinase 1 inhibitors through homology modeling and virtual screening

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Abstract

Entamoeba histolytica, the parasite which causes amebiasis is responsible for 110 000 deaths a year. *Entamoeba histolytica* depends on glycolysis to obtain ATP for cellular work. According to metabolic flux studies, hexokinase exerts the highest flux control of this metabolic pathway; therefore, it is an excellent target in the search of new antiamebic drugs. To this end, a tridimensional model of *E. histolytica* hexokinase 1 (EhHK1) was constructed and validated by homology modeling. After virtual screening of 14 400 small molecules, the 100 with the best docking scores were selected, purchased and assessed in their inhibitory capacity. The results showed that three molecules (compounds **2921**, **11275** and **2755**) inhibited EhHK1 with an I_{50} of 48, 91 and 96 μM, respectively. Thus, we found the first inhibitors of EhHK1 that can be used in the search of new chemotherapeutic agents against amebiasis.

Keywords

Entamoeba histolytica, hexokinase 1, hit discovery, homology modeling, virtual screening

History

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Introduction

Amebiasis caused by parasite *Entamoeba histolytica*, affects more than 10% of the world's population, the untreated infection may lead to severe complications including hepatic amebiasis and intestinal tissue destruction. More than 50 million people worldwide are infected and up to 110 000 of these die every year[1]. Morphologically, *E. histolytica* exists as cyst and as trophozoite, which are the infective and the invasive forms of the disease, respectively[2]. In general, amebiasis can be classified as intestinal, that ranges from asymptomatic to fulminating colitis[3,4], it can also be extraintestinal, where the most common is amebic liver abscess[3,5]. Metronidazole and 5-nitroimidazoles are used as the first line drugs for the treatment of amebiasis[6]; paromomycin, chloroquine, diiodohydroxyquin, diloxanide furoate and emetine have also been used as alternative drugs[6,7]. However, these drugs have important side effects such as neutrocytopenia, dizziness, anorexia, disulfiram-like alcohol intolerance, diarrhea, cardiotoxicity, tenderness, vomiting, nausea, local pain and occasionally encephalopathy and convulsions[6,8,9]. Moreover, they are ineffective against luminal cysts[6]. Additionally, metronidazole has been described as a carcinogenic and mutagenic agent in rodents and bacteria; it is classified as a class B risk factor for pregnancy by the FDA[10–12]. Therefore, there is an urgent need of new drugs for the treatment of amebiasis.

Because *E. histolytica* trophozoites do not have mitochondria, the ameba is totally dependent on glycolysis for ATP supply[6]. Moreover, glycolysis regulation in ameba differs from that in humans. One of the principal differences is the pyrophosphate-dependent enzymes phosphofructokinase (PPi-PFK) and pyruvate phosphate dikinase (PPDK)[13]. Another difference is hexokinase (HK) which is not inhibited by its product glucose-6-phosphate (G6P) like some vertebrate HKs[14], instead, it is inhibited by physiological concentrations of AMP and ADP[13,15]. Furthermore, metabolic flux studies showed that HK controls 73% of the flux in *E. histolytica* glycolysis[16]. Therefore, EhHK is an excellent target in the search of specific inhibitors that can be developed into new drugs for the treatment of amebiasis. HK exists in two isoforms in *E. histolytica*, namely hexokinase 1 (EhHK1) and hexokinase 2 (EhHK2)[15,17]; these two enzymes have very similar molecular weights, 49.8 and 49.4 kDa for EhHK1 and EhHK2, respectively[17]; both enzymes are formed by 445 residues with 89% sequence identity. In this work, EhHK1 was cloned, overexpressed and purified. A tridimensional model of the enzyme was obtained and a virtual screening strategy was applied to discover the first set of EhHK1 inhibitors.

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Scientific Research and Essays

Full Length Research Paper

Regulation of cytokine gene expression during *Brucella abortus* infection

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Toll-like receptors (TLR) play a key role in antimicrobial host defense. Bacterial cell wall components and lipopolysaccharide (LPS) are recognized by macrophages via TLR, resulting in activation of professional antigen-presenting cells, initiation of acquired immune responses and further elimination of the invasive bacteria. TLR2 and TLR4 have been shown to recognize bacterial components. TLR2 is required for signaling by numerous ligands from gram-negative and gram-positive bacteria such as lipoteichoic acids, peptidoglycan and lipoproteins. In contrast, TLR4 fails to confer responsiveness to gram-positive bacteria and their components, but it is the main LPS signaling receptor. LPS is a major constituent of the outer membrane of gram-negative bacteria, such as *Brucella*, and is known to activate neutrophils, monocytes, macrophages, and other cell types to up-regulate expression of adhesion molecules and produce a number of pro- and anti-inflammatory cytokines. This study demonstrates that the attenuated strain *Brucella abortus* RB51 can stimulate cells through TLR4 and MyD88, resulting in NF-κB activation. The virulent strain *B. abortus* 2308 can also stimulate the cells by a MyD88-dependent pathway without involving either TLR4 or TLR2. It also induced NF-κB activation and nuclear translocation, suggesting that *B. abortus* RB51 induces activation of the proinflammatory response by a TLR4-dependent pathway with the subsequent NF-κB activation and nuclear translocation; nevertheless, the 2308 strain induced NF-κB nuclear translocation that was activated by an alternative pathway, different from that induced by TLR.

Key words: *Brucella abortus*, RB51, TLR, NF-κB, transduction signals, cytokines.

INTRODUCTION

Brucellosis is a major zoonotic disease that causes a serious health and economic problem worldwide. In spite of the growing number of countries declared Brucella-free, the disease remains one of the main zoonotic infections throughout many parts of the world with major economical and public health implications. About 500,000 new cases occur annually worldwide with predominance in the Middle East, Mediterranean countries, South

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