Non-essential MCM-related proteins mediate a response to DNA damage in the archaeon *Methanococcus maripaludis*

Alison D. Walters† and James P. J. Chong*

**Abstract**

The single minichromosome maintenance (MCM) protein found in most archaea has been widely studied as a simplified model for the MCM complex that forms the catalytic core of the eukaryotic replicative helicase. Organisms of the order Methanococcales are unusual in possessing multiple MCM homologues. The *Methanococcus maripaludis* S2 genome encodes four MCM homologues, McmA–McmD. DNA helicase assays reveal that the unwinding activity of the three MCM-like proteins is highly variable despite sequence similarities and suggests additional motifs that influence MCM function are yet to be identified. While the gene encoding McmA could not be deleted, strains harbouring individual deletions of genes encoding each of the other MCMs display phenotypes consistent with these proteins modulating DNA damage responses. *M. maripaludis* S2 is the first archaeon in which MCM proteins have been shown to influence the DNA damage response.

**INTRODUCTION**

The eukaryotic minichromosome maintenance (MCM) complex comprises six homologous proteins, MCM2–MCM7, all of which are required for DNA replication initiation and fork progression *in vivo*. MCM genes in eukaryotes have been demonstrated to be essential through the generation of temperature-sensitive and degron mutants [1]. The MCMs appear to act as a nucleation point for the formation of the Cdc45-MCM-GINS (CMG) multi-protein complex necessary for DNA unwinding in eukaryotes [2]. Within the CMG complex, MCMs provide the replicative helicase activity required by eukaryotes during chromosomal DNA replication [3]. Unwinding activity in this complex is likely to be tightly controlled, as evidenced by the number of post-translational modifications reported for the MCM proteins [4–6]. The intracellular concentration of MCMs also has an important influence on the ability of cells to cope with replicative stress. Reduction of MCM concentrations reduces the ability of cells to cope with replicative challenges [7–9]. MCMs are a target of the ATM/ATR DNA damage checkpoint [10, 11], which can be triggered by the Mre11–Rad50 complex binding to double-stranded DNA breaks [12, 13]. Additional evidence suggests that the MCMs, in particular MCM3 [14], may directly influence DNA replication checkpoints to ensure replicative integrity [15–19], although the precise role MCMs play in the modulation of DNA repair pathways is still unclear. Other eukaryotic MCM paralogues have been shown to have a role in the repair of meiotic DNA breaks in mice [20], mammalian DNA mismatch repair [21] and the facilitation of DNA repair at homologous recombination sites [22].

Archaeal MCM homologues have been used as simplified models for understanding the mechanisms employed by the MCM complex in DNA unwinding [23]. Biochemical analysis of archaeal MCMs has led to the identification of a number of motifs that are essential for DNA binding, ATP hydrolysis and DNA helicase activities [24–26]. In all archaea studied to date, with the exception of *Thermococcus kodakarenensis*, a single functional MCM has been identified that forms a homohexameric complex possessing these activities [27].

Members of the archaeal order Methanococcales possess between two and eight MCM homologues [28, 29]. *Methanococcus maripaludis* S2 encodes four MCM homologues [28, 30] corresponding to ORF numbers MMP0030, MMP0470, MMP0748 and MMP1024. We have named these genes *mcmA*, *B*, *C* and *D*, respectively [28]. Homologues of McmA and McmD are conserved in all Methanococcales species and appear to have arisen from an ancient duplication [28]. Phylogenetic analysis shows that the *M. maripaludis* MCMs are more closely related to one another than to MCMs from other archaea (Fig. 1a). While archaea with multiple MCMs have been identified outside...
the order Methanococcales, in most of these species there are truncations or mutations in residues that are essential for DNA helicase activity that result in the presence of only a single functional MCM protein [31, 32]. An exception to this general observation is in T. kodakarenensis, where the genome encodes three MCMs (MCM1–MCM3), all of which are expressed, but only one of which (MCM3) is essential [33]. Deletion of MCM1 or MCM2 in T. kodakarenensis did not affect cell growth or viability, indicating that they are non-essential for DNA replication [33]. As in T. kodakarenensis, multiple sequence alignments of the M. maripaludis proteins with other archaeal proteins show that the motifs known to be required for MCM function are all conserved in McmA and McmD (Fig. 1b). Thus, all four of the M. maripaludis MCMs could potentially function as DNA helicases. McmD possesses additional amino acids between the second pair of cysteines within the zinc finger (Fig. 1b) and a C-terminal 20 aa insert, reminiscent of an insert observed in eukaryotic MCM3 [28]. The four M. maripaludis MCMs co-purify when co-expressed in E. coli, indicating that they can form heteromeric complexes in vitro [28]. M. maripaludis represents an interesting model for studying MCM function not only because it has multiple MCM homologues but also, unusually for an archaeon, a well-established set of genetic tools are available for this organism [34] which allows both genetic and biochemical experiments to be used in the dissection of MCM function.

In this study we demonstrate that at least two of the four M. maripaludis MCMs (McmA and McmB) show robust DNA helicase activity in vitro. We have determined that only mcmA appears to be essential but that mutant strains deleted for non-essential MCMs show changes in cell-cycle distribution and their responses to DNA damage. We have demonstrated that multiple MCM proteins are required for normal proliferation in this organism and that deletion of non-essential MCMs has significant effects on DNA damage responses.

METHODS

Sequence alignments and phylogenetics

Multiple sequence alignments were generated using CLUSTALX [35] and were used to construct a neighbour-joining tree.

Recombinant protein expression and purification

His-tagged proteins were expressed in Rosetta BL21 (DE3; Novagen) at 37 °C or ArcticExpress (RIL; Stratagene) at 12 °C. Expression was induced at 0.8 OD600nm by 0.5 mM IPTG (final concentration). Cells were sonicated in lysis buffer (50 mM Tris pH 8.0, 300 mM NaCl, 5 % glycerol, 5 mM imidazole, 0.1 mM PMSF, 1 µg ml⁻¹ pepstatin, 1 µg ml⁻¹ leupeptin, 1 µg ml⁻¹ aprotonin) with 0.75 mg ml⁻¹ lysozyme and 5 µg ml⁻¹ DNase. Lysate was clarified by centrifugation and bound to 1 ml Talon beads (Clontech), washed with 10 cv of wash buffer (lysis buffer plus 10 mM imidazole) and protein was eluted in elution buffer (lysis buffer plus 150 mM imidazole). Fractions were pooled, diluted 1:3 in dilution buffer (10 mM Tris pH 8.0, 5 % glycerol, 1 mM EDTA, 1 mM EGTA, 1 mM PMSF, 0.1 % β-mercaptoethanol) and loaded on a 1 ml Source Q column (GE Healthcare), washed with 10 cv start buffer and eluted over a 20 cv gradient to 500 mM NaCl. Elution fractions were analysed by SDS-PAGE and concentrated into 10 mM Tris pH 7.5.

Strand displacement assays

Forked substrate DNA was prepared by γ-³²P labelling oligo HS2 (5'-TTTGTGTTTGTGGTTGTTGTTTGTGTTTG}

Fig. 1. Multiple potentially functional MCMs in M. maripaludis. (a) The M. maripaludis MCMs are more related to each other than to other archaeal MCMs. Phylogenetic tree of M. maripaludis MCMs (Mmp) compared to MCM sequences from Methanothermobacter thermotrophicus (Mth), Archaeoglobus fulgidus (Afu), Sulfobolus solfataricus (Sso), Aeropyrum pernix (Ape) and Korarchaeum cryptophillum (Kcr). (b) M. maripaludis MCMs appear to contain all the sequence motifs known to be required for helicase activity. Alignment of the sequences used in (a) in the same order showing conservation of motifs and essential residues that have been experimentally determined to be required for helicase activity. The helix-2 insert (h2-i) is not conserved at amino acid level, but is present in all sequences and shown as a box. Catalytically important amino acids are shown in bold, residues that deviate from typical motifs, but are known to support function, are shaded.
TTTGCCGACGTGCCAGCCAGCGCTCCGTTCC-3’) and annealing to HS1 (5’GGGACCGCTGCGCCCTGACGTCGGCCGTCGCGCCAGCGATGCCGTTTGTTTTGTGTTTGTGTTTGTGTTTT) as described previously [36]. A 10 µl reaction containing HDB [27], 2.5 mM ATP, 150 mM potassium glutamate and 1 mM labelled substrate was prepared on ice. Protein aliquots (10 µl; 0–2400 fmol hexamer) in 50 mM potassium glutamate, 10 mM HEPES pH 7.6 were prepared on ice. The reaction mix (10 µl) was added to each protein aliquot and incubated at 37 °C for 1 h. Substrate alone was boiled for 5 min then placed on ice. Reactions were stopped by the addition of 5 µl 200 mM EDTA, 1 % SDS, 20 % glycerol, 0.4 pmol µl⁻¹ unlabelled HS2 oligo, 1 µg µl⁻¹ proteinase K. DNA was separated on 12 % native polyacrylamide gels, dried and visualized using a phosphor imager (Bio-Rad). Results were quantified using Quantity One software (Bio-Rad).

**Markerless mutagenesis in *M. maripaludis***

Genetic manipulations were carried out using the Mm900 (S2 Δhpt) strain of *M. maripaludis* [37]. Deletion plasmids were constructed by cloning 500 bp of upstream and downstream flanking DNA into the NotI site of pCRPrtNeo, including codons for the five N-terminal and C-terminal amino acids of each MCM to ensure read-through (oligonucleotide sequences available on request) [37]. Transformations and markerless mutagenesis were carried out as described previously [37]. New strains were streak-purified, screened by PCR and analysed by Southern blot.

**Southern blots**

Southern blotting was carried out using DIG-labelling and the detection kit according to manufacturer’s instructions (Roche). Genomic DNA from individual strains was digested with the following restriction enzymes to generate appropriate fragments for probing: mcmA (*PstI*); mcMB (*SacI, PvuII*); mcMC (*PstI, SacI*); and mcMD (*NelI, Xhol*). Regions of interest were detected using digoxin random hexamer-labelled probes to 500 bp flanking regions of each MCM (Fig. S1, available in the online Supplementary Material). Blots were visualized by CSPD (disodium 3-(4-methoxyspiro [1,2-dioxetane-3,2’-(5’-chloro)tricyclo [3.3.1.1^6^] decan]-4-yl)phenyl phosphate) detection (Roche) and exposing to photographic film for 1–5 min.

**Culture and cell sampling of *M. maripaludis***

*M. maripaludis* was cultured in McCas liquid media as described previously [37]. For batch culture of *M. maripaludis*, 21 of modified McCas medium was prepared in a sealed 31 bioreactor (Applikon) as described previously [38]. The medium was inoculated using 5×5 ml cultures of *M. maripaludis* at an OD_{600nm} of 0.7–1.0. After inoculation, OD was measured at 600 nm every 2–5 h. Sodium dithionite was added to samples before OD_{600nm} was measured aerobically.

**Flow cytometry**

*M. maripaludis* culture (1 ml) was centrifuged (16 000 g, 5 min, room temperature). The pellet was resuspended in 100 µl of Tris/sucrose/EDTA buffer (10 mM Tris pH 7.5, 10 mM EDTA, 380 mM NaCl, 200 mM KCl). 1 ml ice-cold (77 % ethanol, 600 mM LiCl) was added; the sample was vortexed and then stored at 4 °C. Before analysis, fixed cells were pelleted (16 000 g, 5 min, room temperature), resuspended in 1 ml buffer A (10 mM Tris pH 7.5, 10 mM MgCl₂), spun and then resuspended in 150 µl buffer A containing 100 µg ml⁻¹ mithramycin A/20 µg ml⁻¹ ethidium bromide. Stained cells were analysed using an Apogee A40-MiniFCM with a 50 mW 405 nm laser. 100 000–500 000 cells were analysed for each sample. Data were processed using FlowJo (Treestar).

**DNA damage**

DNA damage assays were conducted under strict anaerobic conditions. For UV damage assays, 10⁸–10⁹ cells were diluted in McCas medium and spotted on McCas plates. Spots were air dried and then exposed to UV (254 nm). Post-treatment, plates were shielded from visible light. UV dosage was measured using a Blak-Ray UV meter (UVP). For ionizing radiation damage assays, aliquots of cultures were exposed to a calibrated x-ray dose from an x-ray generator. After exposure to x-rays, 10⁶–10⁷ cells were diluted in McCas medium and spotted on McCas plates. Plates were pressurized to 20 p.s.i with a 4:1 ratio of H₂:CO₂ and then incubated at 37 °C for 5 days.

**RESULTS**

**McmA and McmB display in vitro DNA helicase activity**

To investigate whether individual MCMs possessed DNA helicase activity, hexa-histidine-tagged recombinant McmA, McmB and McmC were purified using affinity and anion-exchange chromatography (Fig. 2a). McmD was largely insoluble when expressed recombinantly, even when protein folding was facilitated by the presence of *Oleispira antarctica* chaperones Cpn10 and Cpn60 at 12 °C. Size-exclusion chromatography of soluble Mcms A–C under different salt conditions supports the notion that these complexes might form a range of multimeric complexes in solution (Fig. S2). Walker A motif lysine to glutamate (K>E) mutants were expressed and purified in the same manner and used as negative controls in DNA helicase assays (Fig. 2b–d). The helicase activity of individual MCMs was tested using a strand-displacement assay with a forked substrate containing a 25 bp double-stranded region [36]. Both McmA and McmB showed protein-concentration-dependent helicase activity (Fig. 2b–c). The unwinding activity of McmB at the highest protein concentration (82 % of substrate) was slightly higher than that of McmA (77 % of double-stranded substrate). However, McmB displayed considerably higher DNA unwinding rates than McmA at lower protein concentrations (Fig. 2e). In contrast, we were unable to detect any significant DNA helicase activity in McmC over the same range of concentrations (Fig. 2d).

**McmA is essential**

In order to ascertain whether any of the *M. maripaludis* MCMs were essential, deletions of each of the four
individual MCMs were undertaken using a markerless mutagenesis strategy [37]. Genomic DNA was isolated from the resulting strains and analysed by Southern blotting to confirm whether a deletion mutant could be generated for each MCM gene. Deletion mutants were isolated for mcmB, mcmC and mcmD, demonstrating that these three genes are non-essential (Fig. 3b–d). We were unable to isolate an mcmA deletion strain despite screening more than 75 colonies from three independent transformations, consistent with the hypothesis that this gene is essential (Fig. 3a). This observation is supported by a recent genome-wide transposon mutagenesis study in M. maripaludis that classified McmA as ‘possibly essential’ [39].

Deletion of non-essential MCMs results in proliferation defects

We generated growth curves for each of the Δmcm strains from batch cultures grown in a 3 l anaerobic fermenter to compare to the WT (Mm900, Fig. 4a, b) [37]. In all cases, doubling times of the Δmcm strains were shorter than the WT, although specific growth rates and doubling times of ΔmcmB and ΔmcmD were very similar to those calculated for the WT (Table 1). ΔmcmC displayed an obvious decrease in the calculated doubling time compared to the WT of ~20% (Table 1). Lag phases for all Δmcm strains were longer than observed for the WT (Fig. 4a). Further experiments are required to understand this phenomenon.

DNA content and cell size for samples taken throughout the growth period were analysed by flow cytometry (Fig. 4c–e) and compared between WT and Δmcm cells at similar OD across the entire growth range. The cell-cycle distribution of M. maripaludis is similar to that observed for Methanocaldococcus jannaschii [40]. M. maripaludis cells show a broad distribution of DNA content and cell size, with no distinct genome peaks visible during exponential growth, in contrast to the distinct genome peaks observed for Archaeoglobus fulgidus, Methanothermobacter thermautotrophicus and Sulfolobus solfataricus [32, 40, 41]. This observation supports the previous observation [42], that M. maripaludis cells are highly polyploid under normal growth conditions, as is the case for exponentially growing bacteria [43] and halophilic archaea [44].

Although some consistent minor differences between WT and ΔmcmB or ΔmcmC cells were observed, overall these deletions appeared to have no significant effects on cell size or DNA content compared to the WT (Fig. 4c, d). ΔmcmD cells were larger than the WT in all growth phases. ΔmcmD cells also possessed a greater DNA content than the WT in early and mid-log growth (Fig. 4e). ΔmcmD cells with a very low DNA content increased dramatically in late log/stationary phase to become the dominant population. This phenotype could be indicative of DNA breakage, perhaps caused by incomplete DNA replication, aberrant DNA segregation, defective cell division or an inability to effectively repair DNA damage accumulated during growth.

MCMs mediate a DNA damage response

To determine whether the ΔmcmD cell-cycle distribution differences we observed were due to a defect in the ability of these cells to respond to DNA damage, we subjected WT and mutant strains to increasing doses of UV radiation. Consistent with previous reports [45], we found M. maripaludis S2 cells to be highly sensitive to UV damage (Fig. 5a). This sensitivity was dramatically increased in ΔmcmD but slightly reduced in both ΔmcmB and ΔmcmC.
which were more resistant to low doses of UV damage than the WT. These phenotypes were confirmed by exposing the same strains to ionizing radiation, where \( \Delta \text{mcmD} \) also showed hypersensitivity this type of damage (Fig. 5b).

**DISCUSSION**

We have produced recombinant proteins for three highly similar McmA-type MCMs from *M. maripaludis* S2. McmA and McmB displayed DNA helicase activity but McmC did not. Interestingly, although measurements by size-exclusion chromatography shows complexes of different sizes under different conditions for McmA and McmB, they were still able to unwind DNA. This situation is similar to that described for the eukaryotic MCMs where a complex of MCMs 4, 6 and 7 is sufficient for *in vitro* helicase activity (probably as a dimer of trimers), but the active complex *in vivo* is additionally modulated by the presence of other MCM subunits [46]. *M. maripaludis* encodes multiple RecJ homologues, several of which have been shown to be non-essential, and a single GINS protein, which is probably essential [39]. We have previously reported the recovery of a complex containing all four recombinant *M. maripaludis* Mcm proteins, supporting the notion that a heteromeric complex may be formed *in vivo* [28]. It is also possible that more than one Mcm complex is formed *in vivo*, providing different functions. The absence of helicase activity in McmC and the faster unwinding rate of McmB suggest that additional amino acids to those already identified in the Mcm proteins are critical for modulating helicase activity in complexes formed by individual proteins. A detailed analysis of the McmC sequence compared to McmA/McmB could provide important insights into the modulation of
MCM helicase activity and the molecular mechanisms governing this activity in eukaryotes.

Our results demonstrate that *M. maripaludis* possesses multiple functional MCMs, one of which is essential, with the other three causing defects in cell proliferation and the response to DNA damage when deleted. *mcmA* could not be deleted and displays robust helicase activity *in vitro*. McmB had more vigorous DNA helicase activity than McmA *in vitro* and, when deleted, increased resistance to DNA damage. Δ*mcmD* displayed a faster growth rate than the WT and increased resistance to DNA damage. In contrast, Δ*mcmD* showed a striking increase in DNA damage sensitivity. A previous shotgun proteomics study detected peptides for McmA, McmB and McmD *in vivo* [47]. These data support our findings that McmB and McmD have functional roles *in vivo*. While peptides for McmC were not detected, this does not definitively prove that such peptides were not present.

We have been unable to obtain sufficient soluble McmD to conduct helicase assays, so whether McmD is an active helicase remains unknown. Our previous genome context analysis revealed an upstream ORF of unknown function that is likely to be operonic with *mcmD* in *M. maripaludis* S2 [28]. Interestingly, this ORF is highly conserved throughout the *Methanococcales* (Figs S3 and S4), but not found in any other species. The positioning of this ORF contiguous with

---

**Table 1.** Growth rates of Mm900 (WT) and Δmcm strains calculated from Fig. 4(b)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specific growth rate (µ)</th>
<th>Doubling time (hours)</th>
<th>T₂=ln2/µ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mm900 (WT)</td>
<td>0.0029</td>
<td>3.98</td>
<td></td>
</tr>
<tr>
<td>ΔmcmB</td>
<td>0.0032</td>
<td>3.61</td>
<td></td>
</tr>
<tr>
<td>ΔmcmC</td>
<td>0.0036</td>
<td>3.20</td>
<td></td>
</tr>
<tr>
<td>ΔmcmD</td>
<td>0.0030</td>
<td>3.85</td>
<td></td>
</tr>
</tbody>
</table>

---

**Fig. 4.** MCM deletions result in proliferation defects. (a) Time-course measurements of OD₆₀₀ as an indication of cell number. WT (Mm900, closed circles) or *M. maripaludis* strains harbouring deletions in *mcmB* (open circles), *mcmC* (closed squares) or *mcmD* (open squares) were grown in a 2 l batch culture and sampled as indicated. (b) Exponential growth data from (a) replotted as ln(OD₆₀₀) for the calculation of doubling times (see Table 1). Symbols as for (a), regressions shown as grey dotted lines. (c–e) Flow cytometry indicates that deletion of non-essential MCMs in *M. maripaludis* results in a proliferation phenotype. (c) Δ*mcmB*, (d) Δ*mcmC*, (e) Δ*mcmD*. In each panel the profile for WT cells at a similar OD₆₀₀ is shown in grey, the MCM-deleted strain profile is shown as a black line. Discontinuities at the mid-point in each curve are due to automatic switching between different photomultipliers for detection of small signals in the Apogee flow cytometer used to make these measurements. Within each group of panels, the left column panels show light scatter as an indication of cell size; the right column panels show fluorescence as an indication of DNA content. Event number is normalized. Data are plotted on a logarithmic scale. Numbers indicate the OD₆₀₀ of the deletion strain (top) compared to the WT (bottom).
mcmD is conserved among the mesophilic Methanococcales. It is possible that co-expression of this smaller ORF with McmD would produce soluble protein to allow biochemical analysis.

We have previously noted that McmD possesses a modified zinc finger and C-terminal 20 aa insert and similar features are found in eukaryotic MCM3 [28]. MCM3 has been implicated in the regulation of the eukaryotic MCM complex [4], and has been shown to be specifically phosphorylated by ATM/ATR kinases [10]. An apparent requirement for the specific proteolysis of eukaryotic MCM3 before apoptosis can be induced has also been reported [48, 49]. Thus the notion of a specialized Mmc as a nexus for a modulatory or checkpoint decision is not without precedent. The response of ΔmcmD to UV and ionizing radiation supports the notion that either ΔmcmD or ΔmcmC is important in modulating a response to DNA damage or that McmD is important in controlling the polyploidy observed in M. maripaludis, which in turn could influence the cell’s ability to repair damage through homologous recombination pathways, as reported for Deinococcus. The altered cell size and DNA content of ΔmcmD measured using flow cytometry, supports the hypothesis that McmD may have a role in proliferation control.

ΔmcmB or ΔmcmC strains are more resistant to DNA damage than the WT. This response is reminiscent of the phenotype observed in polyploid Haloferax volcanii when the DNA repair genes mre11 and rad50 are deleted [50]. It has been suggested the Mre11–Rad50 complex delays the repair of damage by homologous recombination to allow DNA repair to occur more rapidly using microhomology mediated end-joining, avoiding the complications inherent in using homologous recombinational repair in a polyploid organism. H. volcanii mre11 rad50 mutants therefore undergo homologous repair more readily than the WT, enhancing cell survival but reducing the recovery rate from DNA damage [50]. ΔmcmB or ΔmcmC strains may bypass the preferred DNA damage response to similarly undergo homologous recombination to repair DNA damage. Whether the DNA repair processes that take place under these circumstances are error-prone or error-free and whether the long-term fitness of ΔmcmB or ΔmcmC strains is reduced remains to be determined.

The responses to deletion of MCM genes in M. maripaludis have allowed us to clearly describe the first example of an archaeal organism where MCMs play a role in the response to DNA damage. This observation indicates that, as in eukaryotes, the multiple MCMs in M. maripaludis have evolved to perform specialized functions. Interestingly, protein interaction studies in T. kodakarensis show that non-essential MCM1 and MCM2 co-purify with proteins with known roles in DNA repair [51]; although a role for these MCMs in DNA repair has not be established. Our data demonstrating that multiple functional MCMs are present in M. maripaludis indicate that this organism provides a useful biochemical and genetic system that could provide further insight into eukaryotic MCM function.

Fig. 5. Δmcm strains show DNA damage phenotypes. (a) WT M. maripaludis (Mm900, closed circles), ΔmcmB (diamonds), ΔmcmC (triangles) or ΔmcmD (open circles) strains were plated at different dilutions before being irradiated with UV light (254 nm) as indicated. Surviving cells were calculated by enumerating colonies formed. The mean and standard errors for three independent experiments are shown. (b) The same strains, indicated by the same symbols as (a), were subjected to ionizing radiation (x-rays) as indicated. ΔmcmD was substantially more sensitive to DNA damage than the WT or the ΔmcmB and ΔmcmC strains, which were more resistant to damage. The mean and standard errors for three independent experiments are shown.

Funding information
The Worldwide Universities Network provided travel grant funds to A. D. W. This work was supported in part by a Biotechnology and Biological Sciences Research Council PhD studentship. J. P. J. C. is a Royal Society Industry Fellow.

Acknowledgements
Thanks to John Leigh and Tom Lie for providing strains, plasmids and expertise in M. maripaludis genetics, and Jo Milner for the loan of the UV dosimeter.

Conflicts of interest
The authors declare that there are no conflicts of interest.

References


**Five reasons to publish your next article with a Microbiology Society journal**

1. The Microbiology Society is a not-for-profit organization.
2. We offer fast and rigorous peer review – average time to first decision is 4–6 weeks.
3. Our journals have a global readership with subscriptions held in research institutions around the world.
4. 80% of our authors rate our submission process as ‘excellent’ or ‘very good’.
5. Your article will be published on an interactive journal platform with advanced metrics.

Find out more and submit your article at [microbiologyresearch.org](http://microbiologyresearch.org).