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Functional Analysis of the Conserved Domains of DEMETER Demethylase

DNA methylation plays critical roles in maintaining genome stability, genomic imprinting, transposon silencing, and development. DNA methylation pattern is maintained by DNA methylation and demethylation processes. Genomic imprinting is established in the central cell by DEMETER (DME) mediated active DNA demethylation before fertilization, and is required for seed viability in Arabidopsis. DEMETER is a glycosylase that works via base excision repair pathway to recognize and replace 5-methylcytosines with unmethylated cytosines. DME encodes a large protein with multiple conserved domains. However, except for the well-characterized glycosylase domain, the functions of these domains have not been elucidated. By studying the function of these conserved domains, we can shed some light on the targeting mechanism of DME. This study will extend our knowledge and possibly enable epigenetic manipulation in the future. Using genetic engineering, we investigated the in vivo function of the different isoforms and truncated versions of DME by complementation assay. In addition, generation of whole genome DNA methylation profile allows us to examine the DME target sites on a single nucleotide base resolution. Here, we show the three conserved domains (termed AGB, for the A, Glycosylase, and B domains) in the C-terminal half of DME are sufficient for DME in planta activity. We found that DME AGB is more efficient in complementing dme-2 seed abortion phenotype than the full length DME. In addition, when CG differentially methylated regions (DMRs) between wild type DME and AGB were compared, the AGB has wider targets and causes deeper demethylation, suggesting a regulatory role of the NTD on DME activity in vivo. Bioinformatics analysis of DME-like proteins uncovered a permutated CXXC motif and a RRM motif, both implicated in epigenetic targeting, within the domain B. Our result suggests that DME AGB contains sufficient information for targeting whereas NTD regulates and fine-tunes its enzymatic activity.

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Assessment of Survival and Virulence of Salmonella in low moisture foods

Low moisture foods (LMF) have been implicated in multiple outbreaks of salmonellosis. However, mechanisms mediating survival and virulence of Salmonella in such foods remain poorly understood. The objective of this study was to assess the survival and virulence of Salmonella in LMF. A two-strain mixture of Salmonella enterica serotypes Typhimurium and Enteritidis was used to inoculate two model LMF, chocolate and in-shell pistachios. Products (100g) were inoculated with 4ml of the Salmonella cell suspension, dried for 1-3h until Aw approximated that of the uninoculated product, aliquoted into 15-ml centrifuge tubes and stored in the dark at 22oC. Salmonella populations immediately after inoculation, after drying and at 1, 4, 6, 10, 15 and 21d were determined in triplicate on non-selective (TSA-YE) and selective (XLD) media. Virulence was tested in the insect Galleria mellonella model by injecting 10 µl of rinsate from products at 1d into the last left proleg of 10 larvae. Rinsate from uninoculated products were also injected as controls. The larvae were incubated at 37oC and larval mortality was daily monitored. The population of Salmonella in the inoculated products was 8 and 9 logCFU/g immediately after inoculation and 7.3 and 8 logCFU/g after drying for chocolate and pistachios, respectively. Populations decreased to 4.5 and 7.5 logCFU/g by 21d in chocolate and pistachios, respectively. Recovery of Salmonella from either product on TSAYE and XLD was similar. Larvae inoculated with 3.4 logCFU/ml of the cocktail had a mortality of 100% after 24h compared to 80% of larvae inoculated with 3.9 logCFU/ml cells from chocolate and 30% for larvae inoculated with 3.7 logCFU/ml cells from pistachios. The mortality of controls was 0 and 10% for uninoculated pistachios and chocolate, respectively. Findings show that Salmonella can survive in chocolate liquor and pistachios and that cells adapted to the LMF environment retained virulence.