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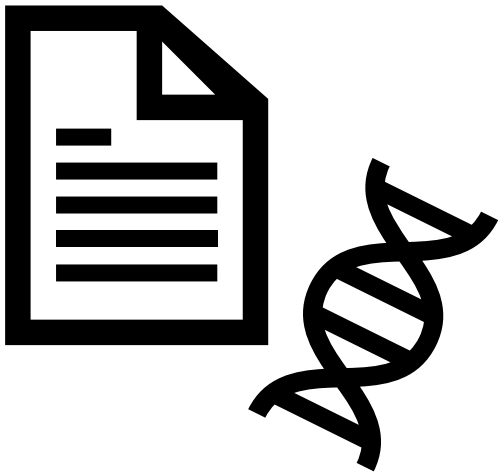
Scientific paper summary



اعداد الطالبات: امجاد المسفر، فاطمة العنزي

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Genetic Determinants of Stress Resistance in Desiccated *Salmonella enterica*

Introduction: (Fatimah Alanazi)

1. *Salmonella enterica* is a significant pathogen that causes foodborne illnesses in both animals and humans. Its ability to survive in dry and harsh environments poses a major food safety concern, particularly in low-moisture foods such as nuts, spices, and dry pet food.
2. Contamination of dry foods by *Salmonella* can result in outbreaks of foodborne illnesses, and current methods for decontamination, such as heat or pressure, are not always effective in these environments. The bacteria's ability to survive desiccation and dry heat makes it a persistent problem.
3. While mechanisms of wet-heat resistance are well-studied, there is limited understanding of how *Salmonella* develops resistance to dry heat. Additionally, it remains unclear how the tolerance to desiccation contributes to dry-heat resistance.
4. This study investigates the genetic determinants of *Salmonella*'s dry-heat resistance using a comparative genomics approach. By identifying the differences in genome sequences between resistant and sensitive strains, the study aims to uncover the genetic factors that contribute to stress resistance in desiccated *Salmonella*.

Materials and Methods: (Fatimah Alanazi)

1. The study utilized a comprehensive approach to investigate the genetic determinants of *Salmonella enterica*'s resistance to dry heat, focusing on a comparative genomic analysis of resistant and sensitive strains.
2. A total of 108 *Salmonella* strains, representing 39 serotypes, were collected from wastewater and genetic stock centers. These strains were screened for their resistance to dry heat, and the 22 most resistant and 8 most sensitive strains were selected for further analysis.
3. The genomes of these selected strains were compared to identify genetic differences. A total of 289 genes were identified as differentially distributed between resistant and sensitive strains. Of these, 28 genes with a potential role in stress resistance were further studied.

4. The expression of these 28 genes was analyzed under different conditions, specifically before and after desiccation, as well as in solid-state cultures on agar plates. This analysis helped identify 15 genes that were significantly upregulated after desiccation or in solid-state cultures.
5. Six of these 15 genes were cloned into two dry-heat-sensitive strains of *Salmonella* using a low-copy-number vector. These strains were then tested for increased resistance to dry heat and high pressure to confirm the role of these genes in stress resistance.
6. The specific conditions for desiccation (24 hours in a vacuum desiccator) and dry-heat treatments (95°C for 1 hour) were established to ensure consistent testing of bacterial survival.

RESULTS AND DISCUSSION (Amjaad ALmisfer)

Variation of the dry-heat resistance of strains of Salmonella.

The study explores the dry-heat resistance of **108 *Salmonella* strains** from **wastewater** and the **Salmonella Genetic Stock Centre** (SGSC), covering 39 serotypes. Key findings include:

1. **Extreme Resistance**: Wastewater isolates showed significant resistance to dry heat.
2. **Genomic Comparison**: Eight resistant wastewater strains were selected for genome sequencing and compared with strains from the stock center.
3. **Resistance Variation**: Resistance varied considerably among strains.
4. **Extended Testing**: Resistance was further tested at 80°C under different water activity levels, showing a broad range of bacterial survival.
5. **Correlation with Other Stressors**: A link was found between resistance to dry heat and high pressure, suggesting shared resistance mechanisms.

Phylogenetic distribution of resistant strains.

- The study investigates the **phylogenetic distribution of *Salmonella* strains resistance to dry heat** and finds **no correlation between resistance and phylogenetic position**. This indicates that resistance traits are likely associated with the **accessory genome** rather than the core genome.

- To explore this, researchers selected the 22 most resistant and 8 most sensitive strains for **comparative genomic analysis**, aiming to identify specific genes in the accessory genome responsible for conferring dry-heat resistance.

Identification of genes that potentially contribute to dry-heat resistance.

- The study identified **353 genes** that were differentially **distributed between the 22 most dry-heat-resistant and 8 most sensitive *Salmonella* strains**, using **Scoary analysis**.

- Of these, **289 genes were present in all resistant strains** but absent from at least 4 of the sensitive ones.

- **22 proteins (genes) related to stress resistance** (oxidative, heat, acid, starvation, and virulence) were selected. These proteins are likely key **contributors** to the observed dry-heat resistance.

Quantification of gene expression.

The study focused on validating the role of **22 genes linked to dry-heat resistance in *Salmonella***.

The findings revealed:

1. Expression Settings: **Gene expression was compared between cells on solid agar and in liquid broth.**
2. Overexpression **Results**:
    - **7 genes** were overexpressed in **both** conditions.
    - **2 genes** were overexpressed only in **solid**-state cultures on agar.
    - **6 genes** were overexpressed only **after desiccation**.
3. Stress and Virulence Genes:
    - Overexpressed genes after desiccation were linked to osmotic and oxidative stress, and virulence.
    - Specific stress-related genes included *ndhI*, *sopD*, *ahpD*, *cysJ*, and *cysM*.
    - Virulence-related genes included *pduF* and *sipA*.

Overall, **15 genes** were differentially expressed due to either growth condition or desiccation, **highlighting their potential importance in *Salmonella*'s survival under stress**.

Genes confer resistance to dry heat or high pressure.

In a study to determine the role of certain genes in conferring resistance to dry heat or high pressure in *Salmonella*, 15 genes were **cloned** into two sensitive *S. Arizona* strains.

The study found:

1. **Increased Resistance**: The introduction of the genes *cysM*, *phoC*, *pspE*, *sopD*, and *uspC* significantly increased resistance to both dry heat and high pressure in the strains.
2. **Specific Protection**: The gene *ahpD* specifically enhanced resistance to **high pressure** only.
3. **Consistent Effects**: The effects of gene overexpression were consistent across both strains tested.

- **These results suggest that these genes play a crucial role in stress resistance mechanisms within *Salmonella*, with potential implications for understanding bacterial survival under adverse conditions and designing strategies to control pathogenic bacteria.**

The findings highlight the complex nature of bacterial resistance and the potential of genetic studies to help manage bacterial threats effectively.

- The study focused on **identifying genes responsible for desiccation and dry heat resistance** in *Salmonella*, **revealing that resistance is not primarily driven by the core genome**, which is common to both *Salmonella* and *E. coli*.

- Instead, **comparative genomics** highlighted **353 genes differentially distributed between resistant and sensitive *Salmonella* strains**, emphasizing the role of the accessory genome.

The study on *Salmonella* identified **several genes linked to dry-heat resistance**, which are involved in managing **different stress responses** such as acid, osmotic, protein homeostasis, and particularly oxidative stress.

Key genes include:

1. **cysM**: Helps synthesize **cysteine** and **utilize thiosulfate**, **reducing oxidative stress** in the periplasm.
2. **pspE**: Acts as a periplasmic **sulfur transferase**, potentially **aiding the cellular response to heat shock.**
3. **PhoC**: Involved in **phosphate uptake**, possibly **supporting membrane synthesis** after stress.
4. **SopD**: Contributes to **membrane fission** during invasion and aids in **desiccation resistance.**
5. **UspC**: Effective against both **dry heat and high pressure**.
6. **AhpD**: Provides **defense against oxidative stress under high pressure**.

- The research also underscores the role of **horizontal gene transfer** in the accessory genomes of Enterobacteriaceae, including *Salmonella*, which is crucial for lifestyle adaptations, enhancing virulence, and conferring stress resistance.

- In contrast to *E. coli*, which can inhabit a variety of environments, *Salmonella* is characterized as an **obligatory pathogen** to humans and animals, reflecting its specialized evolutionary adaptations.

In conclusion

- This study **highlights the significance of accessory genes** that **enhance** oxidative stress tolerance in *Salmonella*, which in turn **increases the bacteria's resistance** to dry heat and high pressure.

- The **overexpression** of **virulence-related genes** post-desiccation may **lower** the infectious dose of *Salmonella* when consumed through dry foods.

- Overall, **understanding** these desiccation tolerance **mechanisms provides insights** into how *Salmonella* manages to survive multiple stressors throughout the food supply chain, offering **potential strategies for better controlling this pathogen in food safety applications**.

THE END.

Reference:

Wang, Z., Zhu, T., Chen, Z., Meng, J., Simpson, D. J., & Gänzle, M. G. (2021). Genetic Determinants of Stress Resistance in Desiccated Salmonella enterica. ***Applied and Environmental Microbiology*****,** ***87*****(23).** **https://doi.org/10.1128/aem.01683-21**