

Short Research Paper

Draft genome sequences of *Salmonella* Oslo isolated from seafood and its laboratory generated auxotrophic mutant

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Abstract

In recent years, the concept of bacteria-mediated cancer therapy has gained significant attention as an alternative to conventional therapy. The focus has been on non-typhoidal *Salmonella* (NTS), particularly *S. Typhimurium*, for its anti-cancer properties, however, other NTS serovars such as *Salmonella* Oslo, which are associated with foodborne illnesses could potentially be effective anti-cancer agents. Here, we report the draft genome sequence of *Salmonella* Oslo isolated from seafood and its laboratory generated auxotrophic mutant.

Key words: Non typhoidal *Salmonella*, *Salmonella* Oslo, Bacteria mediated cancer therapy, whole genome sequence

Introduction

Non-typhoidal *Salmonella* (NTS) is one of the pathogens that frequently cause foodborne infections throughout the world. The pathogenic potential of NTS strains have been well understood [1]. However, its therapeutic potential as an anti-cancer agent remain unexplored. Among more than 2500 NTS serovars, *S. Typhimurium* VNP20009 [2-4], an auxotrophic mutant strain of *S. Typhimurium* A1-R [5, 6], has been successfully studied and was even tested in phase I clinical trial for the treatment of solid tumors [7]. Many features of *Salmonella* namely the ability to thrive in hypoxic environment of the tumor, to induce innate immune response against tumor or its bactofection, and ability to release anticancer genes within tumor were found to be favorable for tumor regression in animal models [8-10]. However, despite the success observed in animal models, the clinical trials didn't yield expected results. This prompted the

researchers to look for alternative NTS strains for their anticancer properties. In this study, the aim was to sequence the genomes of *Salmonella* Oslo isolated from seafood and its laboratory generated auxotrophic mutant. The availability of genetic information provides a basis for further studies, particularly for investigating their role as anti-cancer agents.

Salmonella Oslo was isolated from seafood (squid sample) as per the protocol recommended by the FDA Bacteriological Analytical Manual [11] with minor modifications. Briefly, the seafood sample was pre-enriched in lactose broth, followed by enrichment in selenite cysteine broth and tetrathionate broth. Post enrichment, the sample was streaked on Hektoen enteric agar (HiMedia Laboratory Pvt Ltd, India). During the enrichment and plating steps, the incubation temperature was maintained at 37° C for

16 to 18 h. Colonies with specific morphological features were selected and were subjected to a series of biochemical tests such as indole test, methyl red test, Voges-Proskauer test, citrate test, triple sugar iron agar (TSIA) test, urease and lysine iron agar (LIA) test for conventional identification. The biochemically positive colonies were further confirmed by PCR using genus-specific primer *invA* [12]. Serotyping was done at National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, India.

Lambda red recombinase method [13] was used to generate the auxotrophic mutant of *Salmonella* Oslo by inducing deletions in *argH* and *leuB* genes, which code for arginine and leucine respectively. Biofilm assay was performed using the method described by Stepanovic et al (2004) [14].

To compare the growth kinetics of *Salmonella* Oslo (SO1-wild type) and its mutant (LAT9), a 100 μ l aliquot of overnight culture of SO1 and LAT9 was added to 5 ml Luria Bertani broth (HiMedia Laboratory Pvt Ltd, India) and incubated at 37°C with shaking at 200 rpm. The optical density was measured at 600 nm (OD₆₀₀) at different time points such as 0, 1, 2 to 24 h after incubation and expressed as log (OD₆₀₀ X 1000).

To sequence the genome of SO1 and its laboratory generated mutant, bacterial genomic DNA was extracted using a QIAamp DNA mini kit (Qiagen, Germany). The quality of the extracted DNA was checked by Qubit® and further verified by a bioanalyzer (Agilent technologies). The genomic DNA library was prepared using a Nextera XT DNA library preparation kit (Illumina, Inc, Cambridge, UK). The whole genome sequencing was performed at UCD, Dublin. The raw sequence data were generated using the Illumina MiSeq platform with a depth of 100x. The obtained paired end reads were merged and

the genome was assembled using CLC genomics (version 11) [15]. The processed reads were aligned to the reference genome LT2 strain (LT571437) with Bowtie2 program. The annotation and gene prediction of draft genome was done using the Rapid Annotations Subsystems Technology (RAST) (<http://rast.nmpdr.org/>) [16].

The identification of the isolate as true *Salmonella enterica* serovar Oslo was confirmed by serotyping experiments. As expected, the auxotrophic strain (LAT9) was found to be phenotypically mutant to amino acid arginine and leucine. However, when PCR was performed to confirm the changes in the target genes (*argH* and *leuB*), it revealed no deletions in *argH* and *leuB* genes. This strange observation prompted us to determine the whole genome sequence of wild (SO1) and mutant (LAT9) strain. The generated library produced a total of 1193762 and 1221694 reads for wild type and mutant respectively. The paired end reads of SO1 were assembled into 121 contigs with coverage of 100x. The genome size was calculated at 4,860,262 bp comprising of 4,974 protein coding genes. The GC content of this strain was found to be 52.2%. The analysis obtained from the RAST also revealed 401 subsystems (Fig. 1). The annotated genome had 383 amino acid biosynthesis genes including *argH* and *leuB*. In addition, 78 tRNAs, 11 ncRNAs, 168 pseudo genes were also identified.

Similarly, the paired end reads of LAT9 were assembled into 199 contigs with coverage of 100x. The genome size was calculated at 4,890,414 bp comprising of 5,082 protein coding genes. The GC content of this strain was found to be 52.2%. The analysis obtained from the RAST also revealed 402 subsystems (Fig. 2). The annotated genome had 392 amino acid biosynthesis genes. 79 tRNAs, 11 ncRNAs, 239 pseudo genes were also identified.

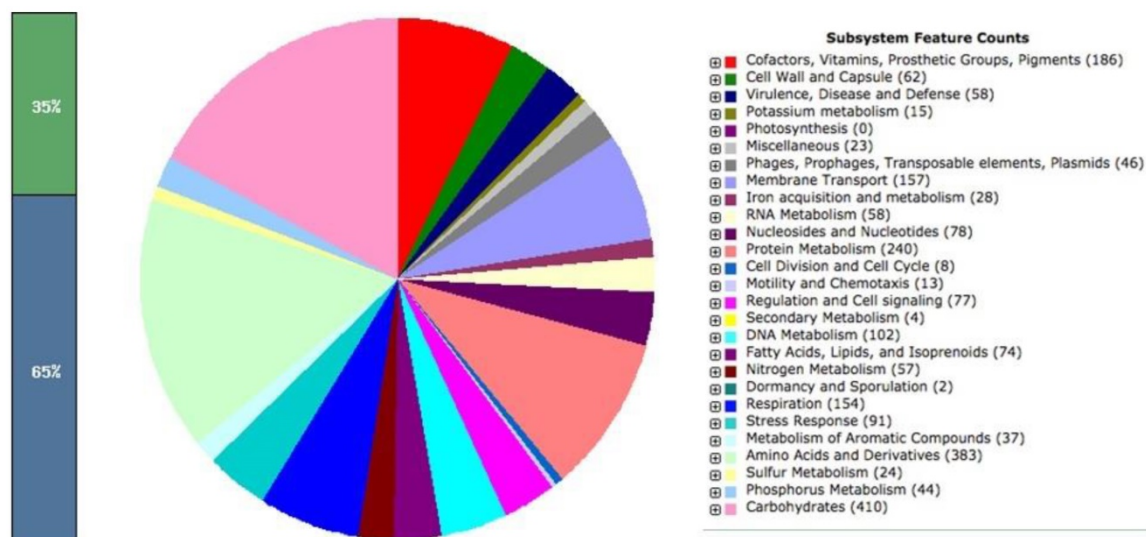


Figure 1. *Salmonella* Oslo (SO1)-WT subsystem feature.

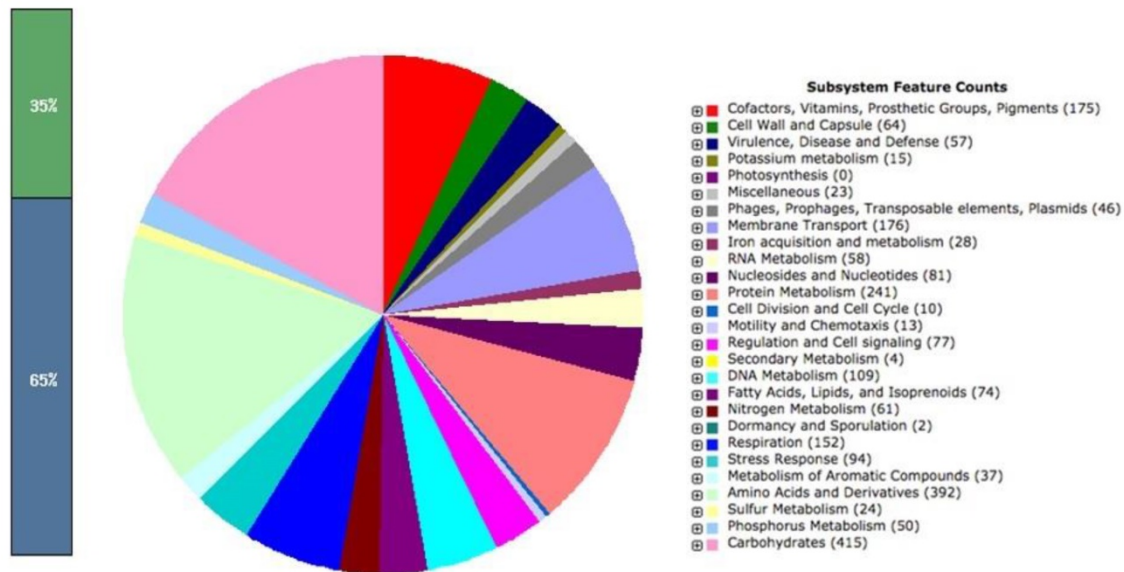


Figure 2. *Salmonella* Oslo (LAT9) subsystem features.

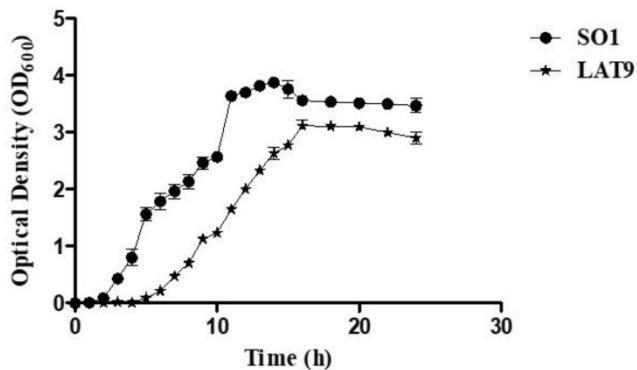


Figure 3. Growth kinetics of SO1 and LAT9.

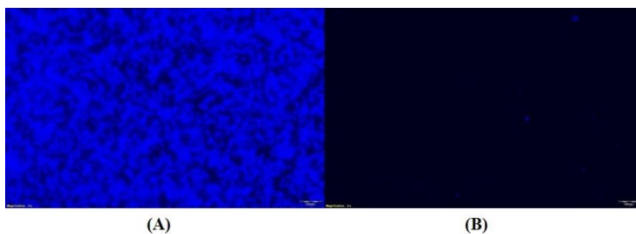


Figure 4. Calcofluor stained images of SO1 (A) and LAT9 (B) strains under fluorescent microscope.

Further, the growth kinetic analysis revealed that the growth of LAT9 was significantly (p -value of >0.001) slower than SO1 (Fig 3). The biofilm forming ability of LAT9 was also significantly reduced when compared with SO1 (Fig 4).

To the best of our knowledge, this is the first draft genome sequence of *Salmonella* Oslo isolated from seafood and its auxotrophic mutant LAT9. Determination of anti-cancer activity of this laboratory generated auxotrophic mutant using cell line and animal models would provide a suitable

alternative to *S. Typhimurium* VNP20009 as candidate strains for bacteria-mediated anticancer therapy.

The whole genome shotgun projects have been submitted to GenBank and the assigned accession numbers are as follows: NZ_SJXK000000000 for SO1 and NZ_SMLR000000000 for LAT9. The version described in the paper represents the first version.

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Competing Interests

The authors have declared that no competing interest exists.

References

- Deekshit VK, Kumar BK, Rai P, Karunasagar I, Karunasagar I. Differential expression of virulence genes and role of *gyrA* mutations in quinolone resistant and susceptible strains of *Salmonella* W eltevreden and Newport isolated from seafood. *J Appl Microbiol.* 2015;119(4):970-80. doi: 10.1111/jam.12924.
- Chen G, Tang B, Yang BY, Chen JX, Zhou JH, Li JH, Hua ZC. Tumor-targeting *Salmonella typhimurium*, a natural tool for activation of prodrug 6MePdR and their combination therapy in murine melanoma model. *Appl Microbiol Biotechnol.* 2013;97(10):4393-401. doi: 10.1007/s00253-012-4321-8.

3. Broadway KM, Suh S, Behkam B, Scharf BE. Optimizing the restored chemotactic behavior of anticancer agent *Salmonella enterica* serovar Typhimurium VNP20009. *J Biotechnol.* 2017; 251:76-83. doi: 10.1016/j.jbiotec.2017.04.006.
4. Coutermarsh-Ott SL, Broadway KM, Scharf BE, Allen IC. Effect of *Salmonella enterica* serovar Typhimurium VNP20009 and VNP20009 with restored chemotaxis on 4T1 mouse mammary carcinoma progression. *Oncotarget.* 2017;8(20):33601. doi: 10.18632/oncotarget.16830.
5. Yano S, Zhang Y, Zhao M, Hiroshima Y, Miwa S, Uehara F, Kishimoto H, Tazawa H, Bouvet M, Fujiwara T, Hoffman RM. Tumor-targeting *Salmonella typhimurium* A1-R decoys quiescent cancer cells to cycle as visualized by FUCII imaging and become sensitive to chemotherapy. *Cell Cycle.* 2014;13(24):3958-63. doi: 10.4161/15384101.2014.964115.
6. Igarashi K, Kawaguchi K, Kiyuna T, Miyake K, Miyake M, Li S, Han Q, Tan Y, Zhao M, Li Y, Nelson SD. Tumor-targeting *Salmonella typhimurium* A1-R combined with recombinant methioninase and cisplatin eradicates an osteosarcoma cisplatin-resistant lung metastasis in a patient-derived orthotopic xenograft (PDOX) mouse model: decoy, trap and kill chemotherapy moves toward the clinic. *Cell Cycle.* 2018;17(6):801-9. doi: 10.1080/15384101.2018.1431596.
7. Fu W, Lan H, Li S, Han X, Gao T, Ren D. Synergistic antitumor efficacy of suicide/ePNP gene and 6-methylpurine 2'-deoxyriboside via *Salmonella* against murine tumors. *Cancer Gene Therapy.* 2008;15(7):474. doi: 10.1038/cgt.2008.19.
8. Jazeela K, Chakraborty A, Karunasagar I, Vijaya Kumar D. Non-Typhoidal *Salmonella*: A Potential Anti-Cancer Agent. *J Appl Microbiol.* 2019. <https://doi.org/10.1111/jam.14297>
9. Pangilinan CR, Lee CH. *Salmonella*-Based Targeted Cancer Therapy: Updates on A Promising and Innovative Tumor Immunotherapeutic Strategy. *Biomedicines.* 2019;7(2):36. doi: 10.3390/biomedicines7020036.
10. Zhou S, Gravekamp C, Bermudes D, Liu K. Tumour-targeting bacteria engineered to fight cancer. *Nat Rev Cancer.* 2018;1. doi: 10.1038/s41568-018-0070-z.
11. Andrews WH, Hammack T. *Salmonella*. In *Bacteriological Analytical Manual US Food and Drug Administration*. Available from: <http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/default.htm>. 2011.
12. Deekshit VK, Kumar BK, Rai P, Srikumar S, Karunasagar I, Karunasagar I. Detection of class 1 integrons in *Salmonella* Weltevreden and silent antibiotic resistance genes in some seafood-associated nontyphoidal isolates of *Salmonella* in south-west coast of India. *J Appl Microbiol.* 2012;112(6):1113-22. doi: 10.1111/j.1365-2672.2012.05290.x.
13. Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences.* 2000;97(12):6640-5. DOI: 10.1073/pnas.120163297
14. Stepanović S, Ćirković I, Ranin L, S vabić-Vlahović M. Biofilm formation by *Salmonella* spp. and *Listeria monocytogenes* on plastic surface. *Lett Appl Microbiol* 2004; 38(5):428-32. DOI: 10.1111/j.1472-765X.2004.01513.x
15. Founoua LL, Founoua RC, Allamd M, Ismail A, Essack SY. Draft genome sequence of a methicillin-resistant *Staphylococcus epidermidis* isolate from swine. *J Glob Antimicrob Resist* 2018;15: 250-251. DOI: 10.1016/j.jgar.2018.10.016
16. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T *et al.* The RAST Server: rapid annotations using subsystems technology. *BMC genomics.* 2008; 9(1):75. <https://doi.org/10.1186/1471-2164-9-75>.