Construction and investigation of plasmid-induced effects on growth of GFP-expressing *Salmonella* strains

Ubaidah Naim Taraq Naem Zia[1], Deepah Samynathan[1], Hasni Idayu Saidi[1] Gayathri Thevi Selvarajah[2] and Cheah Yoke Kqueen[1,*]

[1]*Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*
[2]*Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia*

ABSTRACT

Background: Bacteria such as the *Salmonella* species had been extensively studied not only for its mechanism of pathogenicity, but for its beneficial utilisation in bacterial-mediated tumour therapy. Genetically modified bacterial strain, BDLA *Salmonella* Agona (BDLA *S.* Agona) had shown great promise as tumour targeting and suppressing agent. By, constructing green fluorescent protein (GFP) expressing of this strain, it would be beneficial as it could be used to further study the interaction of the BDLA *S.* Agona strain with various cancerous cells and the host at the systemic level. However, GFP plasmid-induced strains may exhibit growth defects, and this was investigated in this study. **Methods:** The GFP-transformed *Salmonella* strains were constructed, and the replication rate of the strains were investigated using the BacterioScan™ 216R instrument. The replication rate between the strains was compared from the 24-hour data collected and analysed as growth curves. The 24-hour growth curves were constructed using the Log CFU/mL and OD_{650} data collected by the instrument. **Results:** It was observed from the growth curves that the transformation of the GFP plasmid into different *Salmonella* strains did not affect the replication rate of the bacteria. **Discussion and Conclusion:** This finding answers the objective of the study, and it was concluded that incorporation of the GFP plasmid does not cause any negative effect on bacterial growth. GFP-transformed *Salmonella*, specifically the BDLA *S.* Agona strain, could be utilised as a powerful tool for future studies on the mechanism of tumour suppression and real-time *in vivo* biodistribution of the strain.

Keywords: *Salmonella* Agona*; green fluorescent protein; BacterioScan™; growth curve; bacterial therapy*

*** Correspondence**
Cheah Yoke Kqueen
Department of Biomedical Science,
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor Darul Ehsan, Malaysia.
ykcheah@upm.edu.my
Tel: +603-8947 2343

Received: 6 November 2020
Revised: 12 March 2021
Accepted: 2 April 2021
Published: 6 April 2021

doi
https://doi.org/10.28916/lsmb.5.1.2021.73

INTRODUCTION

The second leading cause of death globally, with approximately 9.6 million deaths, or one in six deaths reported was associated with cancer (World Health Organization Agency for Research on Cancer (IARC), 2018). Cancer burdens the individuals, families, communities and health systems by bringing extreme physical, emotional and financial strains (Perry et al., 2019). Conventional anti-cancer therapies available as treatments are such as radiotherapy, chemotherapy and surgery (Farkona et al., 2016). However, these treatments still lacking the capability to eradicate cancer completely and could impose severe side effects on the patient (Kwon & Min, 2013; Liang et al., 2019). These limitations urge the need for the invention of innovative novel anti-cancer treatments that are more specific to eradicate the various subpopulations of cancer cells that exist in tumours while reducing adverse effects on the patients.

The emergence of bacterial mediated tumour therapy (BMTT) dated to the early 20th century when American physician William B. Coley developed the Coley's toxin, which is a bacterial mixture as tumour therapy (Forbes et al., 2018). Dr William Coley hypothesised that bacteria and

bacterial products could have tumour suppressing effects, and he proved this by the excellent results obtained with almost 1,000 treated cases and 150 papers published related to the topic (McCarthy, 2006). The leaky nature of tumour blood vessels due to incomplete endothelial linings allows bacteria in the blood to enter tumours (Baban et al., 2010; Yong et al., 2004). Once bacteria infiltrates and replicates in the tumours, the tumours are starved of nutrients resulting in death of the tumour cells (Wall et al., 2010). In addition to this, the tumour cells also burst following intracellular replication of bacteria or alternatively by induction of apoptosis or autophagy (Forbes et al., 2018; Lee et al., 2014; Uchugonova et al., 2015).

Despite this, his findings were highly controversial and critiqued by many of his colleagues and to make it worse, FDA re-categorised Coley's Toxin as an investigational drug due to lack of safety and efficacy data (Carlson et al., 2020). The last use of this toxin was recorded in the 1980s in China, where it was used as primary therapy for terminal liver cancer patients who received 68 injections of Coley's toxin in 34 weeks, and by the end of the treatment, the large tumours in both lobes were successfully eradicated (Jessy, 2011). A retrospective study which compared the 10-year survival rates of patients by utilising data from the Surveillance Epidemiology End Result cancer registry found that patients receiving modern conventional therapies did not fare better than patients who received the Coley's toxin over 100 years ago (Cann et al., 2003; Murakami et al.,2015).

Even though the success of Coley's Toxin had been overshadowed by the emergence of conventional therapies such as surgery, radiotherapy and chemotherapy, the field of BMTT had once again come into the limelight of cancer research. Significant improvements in molecular biology and microbiology areas of research provide huge possibilities in progress in the field of BMTT. Bacteria species, for example, *Bifidobacterium*, *Salmonella* and *Clostridium* spp. had been studied for its potential as tumour therapy agent and even for imaging purposes (Zheng & Min, 2016). An ideal tumour therapy agent should be inexpensive, target and replicates in the tumour specifically, and have the capacity for therapeutic delivery (Morrissey et al., 2010). *Salmonella*, being an obligate anaerobe, have the advantage to colonise both big tumours with hypoxic centres and small tumours with a non-hypoxic condition (Leschner & Weiss, 2010). *Salmonella* Typhimurium (*S.* Typhimurium) is the most studied strain among the *Salmonella* species, and the VNP20009 strain is the only *Salmonella* strain to be evaluated in phase I clinical study as a treatment of nonresponsive metastatic melanoma or renal cell carcinoma (Wang et al., 2016).

We have previously demonstrated that *Salmonella* Agona (*S.* Agona) has a great potential to be utilised as tumour therapeutic agent as it was shown to exert lesser adverse effect systemically while still having similar tumour inhibition capabilities as the well-studied *S.* Typhimurium (Zia et al., in press). Following this, the *S.* Agona was genetically modified to develop the quadruple knockout ΔsopBΔsopDΔleuBΔargD *S.* Agona (BDLA *S.* Agona) strain. The BDLA *S.* Agona strain had been shown to have better efficacy to suppress tumours and reduced virulence as compared to other wild-type and auxotrophic *S.* Agona strains (Gwee et al., 2019). It is essential to understand the mechanism of action involved to suppress tumours at every level, from the molecular mechanism to biodistribution *in vivo* levels of the BDLA *S.* Agona.

Green fluorescent protein (GFP) labelling is a great invention which enables the transformation of various cells that could be used to visually observe cells for various applications, such as bacteria-cell interactions, migration and biodistribution studies (Phillips, 2001; Schuster et al., 2014). The GFP was isolated from the Pacific Northwest jellyfish, Aequorea Victoria which emits visible green fluorescent light when exposed to a light source, specifically at an excitation wavelength of 396nm and an emission wavelength of 508 nm (Cormack et al., 1996).

GFP is widely used as a fusion reporter gene as a cytological marker to study gene expression or to monitor protein localisation in cells *in vitro*. It also can be used as a marker for tracking bacterial cells, especially to investigate its survival and biodistribution *in vivo*. By utilising long wavelength of UV light, epifluorescence microscopy, laser confocal microscopy or flow cytometry, GFP could be detected easily in all growth phase of bacteria (Ma et al., 2011).

Introduction of the plasmid into bacterial cells are often found to burden the cells, particularly resulting in the reduction of its growth rate (Mi et al., 2016). A study by Hong et al. (2014) observed that introduction and replication of plasmid, particularly those with antibiotic resistance gene burdens the host by lowering ATP concentration and downregulating expressions of genes involved in cellular growth, which results in reduced growth rate. The study concluded that the introduction of plasmid not only causes plasmid replication burden but also affects the ecological fitness of the host in the environment by altering the host chromosomal gene expression and phenotype (Hong et al., 2014).

In this study, the genetically modified BDLA *S.* Agona, unmodified *S.* Agona and *S.* Typhimurium were transformed with GFP plasmid (pGFP) to be used as a tool to study tumour suppressive mechanism of action and real-time biodistribution study of the strains. The objective of this study was to investigate the effect of transformation of the strains with pGFP on the bacterial growth rate.

MATERIALS AND METHODS

Bacterial strains, growth and storage conditions

S. Typhimurium, American Type Culture Collection (ATCC) 14028™ was purchased from ATCC, USA, while *S.* Agona was previously recovered from food source, specifically from indigenous vegetables (Khoo et al., 2009). The vegetables were placed in sterile plastic bag containing 500 mL of sterile buffered peptone water and shaken for 2 minutes, before the samples were suspended to allow the diluent to be collected (Awang et al., 2003).

The BDLA *S.* Agona strain was then constructed from this *S.* Agona strain by knocking out the ΔleuBΔargD metabolic gene from previously double knockout strain ΔsopBΔsopD *S.* Agona, producing the quadruple knockout ΔsopBΔsopDΔleuBΔargD BDLA *S.* Agona strain using Targetron Gene knockout system (Sigma-Aldrich, Germany). The potential L1.LtrB insertion sites were identified using computer algorithm at Sigma-Aldrich Targetron design website and primers were designed to retarget the RNA portion of the intron by primers-mediated mutations according to the manufacturer's protocol with slight modification (Gwee et al., 2019; Khoo et al., 2015).

For overnight culture, the bacteria were cultured in nutrient broth (NB) (Merck KGaA, Darmstadt, Germany) and incubated overnight at 37 °C and 200 rpm. In order to obtain a single colony, the overnight culture was streaked on nutrient agar (NA) (Merck KGaA, Darmstadt, Germany) plate using the four-quadrant method and incubated at 37 °C overnight. The bacterial stock was stored in 20% glycerol-NB at -80 °C for long term storage.

GFP gene transfection

The *Salmonella* strains were grown on the shaking incubator (200 rpm and 37° C) until the mid-logarithmic phase was reached in NB media and harvested at 4° C (Momiyama et al., 2012). The cultures (2.0 × 10^8) were resuspended in 40μL 10% glycerol (Zhao et al., 2005). A volume of 2μL of pGFP (Clontech) vector was added and placed on ice for 5 minutes before electroporation with a Gene Pulser (Bio-rad, Hercules, CA) was carried out at 1.8kV with the pulse controller set at 1,000-Ω parallel resistance (Zhao et al., 2006).

BacterioScan™ analysis

Approximately 2mL of 10^6 *Salmonella* strains were added to the cuvettes, and 24-hour growth analysis was carried out using BacterioScan™ 216R instrument (BacterioScan Inc., St Louis, MO, United States) (Idelevich et al., 2017). The 24-hour bacterial growth patterns between different *Salmonella* strains and the influence of pGFP plasmid transformation was explored using BacterioScan™ real-time analysis, which utilises forward laser scattering technology. This

technology determines the number and size of suspended bacterial cells in a solution by analysing the angular variation in the intensity of the scattered light as a laser beam passes through a liquid sample containing bacteria (Bugrysheva et al., 2016; Syal et al., 2017). Growth curves from the data obtained (Log CFU/mL and OD_{650}) were constructed and compared between the different strain, both transformed and untransformed.

RESULTS

The GFP-expressing strains were observed using a fluorescent microscope to confirm that the transformation was a success. Figure 1 represents the observation of the GFP-expressing BDLA *S.* Agona strain under 400× magnification. As observed in Figure 1, GFP-expressing BDLA *S.* Agona appears to be fluorescent green.

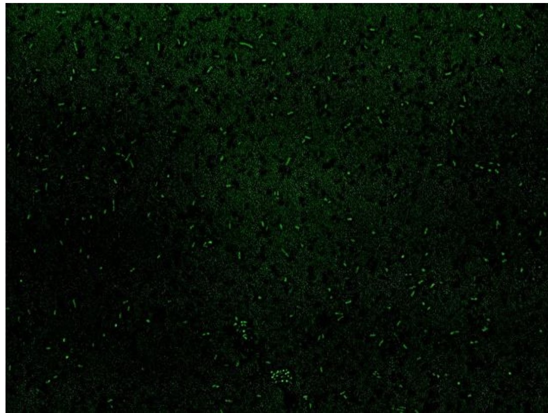

Figure 1: GFP-expressing BDLA *S.* Agona viewed under 400× magnification with a fluorescent microscope.

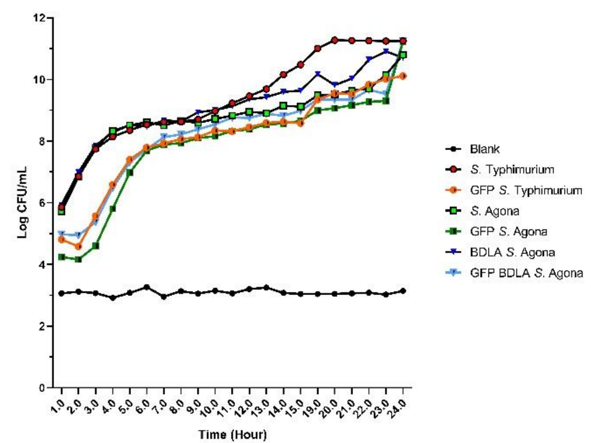

Figure 2: Growth curve (Log CFU/mL) of *Salmonella* strains.

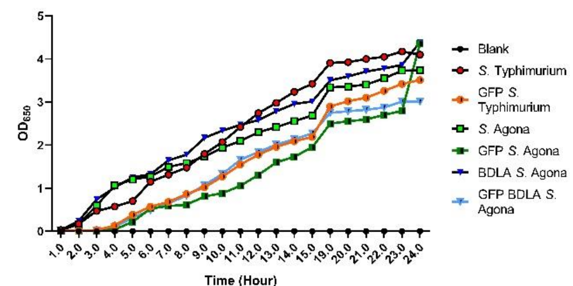

Figure 3: Growth curve (OD_{650}) of *Salmonella* strains.

As this is a preliminary study, the small number of replications for each treatment set means the statistical analysis could not be carried out. The differences between growth rates were investigated by observing the growth rate curve generated by BacterioScan™ for the duration of 24 hours. The data obtained are visualised as Figure 2 and Figure 3. Figure 2 represents Log CFU/mL over time, while Figure 3 represents the OD_{650} for the period of 24 hours.

The growth curves generated for the three parental *Salmonella* strains showed that the three strains have an overall of similar patterns for bacterial replications. A slight difference was observed after the 10-hour mark, where it was observed that *S.* Typhimurium showed a higher replication rate, followed by BDLA *S.* Agona and *S.* Agona. It was also observed from Figure 2 and Figure 3, that the transformed *Salmonella* strain showed reduced bacterial growth compared to the parental strains. After approximately 6 hours, the growth curve of the transformed strains was improved and reached similar capacity as the parental strains, which is observed in Figure 2.

DISCUSSIONS

A study by Rang et al., (2003) demonstrated that incorporation of GFP gene could affect the physiology of the bacterial strain, particularly the bacterial growth rate as it interferes with the cell division of certain enteric bacteria (Rang et al., 2003). Observations on the bacterial growth pattern help determine the plasmid burden effect of the introduction of the pGFP plasmid on the physiology of the bacteria (Ma et al., 2011). Introduction and replication of plasmid burdens the host by lowering ATP concentration and downregulating expressions of genes involved in cellular growth, which results in a reduced growth rate (Hong et al., 2014). Besides that, the stability of the cells might also be influenced as the presence of the new protein itself could lead to protein-protein interaction and further causes crowding of the protein which might destabilise the host (Dave et al., 2016).

The BacterioScan™ 216R instrument was utilised to capture the real-time bacterial concentration (Log CFU/mL) and absorbance detection mode (OD_{650}) readings hourly for a period of 24-hour. Conventionally, it is necessary to generate a curve that relates to bacterial density (OD reading) and bacteria concentration (CFU/mL) to determine the correct bacterial concentration at different time points (Campbell, 2010). This requires the researcher to meticulously read the absorbance and dilute and plate the cultures on an hourly basis. The plates were then incubated overnight, and the number of colonies formed on the plates were counted and compared to the absorbance reading at the particular hour. The BacterioScan™ 216R instrument eliminates the need for such laborious work as it is able to obtain the readings of these parameters at real-time capabilities.

It was observed that the three *Salmonella* strains showed overall similar patterns for bacterial replications and only a slight difference was observed after the 10-hour mark. Different bacterial strains are expected to have slightly different growth patterns, and similar findings were also observed between different *S.* Agona strains, both parental and genetically modified strains in a previous study (Gwee et al., 2019).

As discussed in the results, the transformed Salmonella strains showed slight reduction in bacterial growth compared to the parental strains initially; however, the growth curve of the transformed strains improved and reached similar capacity after 6 hours. This shows that the introduction of the pGFP plasmid did not adversely affect or stunted the replication capabilities of the *Salmonella* strains. These findings were similar to the findings reported by Ma et al., (2011), which reported that the transformation of the pGFP plasmid showed an insignificant effect on the growth of *Salmonella.* In the study, the same pGFP plasmid vector was used to transform *Salmonella, Listeria* and *Escherichia coli* (*E. coli*) 0157:H7 strains and the growth comparisons between GFP-expressing and its parental strains were carried out using the conventional method which required spectrophotometrically monitoring and plating of diluted cultures at regular time interval for 24 hours (Ma et al., 2011). In another study by Allison et al., (2007), it was also observed that there were no significant differences in the doubling time between GFP-expressing *E. coli* and *Pseudomonas putida* and its parental strains. (Allison & Sattenstall, 2007). It is important that the introduction of the

GFP plasmid must not interfere with the bacterial growth to ensure that the studies that utilise the GFP transformed strains are not compromised and reflects the findings of the untransformed bacterial strains.

CONCLUSION

In conclusion, the data presented from this study has shown that the transformation of the *Salmonella* strains with pGFP had minimal effects on the cell physiology, specifically the bacterial replication rate. As GFP expression do not affect the bacterial strains, this allows the use of GFP-labelling for further study of the strains as tumour suppressing agents, specifically to study the mechanism of tumour suppression and real-time study of biodistribution *in vivo*. Future studies are warranted, especially with bigger sample size to analyse and compare the differences between GFP-expressing and its parental strains statistically.

DISCLOSURES

The authors declare there was no potential conflict of interest and disclose no financial interest in the contents of this manuscript.

ACKNOWLEDGMENT

We would like to thank everyone that has been involved in providing their valuable knowledge throughout this study. This research was funded by the Universiti Putra Malaysia Research University Grant Scheme (04-212-2026RU).

REFERENCES

Allison, D. G., & Sattenstall, M. A. (2007). The influence of green fluorescent protein incorporation on bacterial physiology: a note of caution. Journal of applied microbiology, 103(2), 318-324. https://doi.org/10.1111/j.1365-2672.2006.03243.x

Baban, C. K., Cronin, M., O'Hanlon, D., O'Sullivan, G. C., & Tangney, M. (2010). Bacteria as vectors for gene therapy of cancer. Bioengineered bugs, 1(6), 385-394. https://doi.org/10.4161/bbug.1.6.13146

Bugrysheva, J. V., Lascols, C., Sue, D., & Weigel, L. M. (2016). Rapid antimicrobial susceptibility testing of Bacillus anthracis, Yersinia pestis, and Burkholderia pseudomallei by use of laser light scattering technology. Journal of Clinical Microbiology, 54(6), 1462-1471. https://doi.org/10.1128/JCM.03251-15

Campbell, J. (2010). High-throughput assessment of bacterial growth inhibition by optical density measurements. Current protocols in chemical biology, 2(4), 195-208. https://doi.org/10.1002/9780470559277.ch100115

Cann, S. H., Van Netten, J. P., & Van Netten, C. (2003). Dr William Coley and tumour regression: a place in history or in the future. Postgraduate medical journal, 79(938), 672-680.

Carlson, R. D., Flickinger, J. C., Jr, & Snook, A. E. (2020). Talkin' Toxins: From Coley's to Modern Cancer Immunotherapy. Toxins, 12(4), 241. https://doi.org/10.3390/toxins12040241

Cormack, B. P., Valdivia, R. H., & Falkow, S. (1996). FACS-optimized mutants of the green fluorescent protein (GFP). Gene, 173(1), 33-38. https://doi.org/10.1016/0378-1119(95)00685-0

Dave, K., Gelman, H., Thu, C. T. H., Guin, D., & Gruebele, M. (2016). The effect of fluorescent protein tags on phosphoglycerate kinase stability is nonadditive. The Journal of Physical Chemistry B, 120(11), 2878-2885. https://doi.org/10.1021/acs.jpcb.5b11915

Farkona, S., Diamandis, E. P., & Blasutig, I. M. (2016). Cancer immunotherapy: the beginning of the end of cancer?. BMC medicine, 14(1), 1-18. https://doi.org/10.1186/s12916-016-0623-5

Forbes, N. S., Coffin, R. S., Deng, L., Evgin, L., Fiering, S., Giacalone, M., ... & Kaur, B. (2018). White paper on microbial anti-cancer therapy and prevention. Journal for immunotherapy of cancer, 6(1), 1-24. https://doi.org/10.1186/s40425-018-0381-3

Gwee, C. P., Khoo, C. H., Yeap, S. K., Tan, G. C., & Cheah, Y. K. (2019). Targeted inactivation of Salmonella Agona metabolic genes by group II introns and in vivo assessment of pathogenicity and anti-tumour activity in mouse model. PeerJ, 7, e5989. https://doi.org/10.7717/peerj.5989

Hong, H., Jung, J., & Park, W. (2014). Plasmid-encoded tetracycline efflux pump protein alters bacterial stress responses and ecological fitness of Acinetobacter oleivorans. PLoS One, 9(9), e107716. https://doi.org/10.1371/journal.pone.0107716

Idelevich, E. A., Hoy, M., Görlich, D., Knaack, D., Grünastel, B., Peters, G., ... & Becker, K. (2017). Rapid phenotypic detection of microbial resistance in Gram-positive bacteria by a real-time laser scattering method. Frontiers in microbiology, 8, 1064. https://doi.org/10.3389/fmicb.2017.01064

Jessy, T. (2011). Immunity over inability: The spontaneous regression of cancer. Journal of natural science, biology, and medicine, 2(1), 43. https://doi.org/10.4103/0976-9668.82318

Khoo, C. H., Cheah, Y. K., Lee, L. H., Sim, J. H., Salleh, N. A., Sidik, S. M., ... & Sukardi, S. (2009). Virulotyping of Salmonella enterica subsp. enterica isolated from indigenous vegetables and poultry meat in Malaysia using multiplex-PCR. Antonie van Leeuwenhoek, 96(4), 441. https://doi.org/10.1007/s10482-009-9358-z

Khoo, C. H., Sim, J. H., Salleh, N. A., & Cheah, Y. K. (2015). Pathogenicity and phenotypic analysis of sopB, sopD and pipD virulence factors in Salmonella enterica serovar Typhimurium and Salmonella enterica serovar Agona. Antonie Van Leeuwenhoek, 107(1), 23-37. https://doi.org/10.1007/s10482-014-0300-7

Kwon, S. Y., & Min, J. J. (2014). Genetically engineered Salmonella typhimurium for targeted cancer therapy. In Gene Therapy of Cancer (pp. 443-452). Academic Press. https://doi.org/10.1016/B978-0-12-394295-1.00030-5

Lee, C. H., Lin, S. T., Liu, J. J., Chang, W. W., Hsieh, J. L., & Wang, W. K. (2014). Salmonella induce autophagy in melanoma by the downregulation of AKT/mTOR pathway. Gene Therapy, 21(3), 309-316. https://doi.org/10.1038/gt.2013.86

Leschner, S., & Weiss, S. (2010). Salmonella-allies in the fight against cancer. Journal of molecular medicine, 88(8), 763-773. https://doi.org/10.1007/s00109-010-0636-z

Liang, K., Liu, Q., Li, P., Luo, H., Wang, H., & Kong, Q. (2019). Genetically engineered Salmonella Typhimurium: Recent advances in cancer therapy. Cancer letters, 448, 168-181. https://doi.org/10.1016/j.canlet.2019.01.037

Ma, L., Zhang, G., & Doyle, M. P. (2011). Green fluorescent protein labeling of Listeria, Salmonella, and Escherichia coli O157: H7 for safety-related studies. PLoS One, 6(4), e18083. https://doi.org/10.1371/journal.pone.0018083

McCarthy E. F. (2006). The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. The Iowa orthopaedic journal, 26, 154-158.

Mi, J., Sydow, A., Schempp, F., Becher, D., Schewe, H., Schrader, J., & Buchhaupt, M. (2016). Investigation of plasmid-induced growth defect in Pseudomonas putida. Journal of biotechnology, 231, 167-173. https://doi.org/10.1016/j.jbiotec.2016.06.001

Momiyama, M., Zhao, M., Kimura, H., Tran, B., Chishima, T., Bouvet, M., ... & Hoffman, R. M. (2012). Inhibition and eradication of human glioma with tumor-targeting Salmonella typhimurium in an orthopotic nude-mouse model. Cell Cycle, 11(3), 628-632. https://doi.org/10.4161/cc.11.3.19116

Morrissey, D., O'Sullivan, G. C., & Tangney, M. (2010). Tumour targeting with systemically administered bacteria. Current gene therapy, 10(1), 3-14. https://doi.org/10.2174/156652310790945575

Murakami, T., Hiroshima, Y., Zhao, M., Zhang, Y., Chishima, T., Tanaka, K., ... & Hoffman, R. M. (2015). Therapeutic efficacy of tumor-targeting Salmonella typhimurium A1-R on human colorectal cancer liver metastasis in orthotopic nude-mouse models. Oncotarget, 6(31), 31368. https://doi.org/10.18632/oncotarget.5187

Perry, L. M., Hoerger, M., Seibert, K., Gerhart, J. I., O'Mahony, S., & Duberstein, P. R. (2019). Financial strain and physical and emotional quality of life in breast cancer. Journal of pain and symptom management, 58(3), 454-459. https://doi.org/10.1016/j.jpainsymman.2019.05.011

Phillips, G. J. (2001). Green fluorescent protein-a bright idea for the study of bacterial protein localization. FEMS microbiology letters, 204(1), 9-18. https://doi.org/10.1111/j.1574-6968.2001.tb10854.x

Rang, C., Galen, J. E., Kaper, J. B., & Chao, L. (2003). Fitness cost of the green fluorescent protein in gastrointestinal bacteria. Canadian journal of microbiology, 49(9), 531-537. https://doi.org/10.1139/w03-072

Salleh, N. A., Rusul, G., Hassan, Z., Reezal, A., Isa, S. H., Nishibuchi, M., & Radu, S. (2003). Incidence of Salmonella spp. in raw vegetables in Selangor, Malaysia. Food Control, 14(7), 475-479. https://doi.org/10.1016/S0956-7135(02)00105-6

Schuster, D. J., Dykstra, J. A., Riedl, M. S., Kitto, K. F., Belur, L. R., McIvor, R. S., ... & Vulchanova, L. (2014). Biodistribution of adeno-associated virus serotype 9 (AAV9) vector after intrathecal and intravenous delivery in mouse. Frontiers in neuroanatomy, 8, 42.

https://doi.org/10.3389/fnana.2014.00042

Syal, K., Mo, M., Yu, H., Iriya, R., Jing, W., Guodong, S., ... & Tao, N. (2017). Current and emerging techniques for antibiotic susceptibility tests. Theranostics 7(7), 1795. https://doi.org/10.7150/thno.19217

Uchugonova, A., Zhang, Y., Salz, R., Liu, F., Suetsugu, A., Zhang, L., ... & Zhao, M. (2015). Imaging the different mechanisms of prostate cancer cell-killing by tumor-targeting Salmonella typhimurium A1-R. Anticancer research, 35(10), 5225-5229.

Wall, D. M., Srikanth, C. V., & McCormick, B. A. (2010). Targeting tumors with salmonella Typhimurium-potential for therapy. Oncotarget, 1(8), 721. https://doi.org/10.18632/oncotarget.206

Wang, C. Z., Kazmierczak, R. A., & Eisenstark, A. (2016). Strains, mechanism, and perspective: Salmonella-based cancer therapy. International journal of microbiology, 2016. https://doi.org/10.1155/2016/5678702

World Health Organization Agency for Research on Cancer (IARC). (2018). GLOBOCAN 2018: estimated cancer incidence, mortality and prevalence worldwide in 2018. Retrieved from https://gco.iarc.fr/

Yong, A. Y., Shabahang, S., Timiryasova, T. M., Zhang, Q., Beltz, R., Gentschev, I., ... & Szalay, A. A. (2004). Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. Nature biotechnology, 22(3), 313-320. https://doi.org/10.1038/nbt937

Zhao, M., Yang, M., Li, X. M., Jiang, P., Baranov, E., Li, S., ... & Hoffman, R. M. (2005). Tumor-targeting bacterial therapy with amino acid auxotrophs of GFP-expressing Salmonella typhimurium. Proceedings of the National Academy of Sciences, 102(3), 755-760. https://doi.org/10.1073/pnas.0408422102

Zheng, J. H., & Min, J. J. (2016). Targeted cancer therapy using engineered Salmonella typhimurium. Chonnam medical journal, 52(3), 173. https://doi.org/10.4068/cmj.2016.52.3.173

Zhao, M., Yang, M., Ma, H., Li, X., Tan, X., Li, S., ... & Hoffman, R. M. (2006). Targeted therapy with a Salmonella typhimurium leucine-arginine auxotroph cures orthotopic human breast tumors in nude mice. Cancer research, 66(15), 7647-7652. https://doi.org/10.1158/0008-5472.CAN-06-0716

Zia, U.N.T.N., Ng, E.S.N.A., Sidik, M.A., Idris, M.F.M., Khoo, C.H., Sukardi, S., … & Cheah, Y.K. (in press). Tumour Growth Inhibition and Systemic Responses of ΔsopBΔsopDΔpipD Disrupted Salmonella Agona and Salmonella Typhimurium in Mice. Malaysian Journal of Medicine and Health Sciences.

Citation:
Taraq Naem Zia, U. N. ., Samynathan, D., Saidi, H. I., Selvarajah, G. T. ., & Cheah, Y. K. (2021). Construction and investigation of plasmid-induced effects on growth of GFP-expressing Salmonella strains. *Life Sciences, Medicine and Biomedicine*, *5*(1).
https://doi.org/10.28916/lsmb.5.1.2021.73

Copyright © 2021 by the Author(s). Life Sciences, Medicine and Biomedicine (ISSN: 2600-7207) Published by Biome Journals - Biome Scientia Sdn Bhd. Attribution 4.0 International (CC BY 4.0). This open access article is distributed based on the terms and conditions of the Creative Commons Attribution license https://creativecommons.org/licenses/by/4.0/

Life Sciences, Medicine and Biomedicine
ISSN: 2600-7207