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Research Article

## Attenuation of whole cell of *Salmonella sp.*, isolated from a rural community pond water and its appliances in rat model: A preliminary study

Koresh Ranjan Panigrahi, Smaranika Pattnaik\* and Santosh Mohapatra

Department of Microbiology, Gayatri College of Pharmacy, Jamadarpalli, Sambalpur-768200, Odisha, India

### ABSTRACT

There is need for acknowledgment of specific Ag (s), so that a vaccine candidate may be recognized against the dreaded strains like *Salmonella sp.* the causal organism for Typhoid. We have taken an effort to screen an isolated bacterial strain isolated from village pond water for its immune modulating activity. Four groups of mice were taken as a test animal model. Group I was taken as control, Group II was symptomatic; Group III was symptomatic, induced with supernatant of *Salmonella* culture and attenuated with neutral phosphate buffer formalin, while Group IV was symptomatic but induced with crude attenuated whole cells. The rat groups were kept for 28 days with periodical observation and were subjected to Total Leucocyte Count/ mm<sup>3</sup>. The group I, which were found to be healthy with an initial TLC count 7269/mm<sup>3</sup> at the day 1 which could be maintained till the end of the day 28 (7648 mm<sup>3</sup>). Group II and Group III could not maintain the TLC count after day 14. There was a drastic reduction in TLC counts, 5917 /mm<sup>3</sup> for Group II and 5194 /mm<sup>3</sup> for Group III. Group IV could maintain the TLC count from day 1 to day 28. Group II and Group III were unable to survive after day 21. The pond water isolated *Salmonella sp.* attenuated with neutral phosphate buffer formalin had an immune modulatory activity in symptomatic rat models, which could be evidenced from TLC/mm<sup>3</sup> count.

**Keywords:** Village pond water; *Salmonella sp.*; attenuation; TLC count; immunogenicity

### INTRODUCTION

With the increasing antibiotic resistance trend towards *Salmonella* strains, the development of potent vaccine candidates for typhoid fever is a need of the hour (Marathe, 2012). In this context (Garmony, 2002), it had been reported about initial studies in which *S. enterica* var. Typhimurium *aroA* were shown to be attenuated and highly immunogenic in rats. Hence, there is need for screening of newer strains for the antigenicity activity to hold over the disease incidence. Jamadarpalli, a small peripheral village in Western Odisha was chosen for the study as the villagers are deprived of contemporary hygienic facilities due to their poor economic status. The natives of the village use their ponds for multipurpose activities. *Salmonella* strains are notorious as causal organisms of water-borne diseases. Therefore, typhoid fever along with gastroenteritis was observed to be endemic to that area. The orthodox localities believe in traditional black magic. To counteract this rampant situation, the natives should be made into prophylactic for the disease. Vaccination is an effective strategy in the prevention and control over the

disease (Ku, 2005). Therefore, this present pursuit is undertaken to exploit the viable bacterial strains prevailing in pond water to screen for their active immunogenicity to combat against the diseases like typhoid. Keeping this tight spot in mind, this work was initiated to isolate, identify and attenuate the bacterial strains from the village pond water to make into a form of booming vaccine. Here we have used *in-vivo* induced antigen technology (Rollins, 2005).

### METHODS

#### Selection of source of bacterial strain

Multipurpose village pond water used by the inhabitants was taken as source for *Salmonella* strains.

#### Preparation of Media

Nutrient Media in the form of General media and MacConkey media as *Salmonella* as selective media used for this study were prepared according to HI-MEDIA, Mumbai company instructions.

#### Isolation of bacterial strains

Isolation of bacterial culture was performed by "Spread Plate technique" (Pattnaik, 2010). The strains were isolated in mixed culture in General media and were sub cultured in selective media by using "Spread Plate" and "Streaking plate" techniques.

\* Corresponding Author

Email: smaranika2010@gmail.com

Contact: +91- 8895429103

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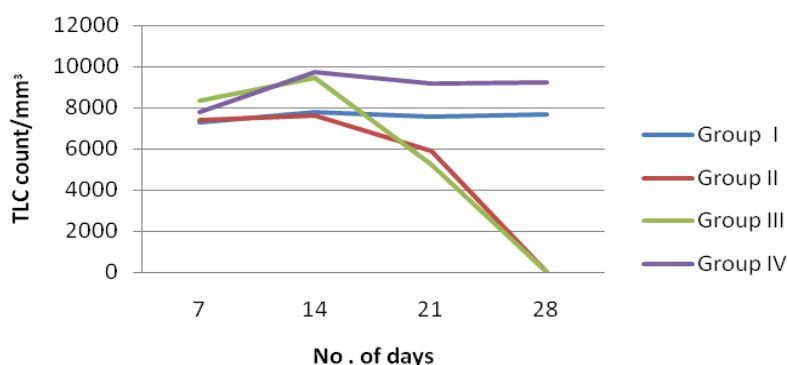
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**Table 1: Depicting the results obtained from preliminary screening for immunogenicity of isolated Salmonella strain (Total leucocytes count/mm<sup>3</sup>)**

Rat groups Days* (Interval of 7 days)	Group I	Group II	Group III	Group IV
BE (1-7days)	7269	7394	8333	7807
DLP1(8-14days)	7789	7621	9440	9755
DLP2(15-21 days)	7566	5917	5194	9204
ALP(22-28days)	7648	NS	NS	9265

\*BE: Before Exposure; DLP: During latent period, DLP1 Injection of attenuated culture, DLP 2: Inoculation with bacterial strain ; ALP: After latent period; NS: Not survived

**Figure 1: Isolated Salmonella colonies from village pond water****Figure 2: Depicts the TLC/mm<sup>3</sup> on test mice group**

Group I: Control, Group II: Symptomatic and without injected with attenuated bacterial cells; Group III : Symptomatic and injected with supernatant of attenuated and centrifuged whole bacterial cells; Group IV: symptomatic mice were injected with attenuated whole cell bacterial cells

#### Identification of bacterial strains

The isolated colonies grown on MacConkey plates were subjected to identification by using Microbiological diagnostic methods. For this purpose, microscopic studies were carried out by Gram staining and Negative staining. The biochemical characterizations were performed by using the regular biochemical test parameters.

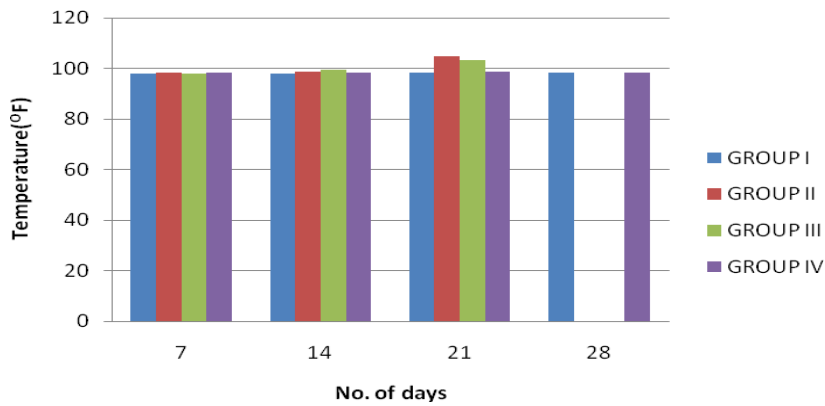
#### Preparation of Phosphate buffer

The saline Phosphate buffer (50mM Po<sub>4</sub> and 0.5 M NaCl 3% neutral PH) was prepared by following stan-

dard protocol. The Po<sub>4</sub> buffer was mixed with formalin at a ratio of 1:4.

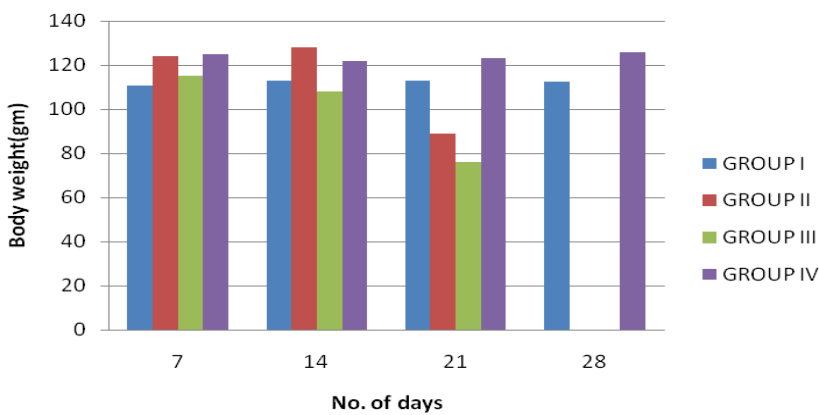
#### Attenuation of isolated Salmonella strain

The isolated colonies of Salmonella strain was inoculated into flasks containing broths were incubated in a rotary shaker cum incubator (REMI) maintained at a temperature 37°C±2 for a period of 12 hrs. This O/N culture of Salmonella strains in broths containing 10<sup>5</sup>CFU/ml was subjected to attenuation by following a modified method (Fadl, 2005). The bacterial broth cultures were assorted with freshly prepared Neutral Po<sub>4</sub> buffer formalin solution (1:1) (Jain, 2001) and incubated at 37°C for a period of 168 hrs.



**Figure 3: Depicts the average body temperature taken from rectum of mice**

Group I: Control, Group II: Symptomatic and without injected with attenuated bacterial cells; Group III : Symptomatic and injected with supernatant of attenuated and centrifuged whole bacterial cells; Group IV: symptomatic mice were injected with attenuated whole cell bacterial cells.



**Figure 4: Depicts the average body weight of rats**

Group I: Control, Group II: Symptomatic and without injected with attenuated bacterial cells; Group III : Symptomatic and injected with supernatant of attenuated and centrifuged whole bacterial cells; Group IV: symptomatic rats were injected with attenuated whole cell bacterial cells



**Figure 5: depicting the four groups of rats. Group I: Control, Group II: Symptomatic and without injected with attenuated bacterial cells; Group III : Symptomatic and injected with supernatant of attenuated and centrifuged whole bacterial cells; Group IV: symptomatic rats were injected with attenuated whole cell bacterial cells**

After the period of incubation with  $\text{Po}_4$  buffer formalin, the broth cultures were made into two parts. One part was subjected to centrifugation (REMI R4) at rate of 3000 rpm to get supernatant while the other part was not centrifuged which was comprised of whole bacterial cells. The supernatant usually devoid of cellular debris but may comprised of cellular extracts like exotoxins which are tarnished for their antigenic activity.

### Screening for immunogenicity of formalin treated Salmonella

For the screening for immunogenicity activity of whole bacterial cell, a modified method of "Induced Ag in Vivo in animal models" (Postol *et al.*, 2013) was used. The immunogenicity activity was estimated in the form of TLC (Total Leucocyte Count) in blood. Total Leucocyte Count (TLC) in blood is an obligatory test which reflects enumeration and status of body's defending cells against antigens. Four groups of albino rats (3rats/group) of age group 6-7 weeks old were taken. The albino rats used for this study were housed by "Animal House" maintained by Gayatri College of Pharmacy, Sambalpur, Odisha. The Animal house is approved with Animal Ethical committee with a registration number 1339/ac/10/CPCSEA, India. The group I included the control group (No symptomatic and no induction), group II included symptomatic, while group III had included symptomatic ones which were induced with supernatant of attenuated whole bacterial cells. The group IV was comprised of symptomatic rats was induced with crude attenuated whole bacterial cells. For induction of attenuated bacterial cells peritoneal injections were made by using sterile hypodermic needles. After a period of 7 days, the rats were examined for progress of disease symptom (if any), with regular checking of pharmacological parameters. For this purpose of TLC/ $\text{mm}^3$ , the blood 0.5ml was drawn from the orbital sinus (Hoff, 2000) of each rat. EDTA (Ethyl di amine tetra acetate) at 100 $\mu\text{g}/\text{ml}$  (w/v) was used as the anticoagulant for the blood. The TLC/ $\text{mm}^3$  count was performed at a local Pathological laboratory, Sambalpur, Odisha.

### RESULTS

The isolated colonies grown on MaConkey agar medium plates (Figure 1) were observed to be *Salmonella sp.* based upon the colony characteristics and routine biochemical tests. From the screening of immunogenicity activity of formalin treated whole cells of a *Salmonella* strain, the Total Leucocyte counts (LTC) in  $\text{mm}^3$  are depicted in Table - 1. It was observed that there was a steady increase in TLC count/ $\text{mm}^3$  with Group IV (Graph-1). Further it was found that the Group II and Group III were unable to survive after day 21 (Figure 2 b and c). The healthy and active group was Group IV which could be comparable with Group I (Figure 2 a and d). In addition to this the body temperature ( $^{\circ}\text{F}$ ) and body weight (gm) so recorded for the different

groups are depicted in Graph 2 and Graph-3 respectively.

Arrows are indicative of isolated *Salmonella* colonies on MaConkey agar plate

### DISCUSSION

From the data depicted in Table-1 it was evidenced that the group I which were found to be healthy with an initial TLC count 7269/ $\text{mm}^3$  at the day 1 which could be maintained till the end of day 28 (7648  $\text{mm}^3$ ). Further it was observed that Group II and Group III could not maintain the TLC count after day 14. Both the groups were found with a drastic reduction of TLC counts (5917 / $\text{mm}^3$  for Group II and 5194 for Group III respectively). Moreover Group IV could maintain the TLC count from day 1 to day 28. The body temperature was observed to be normal for Group I and Group IV while increase in temperature was pragmatic with Group II and Group III. Regarding body weight, both Group I and IV observed to be minimal alteration while Group II and Group III had found to be with significant decrease in their respective weights.

### CONCLUSION

Concisely it may be mentioned here that this study regarding the immunogenicity of Formalin attenuated pond water isolated *Salmonella* whole cells had an immune modulatory activity which was evidenced from TLC/ $\text{mm}^3$  count from test animal models. The regular temperature as well as body weight measurement substantiated the observation. Therefore this small fragment of study may pave a way for development of a *Salmonella* vaccine candidate in future.

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