Summary

For the collection of phage samples, the southern tip of the Indian subcontinent, the State of Tamil Nadu was selected. The sampling sites were selected to cover different geographical regions of the State. A total of 6 sampling sites were selected. Three sites viz., Chennai, Cuddalore and Pudhucherry from the Northern regions, a sampling point at Karur representing the western part of the State, Madurai contributes the central part of the State and Tirunelveli referring the southern part of the State. The host bacterial culture used in the current research was *Salmonella typhi* (MTCC 3917) obtained from Microbial Type Cell Culture Center, Chandhigarh, India. For the isolation of *Salmonella* specific phages, two methods were employed viz., Direct isolation method and Enrichment method. The direct isolation method had failed to produce any plaques on the plaque assay for all the six phages. However in the enrichment assay, the samples contained enriched phages in the order of about $10^2$ to $10^4$. The phage purification was carried out by serial sub culturing of the specific type of plaques in the crude lysate up to the $10^{th}$ passage. The plaques produced by each of the samples have its known unique characteristics.

The purified phages were concentrated using PEG 8000 in the magnitude of $10^4$ on an average. In ammonium sulphate concentration method, the phages were concentrated in the magnitude of $10^2$ on an average. In comparison, the method using PEG stands out.

The host range of the six phages was evaluated against a wide range of bacterial species. The results show that the phages were highly specific for *Enterobacteriaceae*. There were few differences seen among the phages in their host range. Regarding the stability of the phages in different physiological conditions, all
the phages were highly stable at the pH 4, 5, 6, 7, 8 and 9. The phage titre started to decrease at pH above 4 and 10 and almost got completely inactivated at the pH 2 and 11 instantly. Similarly, all the phages were highly stable up to 45°C after which they started to decrease in the titre when the temperature reached 55°C and beyond. They were completely inactivated at the temperatures 65°C, 75°C, 85°C, 95°C & 100°C. On storage in -20°C, phages titres started to decline initially which got stabilized after reaching a threshold titre. All the phages remained highly stable up to 1 hour of UV exposure. The phage STP Pdy although showed some oscillations in its titre also seemed to remain stable.

Different absorption rates were exerted by the phages against the host bacterium. Among the six phages, the phage STP from Madurai showed higher affinity whereas it was very less in the phage STP isolated from Pudhucherry for the host bacterium when compared to the others.

One step growth curve analysis was done to elucidate the life cycle of the phages. The latent period of the phages varied from 10 min to 30 min. The latent periods were of 20, 20, 25, 20, 10 and 15 minutes for the phages STP from Chennai, Cuddalore, Pudhucherry, Madurai, Tirunelveli and Karur respectively. The burst period of the phages lasted up to 35, 35, 45, 35, 25 and 28 min for the phages STP from Chennai, Cuddalore, Pudhucherry, Madurai, Tirunelveli and Karur respectively. The burst size of the phages were 2, 2, 3, 4, 2 and 1.5 log PFU/ml for the phages STP from Chennai, Cuddalore, Pudhucherry, Madurai, Tirunelveli and Karur respectively.

From the DNA isolation and restriction digestion analysis, it can be concluded that the size of all the six phage DNA was approximately around 49 kb in size. This result shows that the phages were genetically similar to one another. They are all believed to contain almost a similar pattern of gene arrangement in their genome.
Even though the phages were isolated from different geographical regions they all
tend to be have a similar molecular make up. Therefore we were unable to diversify
the phages based on the restriction fragment analysis.

The SDS PAGE analysis produced 5 similar structural proteins in all the six
samples. All the 5 protein bands in the gel were believed to be structural proteins.
Similar to the DNA profile, we were not able to diversify the phages based either on
neither their genomic configuration nor using the protein profile of its outer coat.

Lysogenic assays were performed to evaluate the efficiency of the conversion
of lytic phages to lysogens. All the six phages tend to become lysogens during
unfavorable conditions. The suspected lysogens were confirmed by spontaneous
induction of the lysogens into lytic phages.

For the phages to be used as an internal medicine, the evaluation of the
interactions between the phage and the human digestion system is inevitable. For this
purpose, the Stimulated Human Intestine Microbiota (SHIME) was designed. This
system will artificially stimulate the environment present in the human digestion
system. The SHIME system consists of five compartments each representing a
segment or part of the human intestine. The first compartment stimulates the
environment of the stomach and duodenum of the intestine, whereas the second
compartment stimulates the environment of the ileum and jejunum of the small
intestine followed by caecum and ascending colon stimulation in the 3rd compartment.
The 4th compartment stimulates the transverse colon and the last compartment induces
the environment in the descending colon. On introduction of the phages into the
system along with food materials, the phage titre commenced to decrease in the stage
one of the SHIME as the phages were highly stable only up to pH 3 whereas log 2
reductions in the phage titre in the vessel one might be due to the pH of 4 to 2.
Stabilization of the phages occurred on transfer of the phage from the compartment one to two. This confirmed that the acidic environment in the intestine acts as a hurdle for the Phage Therapy.

To overcome the hurdle of the acidic environment in the intestine, the phages were introduced shortly after the introduction of the antacid in to the SHIME system. This method stabilized the phages in the compartment one with a 50% reduction in their titre. Evaluation of the stability of the phages was done against human saliva. The phages were stable up to 10 mins in Human saliva.

The physiological properties of the phages in the gastric conditions were evaluated in \textit{in vivo} condition. For this purpose, animal model Albino rat were used. The test rat feces were processed and confirmed free of \textit{Salmonella} specific phages before the administration of the phages orally. To the healthy rat, phages were administrated orally along with food materials. The feces of the test animals were collected and evaluated for the phage titres regularly. In animals administered with phages without anatacid, 2 log PFU/ml of phages was recovered in its feces. In the animals administered with phages with anatacid, the recovery rate was 4 log PFU/ml. During the entire experimental period all the rat remained active as the control rat.

To evaluate the toxicity of the phage lysate, animal model rat were used. Purified phages were injected into the experimental rat and the physiological and mortality of the animals was studied. All the animals injected with the phages stayed active and no mortality was seen throughout the experiments which ensure that the phage lysates were free of toxic compounds. The sub acute toxicity assay also claimed that the phage suspension is free of toxic compounds.

The immunogenicity of the phages was determined using the rat animal model. All the phages considerably elicited neutralizing antibodies against them on
injection into the rabbit immune system. The neutralizing antibody assay was performed using the serum of the animals injected with the specific phages. All the phages had been completely neutralized in the antiphage serum.

Three novel methods were designed and validated to trace *Salmonella* contamination in tropical rivers using the isolated phages. (Method I: Enumeration of *Salmonella* specific bacteriophage without enrichment, Method II: Enumeration of *Salmonella* specific bacteriophages by enrichment and Method III: Enrichment of the known *Salmonella* specific phage in the water sample) using *Salmonella* specific phages were introduced. The efficiency of the methods to trace *Salmonella* contamination was evaluated against the standard conventional technique (enumeration of the bacterium on semi selective media). Water samples were collected from five different sites in “Chittar” river on two different days. Among the three novel methods, the results of method II & III coincides with the standard conventional method. The method I did not concur with the standard method which failed to trace the presence of *Salmonella* sp. in the water. The methods II & III have successfully mapped the *Salmonella* contamination in the river water. But they were unable to quantify the *Salmonella* sp. Hence, it is recommended that the methods II & III are reliable, cheap and efficient alternative methods to trace *Salmonella* contamination in the tropical river waters.

Regarding the usefulness of the isolated phages in the food industry’s or food processing industries to control *Salmonella* contamination, food materials like chicken, egg and milk were intentionally contaminated with *Salmonella typhi*. These contaminated food materials were treated with phage and antibiotics separately. In the food materials treated with phages, there was a log 4 microbial load reduction in the surface of the chicken, the antibiotics produced log 5 reductions in the microbial
load. In contradiction to these, phages successfully reduced the *Salmonella* contamination up to log 3 in the inner surface of the chicken, whereas the antibiotics produced only log 2 reduction.

The phages were similar in its molecular makeup, but diversified in its physiological properties. They are highly safe to be utilized as an oral drug to treat intestinal Salmonellosis.