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Impact of elevated temperature on *Salmonella typhi*

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Abstract

The purpose of this study was to examine the effect of elevated temperature *on S.typhi*, which is water borne organism and cause the typhoid. It was incubated at various temperatures like 37°C, 42°C, and 47°C for overnight. Then the carbohydrate, lipid and the DNA content in each culture were measured. The results showed that an increase in temperature like 42°C and 47°C produced a marked decrease in the carbohydrates, lipid and DNA content when compared to the culture incubated at 37°C. Therefore slight increase in the temperature caused decrease in carbohydrate, lipid and the DNA content. When the organism is treated at a high temperature that can result in significant decrease in the micro & macromolecules, which ultimately leads to the death of the organism.

Keywords: *Samonella typhi*; Micro and macromolecules; Lipids and carbohydrates.

Introduction

Salmonellas are Gram-negative facultative rods with peritrichous flagella. They are members of the family *Enterobacteriaceae* (Pelczar *et al*., 1993). *Salmonella* infection is the cause of food borne illness in most developing countries. Meat, poultry and dairy products are frequently implicated in outbreaks.

The bacterium *Salmonella typhi* causes typhoid fever (Doughari *et al.* 2007). The bacterium is a motile, non-sporing, non-capsulated bacillus that can be contracted through contaminated water, milk, food or fruits and vegetables or via convalescent or chronic carriers (Duguid *et al*., 1983; Doughari, 2005). It has also been linked with zoonotic transmission via reptiles and common domestic pets (Duguid *et al*., 1983; de Jong *et al*., 2005). Enteric fever (typhoid) is a global bacterial infection with an annual infection rate of 21.6 million and 10% fatality rate (Crump *et al*., 2003). In developing countries, typhoid is more severe due to poor hygiene, indiscriminate use of antibiotics and a rapid rise in multidrug resistance. Resistance to the first line drugs chloramphenicol, ciprofloxacin and amoxycillin has been reported. Currently, 107 strains of this organism have been isolated; many containing varying metabolic characteristics, levels of virulence, and multi-drug resistance genes that complicate treatment in areas that resistance are prevalent. Diagnostic identification can be attained by growth on MacConkey and EMB agars, and the bacteria is strictly non-lactose fermenting. It also produces no gas when grown in TSI media, which is used to differentiate it from other *Enterobacteriaceae*.

Salmonella is the leading agents causing gastrointestinal infections, especially in developing countries. *S. typhi* colonizes only in humans and therefore, the disease can be acquired only through close contact with a person who has typhoid fever or is a chronic carrier. A number of these cases may be associated with consumption of raw vegetables exposed to fecal contamination (Bryan, 1997). Use of raw sewage to irrigate crops is an important mechanism that contributes to propagating conditions for typhoid fever.

Transmission of *S. typhi* has shown only to occur by fecal-oral route, often from asymptomatic individuals. 2-5% of previously infected individuals become chronic carriers who show no signs of disease, but actively shed viable organisms capable of infecting others. The principal habitat of the salmonellae is the intestinal tract of humans and animals. *Salmonella* serovars can be found

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predominantly in one particular host, can be ubiquitous, or can have an unknown habitat. *Typhi* and *Paratyphi A* are strictly human serovars that may cause Grave diseases often associated with invasion of the bloodstream.

On a global scale, at least 16 to 20 million cases of Typhoid fever occur annually, resulting in global mortality of 200, 000 people (Pang *et al.,* 1998). Typhoid fever is a systemic infection in man, which is caused by *Salmonella enterica* serovar and *Typhi* that are strictly adapted to humans or higher primates. The disease is currently rare in the United States and Europe but endemic in Asia, Africa and South America from where it can be imported by foreign travelers. There is an average of 1258.6 cases of Typhoid in Malaysia for the past 15 years (Disease Control Division, Ministry of Health). Food handlers, especially hawkers, and those who are involved in selling food at food stalls were found to be lacking in knowledge, attitude and practice towards food-borne diseases and food safety. A close correlation has been found between the macromolecules of organisms and the rate at which they grow (Sykes & Tempest, 1965). The present study was aimed to see the effect of elevated temperature on the *S.typhi*.

Materials and Methods

Estimation of carbohydrates

1ml of the supernatant was taken. To the supernatant 4ml of Anthrone reagent, (100ml of 95% sulphuric acid was prepared and stored in 4°C for 3 hours. After 3 hours 200mg of anthrone was added and mixed well before use) was added and the blank consisted of 1ml distilled water and 4 ml Anthrone reagent. The sample was taken for spectrophotometric analysis against blank at 630nm in UV-Visible spectrophotometer. Amount of carbohydrate present in the sample was calculated using standard graph.

Estimation of cholesterol

0.1ml of the sample was taken and 0.9ml of precipitating agent (Ferric Chloride precipitating agent - 10ml of stock diluted to 100ml with glacial acetic acid) was added to it. The content was centrifuged at 8,000rpm for 5 minutes and 1ml of the filtrate was collected. To this 1ml of diluting agent and 1ml of conc.H_2SO_4 was added. The blank consisted of 1ml diluting agent (Ferric Chloride diluting agent- 8.5ml stock diluted to 100ml with glacial acetic acid) and 1ml of conc.H_2SO_4. The colour developed was read at 560nm in the UV spectrophotometer and the amount calculated using standard graph.

DNA Isolation

1ml of bacterial culture was taken. Centrifugation was carried out for 5 minutes at 10,000rpm.the pellet was re-suspended with 300µl STET buffer. Then 30µl of RNase/lysozyme was added. Then the mixture was boiled for 1 min 15 sec. Centrifugation was carried out for 15 minutes at 10,000rpm. The supernatant of the flow through was transferred to a 1.5 ml Eppendorf. To the supernatent phenol, extract with 150 µl STET–saturated phenol was added. Then the content was spin down and the supernatent of the flow through was collected. Then 0.6volume isoproponal was added and incubated at RT for 5 min. Centrifugation was carried out for 10 minutes at 10,000rpm. The invisible pellet was washed with 70% ethanol. Then the pellet was re-suspended with 50µl of TE buffer. Then the DNA was resolved on 0.7% agarose gel, stained with Etheidum bromide and

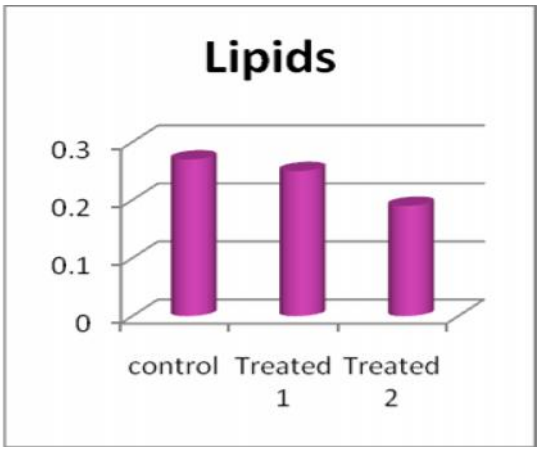

Fig. 1. Lipid content in the control and treated sample

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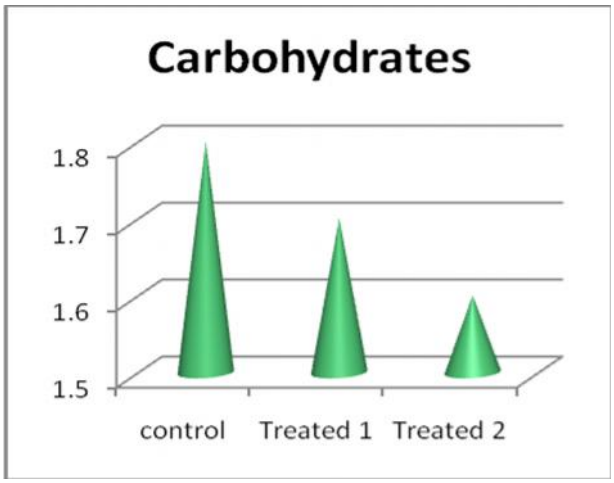

Fig. 2. Carbohydrates content in the control and sample treated

visualized on UV-transilluminator.

Quantification of DNA

0.5µl TE buffer was pipetted directly onto the Nanovue sample plate which act as a blank.Then0.5µl of sample was pipetted onto the Nanovue sample plate and quantified. The purity and concentration were viewed in the display.

Results and discussion

The lipids of microorganisms are sensitive to physical and chemical changes in the environment. Ahmed and Rowbury (1971) found that the lipid composition in bacteria is affected by growth rate, media composition**,** pH, oxygen tension and growth temperature. Fig.1 shows the lipid concentration of *S.typhi* in the elevated temperature. The results show that increase in temperature causes the decrease in the lipid concentration. Earlier studies found that the changes in the lipid content of *B. caldolyticus* at 60°C. Similarly, they found that in *B. f avothermus* the alterations in the lipid were more pronounced when incubated at high temperature. The alterations of the lipid composition were most significant in *B. caldolyticus*, especially at elevated temperature. In contrast, the main changes in *B. flavothermus* were found to occur between 30°C and 50°C.

Carbohydrates serve as the basic building blocks of the cell. It serves as a basic constituent of the genetic material. Cell wall components are highly rich in the carbohydrate moiety. Thus, carbohydrates play an important role in the cell including various carbohydrate metabolisms such as the glycolysis, HMP pathway, gluconeogenesis etc. When this total carbohydrate level tends to increase or decrease it results in metabolic abnormalities. Our results showed that increase in temperature causes the decrease in the carbohydrates (Fig. 2).

Few researches stated that the changes in RNA, DNA, carbohydrate and protein contents of *Aerobacter aerogenes*, which occurred when the incubation temperature of the cultures is increased. He reported that increase in the temperature would result in decrease carbohydrate content. Our results also compromised with his result.

The DNA concentration of *Salmonella typhi* treated at various temperatures is shown in the (Table 1 and Fig. 3). It was found that the DNA concentration was found to decrease when the temperature decreases.

Fig. 3. Showing the DNA concentration in control, treated 1, treated 2 samples

John Sommerville (1982) found that when paramecia grown at 24°C are transferred to 32°C DNA and protein synthesis continue uninterrupted but at higher rate, rapidly. This clearly shows that the increase in temperature will result in decrease in the DNA content,

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which ultimately leads to the DNA damage.

Table.1. Depicts the culture incubated atvarious temperature

S.No	Culture condition & Designation	Temperature (^{o}C)	Time Period of incubation
1	C (control)	37	overnight
2	T1 (culture condition I)	42	overnight
3	T2 (culture condition II)	47	overnight

Conclusion

Global warming is an important issue in contemporary times. It affects the ecosystem largely; it may be positive or adverse. The project carried out was a preliminary effort to show how the process of global warming affected the particular species ie. *S.typhi,* which was exposed to three different temperatures 37ºC, 42ºC, and 47ºC. Due to elevation in the ambient temperature the carbohydrate, lipid and DNA content in the species was affected drastically. The present study reveals that the elevated temperature significantly affected the organism *S.typhi.* Because of it, the disease caused by this organism can be prevented when treated at high temperature.

References

1. Ahmed N and Rowbury RJ (1971) Temperature-sensitive cell division component in a mutant of Salmonella typhimurium. *J Gen Microbiol*., 67(1), 107-15.
2. Bryan (1977) Diseases transmitted by food contaminated by wastewater *J. Food Prot,* 4, 45-56.
3. Crump JA, Youssef FG, Luby SP, Wasfy MO, Rangel JM, Taalat M, Oun SA and Mahoney FJ (2003) Estimating the incidence of typhoid fever and other febrile illnesses in developing countries. *Emerg Infect Dis*., 9(5), 539-44.
4. Doughari JH (2005) A comparative study on effects of crude extracts of some local medicinal plants and some selected antibiotics on *Salmonella typhi*. *Thesis Federal University of Technology,* 5, 1-4.
5. de Jong B, Andersson Y and Ekdahl K (2005) Effect of regulation and education on reptile-associated salmonellosis. *Emerg Infect Dis.*, 11(3), 398-403.
6. Doughari JH, Pukuma MS and De N (2007) Antibacterial effects of Balanites aegyptiaca L. Drel.and Moringa oleifera Lam. on Salmonella typhi. *Biotechnol,* 6, 2212-2215.
7. Duguid JP, Marmion BP and Sawin RHA (1983) Mackie and McCartney 13th edn. ELBAS Churhill Livingston. *Medical Microbiology,* 314-323.
8. John Sommerville (1982) Gene Structure and Expression, *Biophysica Acta (BBA),* 698,158-166
9. Pang T, Levine MM, Ivanoff B, Wain J and Finlay BB (1998) Typhoid fever - important issues still remain. *Trends in Microbiology,* 6, 131–133.
10. Pelczar MJ, Jr Chan ECS and Krieg NR (1993) Microbiology Concepts and Application. 1st ed.
11. Sykes J and Tempest DW (1965) The effect of magnesium and of carbon limitation on the macromolecular organisation and metabolic activity of Pseudomonas sp., Strain. *Biochim. Biophy,* 9, 103-105.