



Impact of Hyperthermia on Gut Microbial Adaptation: Role of Thermophilic Bacteria in Host Physiology

Sugandha Jaiswal, Vinod Kumar Nigam, and Rakesh Kumar Sinha

Department of Bioengineering and Biotechnology, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, 835215, India; sugandhaoffice96@gmail.com

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Background: Heat stress is one of the most challenging environmental conditions, responsible for impaired growth and reproduction in living systems. It also leads to altering the release of different biochemicals responsible for controlling the metabolic pathway. The objective of the present research is to determine whether the thermophilic gut microbes that survive in hyperthermic rats exhibit promising signs of beneficial effects, potentially modulating host physiology for therapeutic applications.

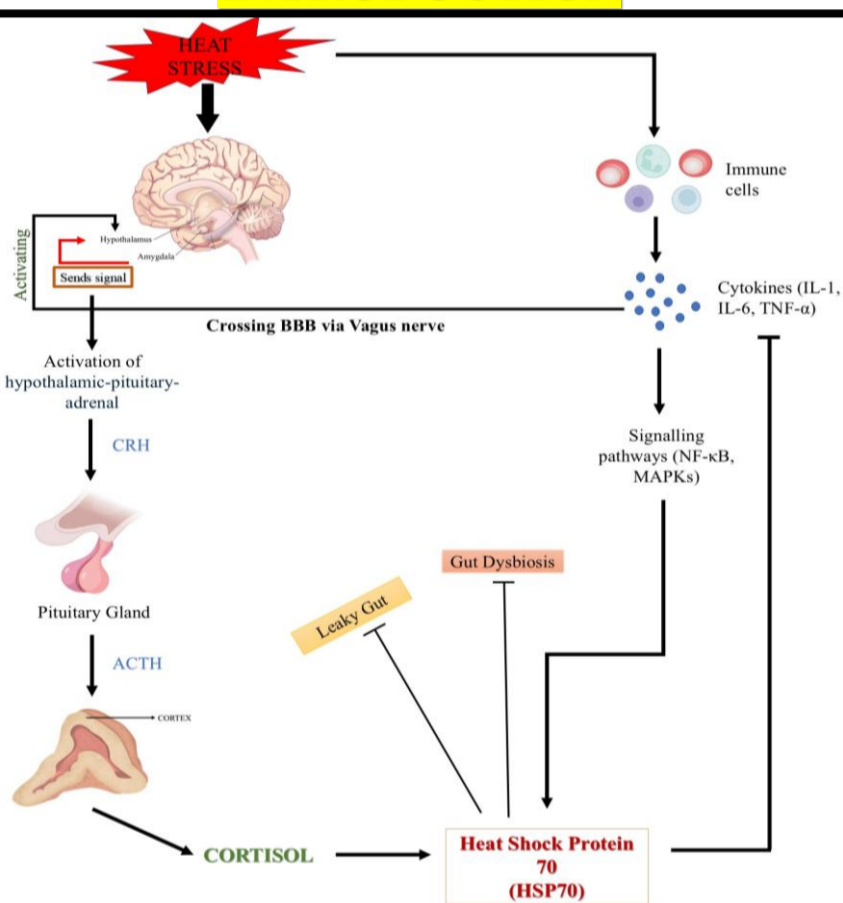
Methods: Five White Wistar rats were exposed at $42\pm 1^\circ\text{C}$ inside a closed chamber for the induction of hyperthermia. The rectal temperature was recorded before and after the heat exposure. The sample from the colon of sacrificed rats was collected under sterile conditions for the isolation of gut bacteria on a nutrient agar plate at 50°C , 60°C , and 70°C . The sample was incubated for 24 hours, and isolates were further purified. The proteolytic, amylolytic, cellulolytic, and xylanolytic activities were measured via the plate assay, and the enzymatic index was calculated. Total protein and estimation of Heat Shock Protein 70 (HSP70) were also quantified.

Results: Initially, the rats' rectal temperature was $37.1\pm 0.2^\circ\text{C}$, but after exposure to heat temperature was $40.8\pm 0.2^\circ\text{C}$. The number of purified isolates was recorded, i.e., at 50°C (04), at 60°C (01), and 03 at 70°C , respectively. Among eight isolates, *Bacillus licheniformis* (50°C) showed all four enzymatic activities with a higher enzymatic index. Further, this novel isolate also exhibited maximum concentration of HSP70.

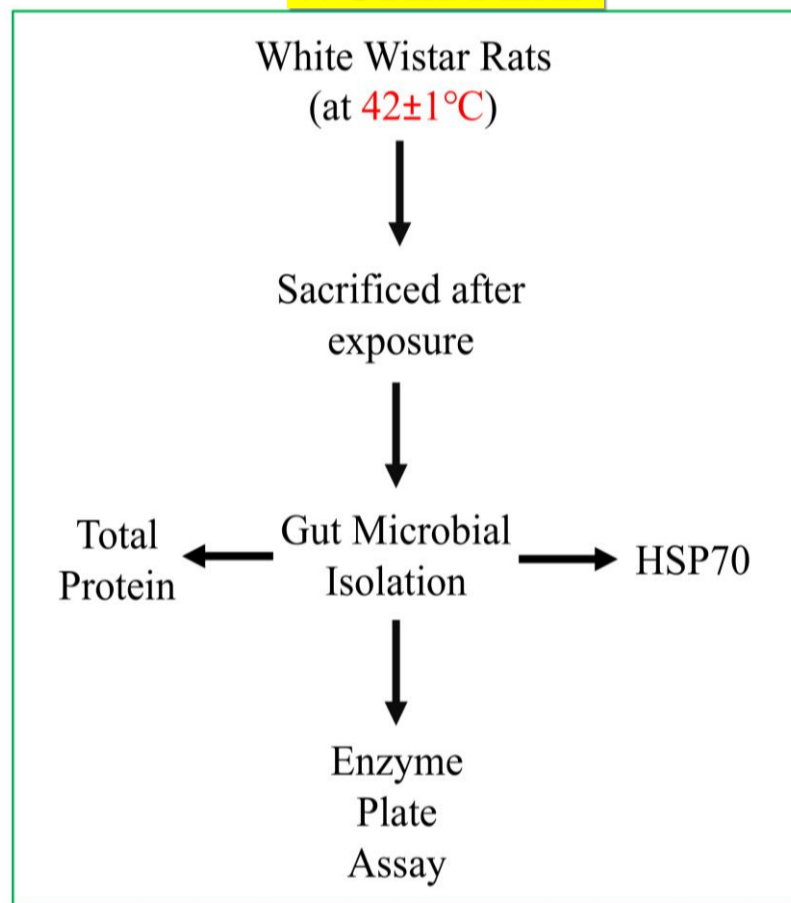
Conclusions: This study revealed the survival of a novel bacterium (*B. licheniformis*) capable of producing key metabolites, highlighting its significance in supporting host physiology and heart health. As a probiotic, it may serve as a potential therapeutic bridge connecting HSP70, cardiac function, and gut health.

Keywords: *Bacillus licheniformis*; enzymatic activity; gut microbiota; HSP70; hyperthermia

INTRODUCTION



WORK PLAN



RESULTS

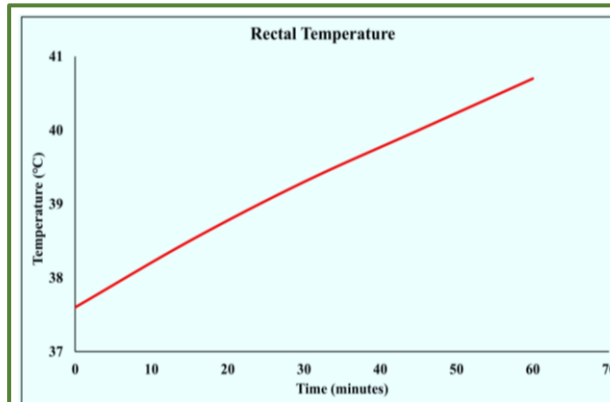


Figure 1: Increase in rectal temperature with the duration of exposure

Table 1: Number of isolates obtained at different temperatures in control and hyperthermic conditions

S.NO.	TEMPERATURE (°C)	Control Isolates	Hyperthermic Isolates
1.	50	5	4
2.	60	3	1
3.	70	No isolates	3

Table 2: Enzymatic Index of all isolates at three different temperatures exhibited different enzymatic activities

S. No.	Temperature (°C)	Code Name	Gram's Stain	Protease activity	Amylase activity	Cellulase activity	Xylanase activity
1.	50	JSNBEBT50°1	+ve	6.33	1.13	5.37	1.50
		JSNBEBT50°2	+ve	2.70	-ve	-ve	-ve
		JSNBEBT50°3	+ve	2.81	-ve	-ve	-ve
		JSNBEBT50°4	+ve	1.26	-ve	-ve	1.28
2.	60	JSNBEBT60°1	+ve	2.00	-ve	-ve	1.36
3.	70	JSNBEBT70°1	+ve	1.14	-ve	-ve	-ve
		JSNBEBT70°2	+ve	1.66	1.57	-ve	-ve
		JSNBEBT70°3	+ve	1.50	1.33	-ve	-ve

METHODOLOGY

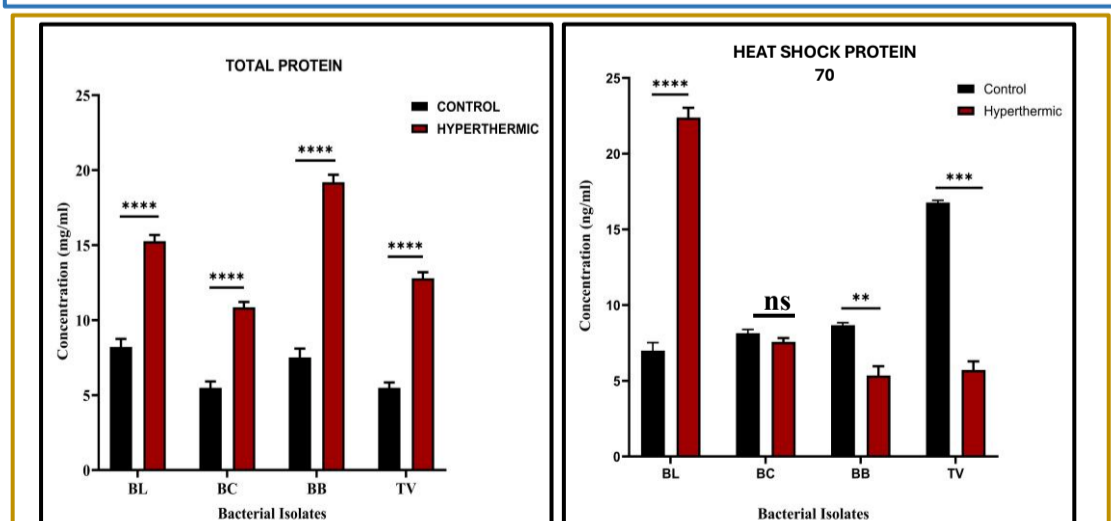
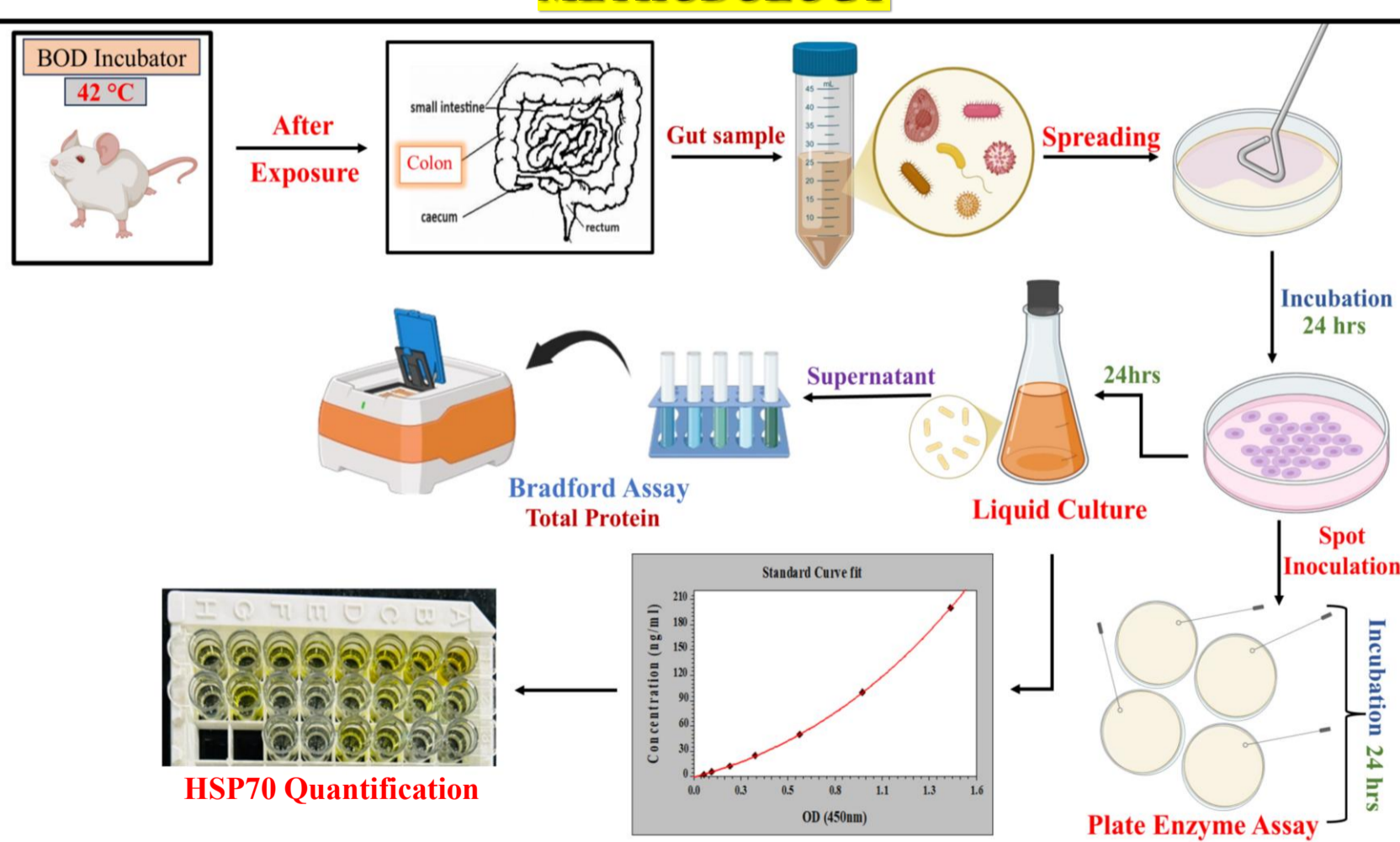


Figure 2: Graph showing the estimation of total protein and HSP70.

CONCLUSIONS

- The body temperature increased with the time of exposure and reached the state of hyperthermia as it crosses 40°C .
- Table 1** reveals three isolates were obtained at 70°C in hyperthermic conditions compared to control.
- All the isolates showed a highly significant increase in the total protein concentration, however *JSNBEBT50°1* produces significantly high amount of HSP70 compared to all the obtained common isolates.
- JSNBEBT50°1* was the only isolate showing all the enzymatic activities and a good range of EI index that sheds light on the metabolic capacity of the microbes.
- From the above results, *Bacillus licheniformis* (*JSNBEBT50°1*) was found to be the best candidate for further selection to evaluate the role of microbiota in pathophysiological conditions, such as in thermoregulation and/or other cardiac conditions.

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**BIRLA INSTITUTE OF TECHNOLOGY, MESRA
RANCHI, INDIA- 835215**