APPLICATION NOTE

Thermal Analysis

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Metabolic Heat Associated with Viable Escherichia Coli

Abstract

Biological samples can present various problems for micro-calorimetric measurements stemming from the unique physiology of living cells. Potential problems include rapid changes in PH, substrate exhaustion, extended lag phase associated with substrate changes, metal ion toxicity, sub-optimal oxygen concentrations, and possible thermal shock associated with sample preparations. For improved results, various instrumental factors need to be optimized to meet the narrow physiologic requirements of living cells. We evaluated approximately 10E8 and 10E5 Escherichia coli (ATCC Strain 29194) grown in tryptic soy broth for 6 and 2 hours respectively. Log phase growth of 10E5 E. coli cells produced approximately 0.9 milliwatts of heat over 25 minutes as measured on a PerkinElmer power-compensated Differential Scanning Calorimeter. Physiologic factors that interfere with the thermal output were investigated including substrate exhaustion, substrate switching, metal toxicity, and thermal shock. In addition strains bearing antibiotic resistant plasmids were compared to strains lacking such plasmids. Substrate exhaustion and metal ion toxicity appear to be important factors that cause a rapid drop in measurable thermal output in E. coli. These observations should be considered when designing applications such as antibiotic susceptibility testing or detection of plasmid bearing strains.

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Introduction

Micro-calorimetric measurements of metabolic heat derived from viable bacterial cells has found application in the food, pharmaceutical, and medical diagnostic industries as well as in the studies of environmental and soil microbiology.[1,2] The method shows particular promise for the analysis of slow growing microorganisms where typical bioassays for drug susceptibility may require weeks or months.[3,4] Typical bioassays used in clinical laboratories rely on highly reproducible physiologic conditions to allow interpretations of bacterial growth response; for example changes on growth in response to an antimicrobial agent require precisely reproducible cation concentrations. The physiology of bacterial respiration has been well studied.

10E6 E. coli consume approximately 125 micro-liters of oxygen at 37 ˚C under aerobic conditions.[5] These physiologic requirements have not been extensively studied in the effect of substrate exhaustion, metal ion toxicity, thermal shock, and substrate switching on bacterial respiration as measured by the heat release in a power compensation DSC.

Experimental

Strains: Escherichia coli strain ATCC 29194 (Figure 1) was used in studies of heat shock, metal ion toxicity, and substrate exhaustion. A sucrose positive laboratory strain (H1) was used to demonstrate substrate switching. A second laboratory strain (P1) with and without 12 kb plasmid was used to demonstrate the effects of plasmid on thermal output. All strains were grown overnight in tryptic soy broth and then were sub cultured in tryptic broth for 2 hours at 37 ˚C with an oscillatory shaker at 100 cpm. Six hour cultures were prepared in experiments on substrate exhaustion.

Thermal Analysis: Micro-calorimetric measurements were made under isothermal conditions at 37 ˚C except where mentioned in the text. Micro-calorimetric measurements were made using a PerkinElmer power compensated DSC-7. This DSC's low mass furnaces and power compensation gave outstanding sensitivity to small changes and the instrument is well known for its isothermal stability. The reaction cells were held under nitrogen, air or oxygen atmospheres. The DSC was held at 37 ˚C and baseline subtraction was used. Runs were performed in triplicate and averaged to account for biological variability. Baselines showed a drift of +0.143 mW over 25 minutes with substrates in both cells. All other heat flows reported in this work are exothermic. A high pressure accessory from PerkinElmer was used for evaluated pressure work. Bacterial samples were assayed in tryptic soy broth and compared to a reference cell containing uninoculated tryptic soy broth and substrate. Unless otherwise specified, all sample volumes were 50 micro liters of solution containing 10E7 E. coli in log phase growth. All samples were run in aluminum pans unless otherwise noted.

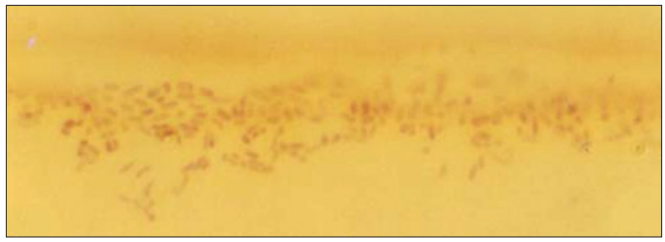

Figure 1. Escherichia coli.

Results

Initial studies on 10E5 E. coli produced approximately -0.9 mW of heat over a 25 minute interval. This is in contrast to where the same innoculum of cells grown at 37 ˚C were shocked by exposure to 40 ˚C. Apparent metabolism is reduced to -0.05 mW in 25 minutes and does not recover over the time interval studied.

Figure 2 shows a higher degree of thermal output (-29 mW) associated with a heavy innoculum of 10E7 cells. The slope shows a gradual decrease with time. When 10E7 cells are grown under nitrogen atmosphere, less heat (-12 mW) is generated than when an air purge is used. This is shown in Figure 3. Use of higher concentration of oxygen did not increase the heat generated as neither pure oxygen nor 10 bar oxygen pressure caused an increase in metabolic heat.

An innoculum of 10E7 cells from an 18 hour old culture was prepared and would be expected to be in a stationary state of growth. In Figure 4, this stationary phase growth and fresh media containing a low amount of glucose (0.01%) were mixed 1:1. Initial heat generation was slow but a brief burst of metabolism occurred as the glucose was utilized and then exhausted. It might be anticipated that induction of metabolic pathways required for the utilization of different substrates could generate a similar lag phenomenon but using sucrose and maltose substrates, we were unable to demonstrate any lag in heat production within the time frame studied.

The selection of different sample pans has a significant effect on the thermal output from the E.coli. When aluminum pans were used, 10E7 cells produced approximately -29 mW of heat in 15 minutes. In contrast, when stainless steel or gold pans were used, the thermal output immediately dropped to -1.9 and -0.6 mW respectively. A summary of effects is presented in Table 1.

Discussion and Conclusions

In E. Coli, 42 ˚C typically induces the expression of heat shock proteins that allows the organism to survive at adverse temperature.[5,6] During the DSC scans, itd metabolism was observed to promptly decrease even at temperatures well within the growth range of E. coli. This prolonged period required for adjustment to a new temperature is referred to as thermal shock. In our experiments, metabolism decreased by approximately 20 fold on exposure to 40 ˚C.

A heavy innoculum produced greater thermal output than a light innoculum but the slope gradually decreased presumably due to substrate exhaustion. In the design of thermal assays, this would mean that the ratios of low innocula to high innocula might not yield strictly proportional thermal gain. In an extreme example some lag time might be required before the substrate can be utilized (Figure 4). Thus proportional or kinetic antimicrobial methods might require strict standardization of the innocula when performing thermal methods. We were unable to demonstrate any lag with substrate switching. The complex tryptic soy broth may provide other metabolic substrates used in addition to and perhaps in preference to the sugars tested.

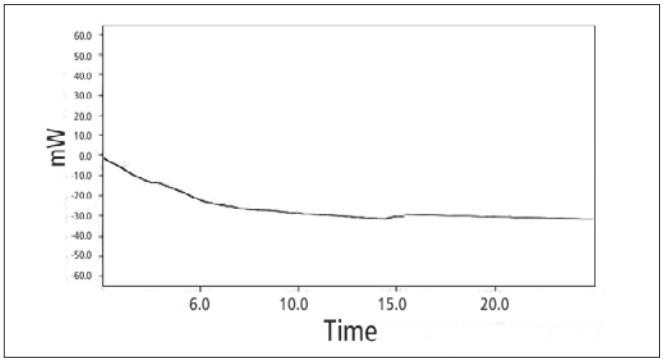

Figure 2. DSC on 10e7 E. coli in air.

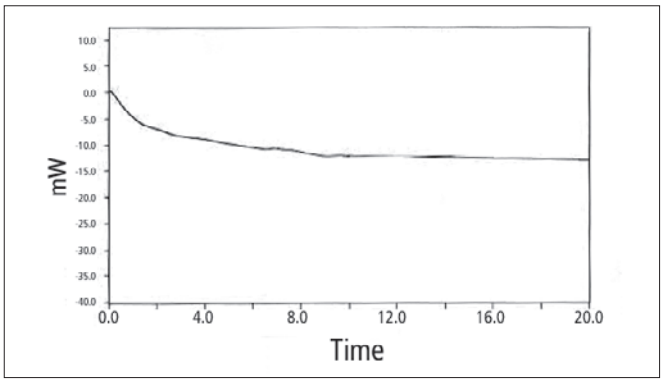

Figure 3. DSC on 10e7 E. coli in N_2.

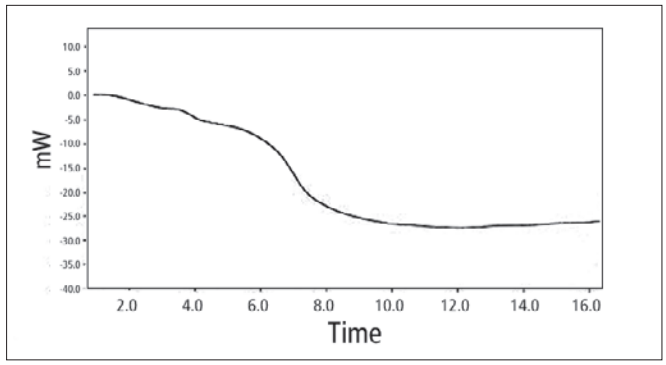

Figure 4. Substrate-induced lag time.

We tested an E. coli strain containing a 12 kb plasmid and a variant of the same strain that had lost the plasmid.[7] Only a 10% increase in thermal output was seen. However, unlike the other experiments where conditions were compared using the same innocula, this required using 2 different innocula. Thus the 10% difference could be due to the effect of the plasmids on metabolic efficiency or a mismatch in innoculum size. Further work is needed to validate this.

Perhaps the most significant condition affecting the physiology of the viable E. coli was the choice of sample pan material. Both stainless steel and gold pans proved to be highly inhibitory to bacterial metabolism. The toxic effect of heavy metals in E. coli is well known and it appears even brief contact of the aqueous solutions with the stainless steel and gold pans results in sufficient metal ion concentration to inhibit E. coli metabolism.

In summary, of the several factors studied heavy metal ion contamination and substrate exhaustion were the most important factors in determining the metabolic response of rapidly growing E. coli in a thermal analysis format. These physiological factors must be considered in future applications with live microorganisms since high concentrations of the organism are used to maximize the thermal signal in a rapid bioassay format.

***Table 1.* Summary of effects on metabolic heat.**

E. coli	Gas	Pan	Temp.	Heat Flow[1]
10E5	air	Aluminum	37	-0.9
10E5	air	Aluminum	40	-0.05
10e7	air	Aluminum	37	-29.1
10e7	N_2	Aluminum	37	-18.3
10e7	O_2	Aluminum	37	-17.6
10e7	O_2, 10 bar	Aluminum	37	-19.2
10e7 with plasmids	air	Aluminum	37	-32.1
10e7	air	Stainless steel	37	-1.9
10e7	air	Gold	37	-0.6
baseline	air	Aluminum	37	+0.1

[1] *heat flow as mW per 25 minutes, averaged from three runs.*

References

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