USE OF BATCH ISOTHERMAL MICROCALORIMETRY FOR GROWTH MEDIA OPTIMIZATION

INTRODUCTION

One of the fundamental steps of probiotic product production is media optimization. The expanding species and strains of probiotics demand a wider selection of different growth media. Achieving the best growth conditions for traditional probiotics like *Lactobacillus* spp and *Bifidobacterium* spp is already challenging. However, new microbial species, known as next-generation probiotics, pose even more challenges for the industry and often involve extensive growth media screening.[1] Furthermore, media optimization is a balancing act between bacterial needs and the cost of the formulations. With the new formulations comes the need for new technologies to evaluate the suitability in an easy, fast and cost-effective way.

Biologically and economically suitable media need to contain carbon, nitrogen and mineral sources or other micronutrients at certain concentrations for any given strain and species. A variety of methods are used for screening and determining the most suitable formulations, each with their own advantages and disadvantages. Classical plating and optical density measurements focus on biomass production but do not give information about vitality and metabolic yield of the microorganisms. Furthermore, these methods are sensitive to clumping and media turbidity. To visualize the live-dead portions of the biomass, flow cytometry is often used. Although fast, it only provides a snapshot of the situation and requires sampling over time. In addition, acid production of bacteria could be followed but it would not be suitable for all species.

Biocalorimetry has been employed in wide range of microbiology applications. Isothermal microcalorimeters such as calScreener measure heat flow between the sample and its surroundings. All living organisms and their biological processes produce heat, and it is this that is measured in calorimetric assays.[2] The independence of isothermal calorimetry from media turbidity and kinetic data and its requirement for minimal hands-on time makes it a versatile tool for probiotic companies and academic groups.[3 4]

METHODOLOGY

The first step in creating the optimal media for probiotic bacteria involves selecting the right components. Each strain of bacteria has preferred carbon and nitrogen sources which must be considered alongside the requirement for large-scale production.

The workflow for nutrient screening with a batch calorimeter is straightforward. Probiotic bacteria from appropriate stock or starter cultures are inoculated into various media formulations on a calorimetric plate. The plate is inserted into a calorimeter, and after a suitable equilibration time the heat flow can be continuously measured in real-time. The run time is not limited and data can be collected for as long as necessary (Figure 1). In our study, we used an MRS broth base without a carbon source and added single carbon sources at a 2% concentration to investigate the metabolic activity of *Lactobacillus plantarum*.

RESULTS

Our results showed that the addition of glucose led to the highest metabolic activity. It was also noted by observing the steepest slope for the exponential growth phase that the growth rate was highest under this condition (Fig. 2A). In contrast, lactose resulted in a lower maximum metabolic output, although it produced a similar biomass. The first bump in the heat flow curve suggests a change in metabolic activity, likely due to catabolite repression and a switch to using lactose. In terms of total heat that the bacteria can produce, all substrates were fairly similar, reflecting the similar amounts of biomass they produced (Fig 2B).

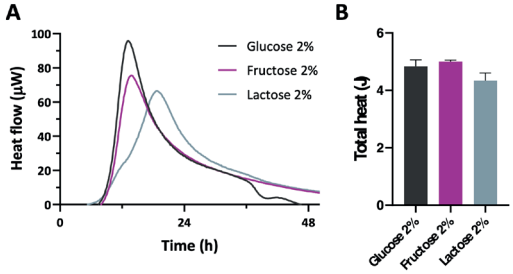

Figure 2 Metabolic activity of L. plantarium grown in MRS supplemented with different carbon sources: 2% glucose, fructose, or lactose. A: The curves represent heat flow over time at 37°C. B: Total heat generated during the experiment.

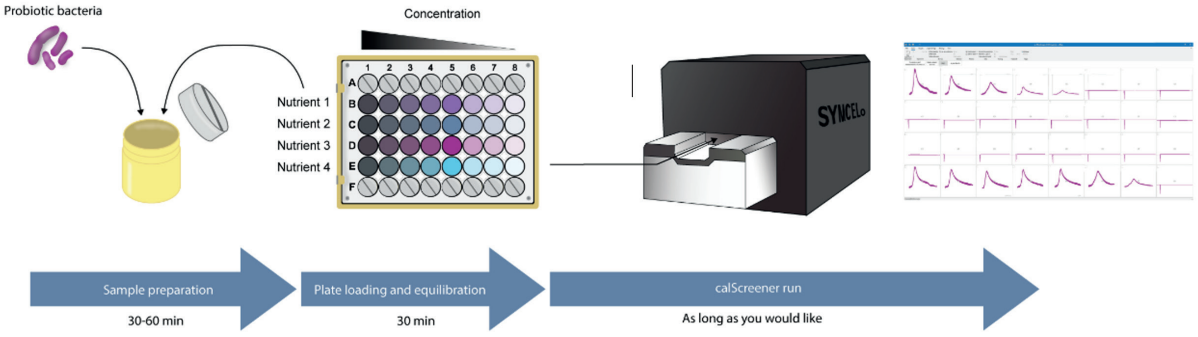

Figure 1 Workflow of media optimization

After the components for the growth media have been selected they need to be optimized for the amount added. More is not always better, as it might inhibit growth or be economically wasteful. Here we look at the effect of adding additional carbon sources to *Staphylococcus aureus* culture in MHB broth. The addition of glucose boosts metabolic activity and results in higher maximal metabolic activity. However, 0.5% is enough to achieve that peak and additional glucose would have no further effect and would be wasteful.

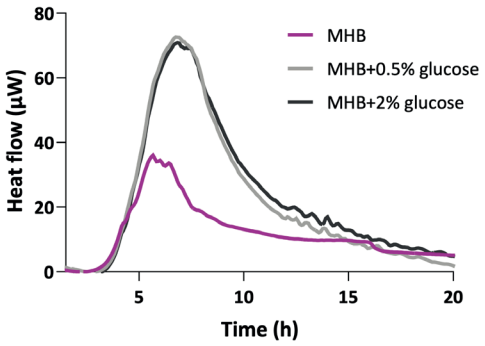

Figure 3 Addition of glucose to *S.aureus* in MHB.

IMPLEMENTATION CASES

Using biocalorimetry would be especially useful for cases when components lead to turbidity of growth media or aggregation of cells. It is also suitable for studying anaerobic bacteria without the need to place the entire machine inside the anaerobic chamber. Furthermore, the batch format allows the checkerboard method to screen for best combinations and synergies for media components.

Metabolic readout provides information on metabolic activity and viability as well as growth, which can help understanding of other aspects of probiotics beyond just biomass production. Duboux et al showed that slower growth using more complex sugars could make bacteria more stress tolerant. Additionally, kinetic growth data could suggest the best time to harvest the sturdiest bacteria.

Optimization of probiotic growth and cost-effectiveness with the calScreener

Using the calScreener for media optimization offers significant advantages for the probiotics development process. This tool offers real-time measurements of heat flow, enabling dynamic monitoring of probiotics' metabolic responses to various media formulations. Such continuous assessment captures subtle changes, facilitating the identification of optimal media that promote higher metabolic yield and faster growth compared to traditional biomass focused methods like plate counts or optical density measurements.

The calScreener's versatility to test various conditions, including opaque, viscous, or solid media under aerobic and anaerobic conditions, ensures comprehensive evaluation of growth conditions. Integrating calScreener data with parallel flow cytometric sampling verifies biomass production and metabolic activity, providing a robust and efficient method for media optimization.

By adopting the calScreener, researchers can accelerate the screening process, reduce costs, and improve the accuracy of media development, ultimately enhancing the efficiency of probiotics production and supporting the creation of cost-effective, optimized growth media.

References

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