Food Control 29 (2013) 290–299

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Predictive microbiology theory and application: Is it all about rates?

Tom McMeekin*, June Olley, David Ratkowsky, Ross Corkrey, Tom Ross

Food Safety Centre, Tasmanian Institute of Agriculture/School of Agricultural Science, University of Tasmania, Hobart 7001, Tasmania, Australia

ARTICLE INFO

Article history:
Received 9 March 2012
Received in revised form
23 May 2012
Accepted 1 June 2012

Keywords:
Predictive microbiology
Systems biology
Biological rates
Stringent response
Persister cells
Antibiotic-resistance
Biocide-resistance
"Source–sink"
Growth rate –temperature response
Bioinformatics
Protein-folding-thermodynamics
Mechanistic model

ABSTRACT

We review early work on the microbial growth curve and the concept of balanced growth followed by commentary on the stringent response and persister cells. There is a voluminous literature on the effect of antibiotics on resistance and persistence and we call for a greater focus in food microbiology on the effect of biocides in the same context. We also raise potential issues in development of resistance arising from "source–sink" dynamics and from horizontal gene transfer. Redox potential is identified as crucial in determining microbial survival or death, and the recently postulated role for reactive oxygen species in signalling also considered.

"Traditional" predictive microbiology is revisited with emphasis on temperature dependence. We interpret the temperature *vs* growth rate curve as comprising 11 regions, some well-recognised but others leading to new insights into physiological responses. In particular we are intrigued by a major disruption in the monotonic rate of inactivation at a temperature, slightly below the actual maximum temperature for growth. This non-intuitive behaviour was earlier reported by other research groups and here we propose that it results from a rapid metabolic switch from the relaxed growth state to the stringent survival state.

Finally, we envision the future of predictive microbiology in which models morph from empirical to mechanistic underpinned by microbial physiology and bioinformatics to grow into Systems Biology.

1. Introduction

This paper is based on a presentation of the same title given at 7ICPMF in September 2011 (McMeekin, Olley, Ratkowsky, & Ross, 2011). In the presentation we discussed rates and time scales in microbiology, early studies on microbial growth rate curves and the concept of balanced growth. This was followed by consideration of the stringent response and persister cells, topics which are under-represented in the food microbiology literature. Attention was then focused on modelling studies with emphasis on temperature dependence models. Finally, we asked if the quantitative information embedded in predictive models could be effectively integrated with microbial physiology and "omics" technologies.

In answer to the question posed in the presentation title, "… is it all about rates?" we were confident to respond positively when the question addressed the use of predictive models to estimate the safety and shelf-life of foods. However, our response was equivocal when the question was framed in the context of integrating predictive models with microbial physiology studies and the data deluge emanating from "omics" studies. Here we extend the scope of the 7ICPMF presentation by incorporating recent literature (much of it published since September 2011) in an attempt to provide a definitive response to the objective of successful integration of traditional and futuristic predictive modelling.

2. Rates: all pervasive and all persuasive?

Time scales in microbiology range from milliseconds for enzyme catalysed reactions (Stockbridge, Lewis, Yuan, & Wolfenden, 2010) to doubling times of 7 min for *Clostridium perfringens* to days, weeks or months for psychrophiles growing under optimal conditions to more than 3.5 billion years for life to reach the current level of adaptive evolution. The last time frame has been achieved only as a result of the sub-second rates of enzyme catalysed reactions. For example the enzyme catalysed decarboxylation of orotdine-5′-phosphate, the final step in the synthesis of pyrimidines and thus nucleic acids, has a half-life of 0.017 s. Without the enzyme the half-life of the same reaction at 20 °C is 78 million years (Stockbridge et al., 2010).

In microbiology we consider rates, with time as the universal denominator to describe the development and decline of microbial populations. Much of the early work on microbial growth was carried out by the Paris School (Monod, 1942, 1949) and the Copenhagen School of Maaløe, Kjeldgaard, Neidhardt and Schaechter. Schaechter

* Corresponding author.
E-mail address: tom.mcmeekin@utas.edu.au (T. McMeekin).

0956-7135/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodcont.2012.06.001

(2006) provided an interesting retrospective of that group's research drawing particular attention to the concept of balanced growth, defined by Campbell (1957) as the condition in which all cell constituents increase in proportion over the same time interval. Thus, cell composition varies with growth rate with higher rates of growth requiring more DNA, RNA and proteins (Kjeldgaard, Maaløe, & Schaechter, 1958; Schaechter, Maaløe, & Kjeldgaard, 1958). An important conclusion drawn by the authors was that growth rate is the primary factor determining the physiological state of cells.

Schaechter (2006) also argued strongly for precision to be maintained in the design and execution of experiments to ensure balanced growth commenting that: "Not infrequently articles published in the literature include only a casual mention of how the cultures were grown….an indication of indifference is the use of terms like "mid-log" phase. This is nearly meaningless. It is the mid-point of what? …. conditions and a growth curve should be published in detail in papers that describe bacterial growth." Prerequisites for specification of the physiological state include exact definition of media composition, inoculum size and time/temperature combinations for preparation of starter cultures.

To understand more about the origins and significance of the Schaechter–Maaløe–Kjeldgaard experiments and the excitement generated by the pioneering research of the Copenhagen School, Cooper (1993) is a "must read" paper in which the author provided insights into the experiments, the researchers, and the experimental process. The experimental process also attracted comment from Neidhardt (1999) who noted that: "analysis of problems relating to the growth rate are as reductionist as any…. Yet, when growth is the ultimate interest, one cannot delve into single enzymes, genes or pathways without returning to the whole cell and [its] coordinated operation." In other words the cell is not simply a bag of enzymes.

Discussing the concept of "solving the cell" Cooper (1993) opined that the Copenhagen school markedly advanced understanding of microbial physiology. Now this pursuit has moved to the next level with the widespread adoption of molecular techniques in physiological studies as predicted by Schaechter (2006) in the title of his publication "From growth physiology to systems biology." Balanced growth also provides a physiological interpretation of transition from the lag phase to the exponential growth phase and then to the stationary phase of growth, respectively as a nutritional up-shift and a series of nutritional down-shifts. While the lag and stationary phases are regions of zero net growth rates for populations of cells, it is important not to lose sight that zero rates have important physiological and practical implications. Another member of the Copenhagen School, Neidhardt (1999), wrote about the "obsession" with dN/dt (i.e., population growth rate) but did not subscribe to the view that emphasis on growth had delayed work on the stationary phase. On the contrary he observed that understanding growth was a prerequisite for studying the physiology of the stationary phase noting that "the physiology of non-growth is not the absence of the physiology of growth."

Others argue that the "real action" in the bacterial population growth cycle is confined to resolution of the lag phase and the exponential growth phase where the maximum growth rate is reached. This may be so for some spoilage and pathogenic bacteria where the maximum population density (MPD) in a food will be attained after spoilage has occurred or safety is compromised. But, with these organisms, as well as with probiotic and protective cultures, full growth curves also indicate the MPD and provide information on potential interactions between components of mixed populations. An example of a non-specific interaction is the effect described by Jameson (1962) who observed that the first group of organisms to reach its MPD would cause competitors to move from the exponential to the stationary phase i.e. from a rapid rate to a zero net growth rate. Despite that the lag and stationary phases of growth are regions of zero or very slow growth they are crucial to the continuation of a lineage as will be evident when the physiology of the stringent response and of persister cells are considered. The physiology of the lag phase identifies it as a distinct growth phase that prepares cells for exponential growth (Rolfe et al., 2012).

3. The stringent response – paradigm lost in food microbiology

Even major changes in the physiology of the cell can occur in seconds. A good example is the transition from the relaxed response state to that of the stringent response and the converse switch which occur in 20–30 s (Cashel, 1975). This is equivalent to the half lives of guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (ppGppp), the alarmones that give an unequivocal clue to cells to switch from one state to the other (Lund & Kjeldgaard, 1972). Thus, in a few seconds the purpose of cellular metabolism is totally reversed from a focus on growth (the relaxed state) to a focus on survival (the stringent [response] state; SR).

The SR has been shown to play a significant role in processes as different as biofilm formation, quorum sensing, antibiotic resistance, and virulence regulation (Navarro Llorens, Tormo, & Martinez-Garcia, 2010). Perhaps, it also provides an explanation for the Jameson Effect? Here we posit that the component of a mixed culture to approach MPD first produces sufficient ppGpp not only to signal its entry into the SR state but also that of its competitors. Relief from the SR state can be achieved by inoculation into a fresh batch culture or prevented by growth in continuous cultures in which the alarmone is continually diluted and nutrients are continually added. However, if a continuous culture is set up in a retentostat (a chemostat with 100% feedback of biomass) very slow or zero growth rates ensue (Chesbro, Evans, & Eifert, 1979; Goffin et al., 2010). This phenomenon can again be attributed to the programmed objective of the stringent response physiological state: survival at any cost. During transition from the exponential to the stationary phase the cytoplasmic concentration of the general stress factor (RpoS) increases in Gram negative cells (Gentry et al., 2003; Lange & Hengge Aronis, 1991, 1994) and cell density and quorum sensing compounds have been proposed to have a role in the mechanism of transition (Hengge-Aronis, 2002). However, Ihssen and Egli (2004) argued that, as other factors (growth rate, carbon source availability and metabolite concentration) also change during the transition, quorum sensing alone is insufficient to explain the transition. They concluded, on the basis of both batch and continuous culture experiments, that RpoS expression is not controlled by quorum sensing, that specific growth rate plays a prominent role and that ppGpp is a possible intracellular signal linking RpoS and specific growth rate. From an ecological standpoint this "egoistic" strategy of self-determination by individual cells was deemed to be eminently sensible compared with the more risky option of depending on signals from other cells. The line of reasoning developed by Ihssen and Egli (2004) was supported by Potrykus, Murphy, Philippe, and Cashel (2011) who concluded that ppGpp is the major source of growth rate control in *Escherichia coli*.

4. Persister cells – "stealth bombers" in the microbial survival armoury?

Cells with slow or zero growth rates confer a very distinct competitive advantage on microbial populations that, as a result of minimal metabolism, are extremely difficult to inactivate. The term, "bacterial persistence," was introduced by Bigger (1944) who reported the inability of ampicillin to "sterilize" cultures of