Bioengineered yogurt: 'open source' medicine.

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Editorial

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INTRODUCTION

Among food ingredients, yogurt is unique in being among the few that contains active bacteria. The commensal bacteria found in yogurt are beneficial to the health of the gut and have been shown to possess probiotic effects. Yogurt is easy to 'manufacture', the process essentially involving adding yogurt to milk and letting stand for a period of hours.

Genetically modified bacteria have a long history of use as gene delivery vectors primarily by intravenous administration, some progressing to clinical trials. Several molecules have been bioengineered into *lactobacilli* including cyanovirin, interleukin-10, beta-carotene, cytosine deaminase and the extracellular domain of TRAIL (tumor necrosis factor related apoptosis inducing ligand). The discovery that a food grade bacterial vector can be ingested and can subsequently translocate out of the gastrointestinal tract to successfully deliver the gene of interest is relatively recent (1). It could have wide ranging implications to be applied to foods containing live bacterial cultures such as yogurt. In particular, the finding suggests that genetically engineered (GM) bacteria in yogurt need only be ingested in sufficient amounts to systemically deliver the product(s) of the engineered genes. Furthermore, the predominantly anaerobic and facultatively anaerobic bacteria in yogurt preferentially colonize hypoxic and necrotic regions such as those found in tumors although other mechanisms are active in such tumor homing as well (2).

The democratization of synthetic biology is evidenced by the presence of non-profit foundations such as the International Genetically Engineered Machines (iGEM®) foundation, the BioBricks® foundation and their information dissemination apparatus consisting of websites such as http://www.partsregistry.org from where DNA sequences of enzymes such as cytosine deaminase can be obtained (Figure 1). Commercial organizations can synthesize DNA cheaply and mail it as a plasmid (along with the obligatory antibiotic resistance gene coded in) in the form of a lyophilized powder. For example, Gen-Script® can synthesize the gene in a pUC vector that contains the amp^R gene for $0.29 per base pair. Anyone with a rudimentary knowledge of electrical circuits and components can build an elecroporation

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BBa_J32019 Part-only sequence (1306 bp)

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atggcgaataacgctttacaaacaattattaacgcccggttac
caggcgaagaggggctgtggcagattcatctgcaggacggaaa
aatcagcgccattgatgcgcaatccggcgtgatgcccataact
gaaaacagcctggatgccgaacaaggtttagttataccgccgt
ttgtggagccacatattcacctggacaccacgcaaaccgccgg
acaaccgaactggaatcagtccggcacgctgtttgaaggcatt
gaacgctgggccgagcgcaaagcgttattaacccatgacgatg
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cggcattcagcatgtgcgtacccatgtcgatgtttcggatgca
acgctaactgcgctgaaagcaatgctggaagtgaagcaggaag
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acctcacgcctgttccgcttgctgaaaatgtccggtattaact
ttgtcgccaacccgctggtcaatattcatctgcaaggacgttt
cgatacgtatccaaaacgtcgcggcatcacgcgcgttaaagag
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caaggacgttgaatttgcaggattacggcattgccgccggaaa
cagcgccaacctgattatcctgccggctgaaaatgggtttgat
gcgctgcgccgtcaggttccggtacgttattcggtacgtggcg
gcaaggtgattgccagcacacaaccggcacaaaccaccgtata
tctggagcagccagaagccatcgattacaaacgttgaacgact
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Figure 1 The DNA sequence of the enzyme cytosine deaminase, as downloaded from http://www.partsregistry.org

apparatus (Figure 2) and transfect store bought yogurt with the gene of interest. Colonies can be easily cultured under anaerobic conditions (obtained by the extinguishing of a lighted candle in a snap closed plastic jar for example) in an incubator (a closed polystyrene box with a lighted bulb of appropriate wattage) on commercially available agar plates in the presence of an antibiotic. The colonies that grow are those that contain the transfected bacteria. These can be harvested after growing for several generations and added to milk to make bioengineered yogurt.

Once the genetic sequences of interest have been identified, which is no small task, the obstacles to this egalitarianisation of synthetic biology are mainly procedural, rather than conceptual, and limited only by the perseverance of the experimenters, rather than by their knowledge. An extensive body of 'tool kits' already exists, which can be modified or expanded to suit particular applications, much like object oriented programming languages have made it possible for even computer programming illiterates to write and design 'apps'. 'Garage bioengineering' enthusiasts have synthesized the usual motley of gimmicks, as would be expected of any movement in its infancy, such as glow-in-the-dark yogurt, and beta-carotene yogurt. If and when the community matures, more esoteric and useful applications can be designed, such as a GDEPT (gene delivery enzymatic prodrug therapy) that would deliver the enzyme, e.g., cytosine deaminase specifically to tumor cells by eating bioengineered yogurt followed by the ingestion of 5-fluorocytosine (which can be obtained for $200/kg at http://www.alibaba.com), which would be converted to the tumoricidal drug, 5-fluorouracil, only in or in the vicinity of expressed cytosine deaminase in tumor cells. This model has been tested successfully in murine models using an attenuated strain of *Salmonella typhimurium* (3).

The ease with which commensal bacteria in yogurt can be bioengineered to express an array of biotherapeutics, coupled with altruistic distribution systems that can easily include those, most likely to benefit (think expression of antiplasmodial peptides in the *Bifidobacterium* genus so that a herder in the Sahel can inoculate this yogurt culture into goat milk thus making 'antimalarial yogurt' for free for his entire village. This could potentially go on as long as the plasmid is stable) and could lead to a revolution in the way medicines could become 'open sourced'. Antiplasmodial peptides such as scorpine and plasmodium enolase-plasminogen interaction peptide (EPIP) have successfully been cloned into *Pantoea agglomerans* and demonstrated efficacy in mosquito models (4). If bacteria can be engineered to produce the antimalarial drug, Artemisinin (as opposed to its precursor, artemisinic acid (5) malaria related deaths could be significantly reduced (although at the expense of producing resistance, see below).

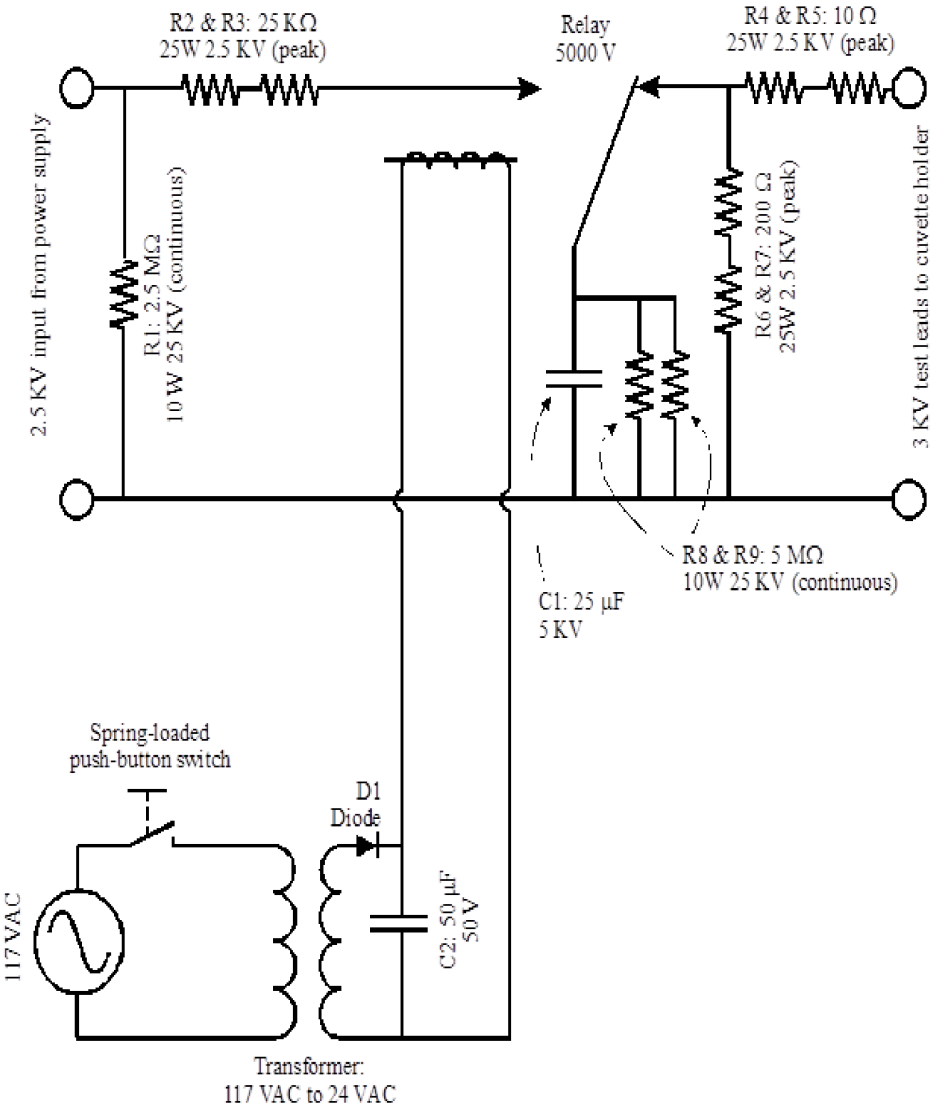

Figure 2 Circuit diagram of an electroporation apparatus, as downloaded from the website http://ebookbrowse.com/electroporator-doc-d25770175 .(The website does not have a digital object identifier (DOI) associated with it hence there is no guarantee that the link will be active in the future.)

Some may express horror at the suggestion of such egregious self-medication, especially in an era where medicines are highly regulated. Opinion is, however, not independent of economic and health status. One third of all Americans, have at some point taken recourse to 'alternative medicines'. The lure of such 'open source' bioengineered cures may be, in no small part, due to its legitimacy based on claims of evidence based 'mechanism of action', universality and ease of availability, perpetuity, and cost. Furthermore, because the science of genetics is, for the most part, algorithmically compressible, bioengineered products can be easily understood in terms of simplified semantic slang, making them all the more accessible, less daunting and deemed safe for use by the layperson.

The greatest danger of unregulated bioengineered products including bio-engineered yogurt is the difficulty of containment. Once a particular gene modified yogurt product is made available, there seems to be no

method to put the 'genie' back in the box. It may 'live on' for several years (depending on the stability of the transformed bacterial cell) and contaminate non-transformed sources and other organisms via horizontal gene transfer. Amongst other attempts, one promising approach is to insert the therapeutic gene in place of a key bacterial thymidylate synthase (thyA) gene, which is essential for DNA replication and survival outside the thymine rich environment of the human host. Such an approach has been successful in constructing genetically modified (GM) strains of the dairy bacterium *Lactococcus lactis* (6).

The other, eminently valid objection to DIY GM yogurt, is making non-standardized medicines available across the globe. Bioengineered medicines, especially those that can potentially be 'home-cultured' indefinitely do indeed possess non-standardized or sub-optimal dosing properties. Such variable dosages, especially of single compounds (antimalarial therapies containing artimisinin are recommended to be administered in combination with other antimalarial drugs to reduce resistance) may increase pathogen resistance.

If gene sequences can be used analogously to a Creative Commons attribution generic license (free to copy, distribute, transmit, adapt and make commercial use of), the hurdle to 'open sourced' medicine could be significantly reduced. Even if gene sequences could be patented, would these patents deter bioengineering enthusiasts? How can patents be enforced when each step of the distribution chain potentially is an infringing event (GM yogurt culture added to milk by villagers, shantytown dwellers, goat herders or migrant workers) and the source is virtually non-traceable?

The model of anyone, anywhere making bioengineered pharmaceuticals is a scenario that regulatory agencies are sure to look askance at. There is arguably not much that can be done to institutionalize such a ubiquitous phenomenon except for the restraint and common sense exercised by the community. It is interesting to compare 'open sourced medicine' with its altruistic counterpart, the Linux computing community. The latter has evolved into an, as yet still free, operating system (Ubuntu® 12.04 LTS, Precise Pangolin) that is arguably on par with commercial operating systems. Pharmaceutical for-profit organizations will need to deal with the 'creative destruction' that this truly 'mass manufacture' model portends.

We could encounter 'bioengineered yogurt' in village schools or the local village store, or as part of standard medicines issue for non-profit organizations such as Médecins Sans Frontières, in sanitation poor locales such as *favelas*, townships, or hutment colonies where it would make the most difference to the health of residents. Would there exist 'bio-depot' stores where one could buy plasmid expression cassettes, different strains of *bifidobacteria* or culture media and could get a DNA sequence synthesized? Would various 'flavors' of 'bioengineerd yogurt' be available at auction on ebay, with promises to cure diabetes, cancer, obesity or coronary disease? These are heady and exciting times for synthetic biology when new frontiers are being opened, elitist dogmas of synthetic biology are being demystified, the power of creation is gleefully being utilized by a small, but rapidly growing, populace of enthusiasts, hobbyists, amateurs and entrepreneurs. What comes out of this primordial soup is anybody's guess. It may be pertinent to quote the Bachman-Turner Overdrive band in 'You ain't seen nothin' yet', with regard to the magnitude of changes yet to come.

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