Scientific Rationale and Evidence for the Form and Function of Probiotic Mixed Culture Liquid Fermentations

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BioBrew Ltd products are multi-strain liquid probiotic fermentations containing yeasts and lactobacilli, manufactured to high feed grade standards.

What are Probiotics?

The FAO/WHO consultation in 2002 define probiotics as "*live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host*" (FAO/WHO, 2002).

Sanders (2008) goes further, defining, in part what probiotics are not:

"*The internationally endorsed definition of probiotics is live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Other definitions advanced through the years have been restrictive by specification of mechanisms, site of action, delivery format, method, or host. Probiotics have been shown to exert a wide range of effects. The mechanism of action of probiotics (e.g., having an impact on the intestinal microbiota or enhancing immune function) was dropped from the definition to encompass health effects due to novel mechanisms and to allow application of the term before the mechanism is confirmed.*

Physiologic benefits have been attributed to dead microorganisms. Furthermore, certain mechanisms of action (such as delivery of certain enzymes to the intestine) may not require live cells. However, regardless of functionality, dead microbes are not probiotics.

The term "probiotic" is sometimes erroneously used as a synonym for putatively beneficial members of commensal microbiota. The context for this misuse is the assertion that certain dietary or environmental factors may "encourage your native probiotics." Members of human commensal microbiota are often sources from which probiotics are isolated, but, until such strains are isolated and then adequately characterized for content, stability, and health effects, they are not probiotics."

Lactobacilli as Probiotics

The genus *Lactobacillus* contains about 80 species inhabiting niches as diverse as the honeybee stomach and the surface of leaves (Tannock, 2004). Some species have had an intimate relationship with human food and have been used for preservation and fermentation of foods as diverse as sake, sourdough, saukraut, yoghurt and sausages (Tannock, 2004).

Metabolically and nutritionally Tannock (2004) summarizes them well "They are strictly fermentative, aerotolerant or anaerobic, aciduric or acidophilic, and have complex nutritional requirements (carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, vitamins). Using glucose as a carbon source, lactobacilli may be either homofermentative (producing more than 85% of fermentative products as lactic acid) or heterofermentative (producing lactic acid, carbon dioxide, ethanol, and/or acetic acid in equimolar amounts)." These metabolites are, themselves of nutritional value and will be discussed later.

A distinction needs to be made between the use for food fermentation and the probiocity of the organisms and Hussain (2014) summarizes this nicely:

"*Production of a product with specific sensory traits and consumer acceptability is the major objective of microorganisms used in fermented food products. Contrary to this, the essence of organisms' presence in a probiotic product is associated with interventions for improved health and wellbeing of the host* (animal or human)."

Isolates of lactobacilli with probiotic characteristics go through a screening process of which passage through the gastro intestinal tract is the first hurdle. Specific isolates have specific benefits to the digestion and immune status of the host animal. There are currently available on the New Zealand GRAS (**g**enerally **r**egarded as **s**afe) register a list of 11 species i.e.:

Lactobacillus acidophilus
Lactobacillus bifidus
Lactobacillus brevis
Lactobacillus buchneri
Lactobacillus bulgaricus
Lactobacillus casei
Lactobacillus delbrueckii subspecies *lactis*
Lactobacillus fermentum
Lactobacillus plantarum
Lactobacillus rhannosus
Lactobacillus salivarius
(http://www.foodsafety.govt.nz/registers-lists/gras/onc.htm)

BioBrew Ltd has recently added another species
Lactobacillus reuteri

It must be noted that not all strains of these species show probiotic effects and that each isolate must be individually assessed for probiocity.

Yeasts as Probiotics

Saccharomyces cerevisiae is the common yeast of beer, wine and bread. *S. cerevisiae* has been used as a digestive enhancer and specific strains, usually of *S. cerevisiae* ssp. *boulardii*, have been used in ruminant and monogastric animal nutrition.

Effects of Probiotic Organisms

The gut of mammals and birds is a complex microbial environment and shows strong ecological resilience or homeostatic properties (Tannock, 2004). Probiotic organisms are considered to be transitory in the gut environment as they typically disappear from stool samples shortly after administration has ceased (Tannock, 2004).

While the residence time in the gut may be relatively short compared with the established microflora, there is ample scientific evidence that probiotic organisms affect the host animal in a number of ways including e.g. stimulating gut epithelial cells to replace and shed (Tannock et al, 2014), reduced gut inflammation (Tannock, 2004), changes in gene expression within the gut lining (Tannock et al, 2014), producing bacterocin like substances to directly inhibit pathogenic organisms (Mørtvedt et al, 1991), improved host immune status and protection from specific gut pathogens (Hamilton-Miller, 2003).

Fresh vs Freeze Dried

Very little work has been done analyzing the difference between the use of freeze dried powder verse using fresh, active probiotics in animal husbandry. Indeed there appears to be very few comparisons in the literature for human use either. This is unsurprising when we consider that most commercial "products" are sold in a dry "powdered or pelleted" form and the companies selling these products have little incentive to draw attention to the differences between preserved and fresh products.

A comparison in humans using the probiotic *Lactobacillus* GG concluded that in the active fermented form "the lowest colonizing dose was 100 million living bacteria, but in dry pharmaceutical preparations (food supplements) a daily dose needed was 10 billion colony-forming units." (Salexlin, 1996). Two orders of magnitude more freeze dried CFU are required for a probiotic effect and this makes sense given that freeze-dried *Lactobacillus* take an hour or more to "wake up" and are more easily damaged by stomach acids (Craig Bunt, pers comm.)

Note: BioBrew products are fresh and actively metabolizing when fed to stock.

Single vs. Multi Strain Probiotics

Professor Xiyang (Kent) Wu, (The Department of Food Science and Engineering, Jinan University, Guangzhou, China), presented recently at Asia-Pacific Workshop 20-21 October, 2014. (Centre for Food Research and Innovation, Lincoln University, New Zealand) on the topic of developing multi strain DFM (direct fed microbials, a term used for animal feed where the word "probiotic" is regulated).

To quote from his abstract: "*combinations of different probiotic organisms have an advantage over the use of any single culture alone*." (Wu, 2014)

Note: BioBrew products contain a range of probiotic organisms in a mixed fermentation

Selection Criteria

When assembling and formulating a probiotic product (or DFM) it is important to know that the microorganisms survive the production process (Bunt, 2014). Indeed many products and formulations for human consumption fail to live up to the label claims (Nezhad, 2014). Many probiotic isolates are put through damaging production and manufacturing processes. This requires they be screened for the ability to withstand this whilst retaining their probiotic characteristics.

Note: BioBrew products contain a range of probiotic organisms that are tested for viability both during production and through to the end of shelf life

Quality Assurance Criteria

"*Key quality indicator for quality of a probiotic product is maintenance of viability of the probiotics*." (Hussain, 2014) This is an area of particular importance as seen in the studies by Nezhad (2014) and, in New Zealand agricultural preparations Bennet, et al (2013).

Evidence of robust QA systems and criteria were recently presented at Asia-Pacific Workshop 20-21 October, 2014. Centre for Food Research and Innovation, Lincoln University, New Zealand (Prassinos et al, 2014)

Note: BioBrew Ltd has an active QA system that tracks batches and quality throughout the production and sales cycle.

Probiotics and Organic Acids as an Alternative to AGPs (Antimicrobial Growth Promoting agents)

Europe has been without the use of AGPs for longer than any other part of the world. When ranking various alternatives to AGPs on a scale of 1-5 (1 least effective, 5 most effective) European feed professionals ranked organic acids at 3.7 and probiotics at 3.0, while in the Asia/Pacific region the rankings were organic acids at 3.8 and probiotics at 3.7 (Riley, 2014).

One of the confounding factors in the ranking of these probiotics has been the variability of formulations and diverse modes of delivery (William Riley, pers comm.)

Note: BioBrew products contain a range of organic acids as the byproduct of metabolism during the culturing of our mixed probiotic fermentations. These organic acids help maintain a stable environment for improved shelf life as well as directly conferring benefits to the target animals

The Evidence for Quality and Efficacy of BioBrew Products

In 2013/14, a Masters student co-sponsored by BioBrew and the New Zealand Government through Callaghan Innovation, to examine the population ecology, examine metabolites produced, and systemize BioBrew's QC (quality control) measures into a robust QA (quality assurance) system. The student (Nagaiah Koneswaran) undertook the work at the BioBrew facility in Christchurch, NZ and was co-supervised by Dr Malik Hussain and Dr Craig Bunt, of Lincoln University and Don Pearson from BioBrew Ltd.

Quality Control

The quality control system developed involves the sampling and recording of time, temperature, pH and the testing of samples for cfu (colony forming units following serial dilution) taken throughout the production cycle

Lactobacilli Numbers

Lactobacilli cfu numbers are consistently in the close vicinity of 10^9/ml (and 10^{10} is not unheard of) and we certify our products to at least 10^8/ml at the end of shelf life

Organic Acids and Volatile Organic Compounds

A range of volatile and soluble compounds were tested for using SPME/GC-MS.

Relative amounts (µg/L) of major volatile compounds identified by the SPME/GC-MS from BioBrew products

	Days since bottling			
Metabolite	7	14	28	42
Ethyl acetate	90	110	219	229
Ethanol	1196	1252	1244	975
Ethyl butyrate	464	566	534	682
Isobutanol	41	36	51	29
Isoamyl acetate	27	29	57	38
Isoamyl alcohol	427	463	517	341
Acetic acid	311	312	392	458
Butyric acid	2723	2819	2331	2331
Phenylethyl Alcohol	237	237	266	158
Phenol	83	67	64	67

In addition ethanol and lactic acid levels were tested using commercial test kits.

	Average Levels (mg/100ml)
Ethanol	419
D-lactic acid	154
L-lactic acid	183
Total lactic acid	337

Efficacy Trials and Case Studies

Calf Trial Spring 2013

In the spring of 2012 an ambitious calf trial was undertaken in southern South Island, New Zealand. The aim of the experiment was to test if a fresh, locally produced, liquid agricultural probiotic product (BioBrew) would increase the growth rate of semi-extensively hand reared dairy calves (Bennett and Deverson, 2013).

The experiment involved 3 commercial dairy farms and some 300 calves. There were 20 pens (approximately 15 calves per pen), matched in pairs, with one of the pair receiving the liquid probiotic in milk or milk replacement and the other receiving only the milk or milk replacement (Bennett and Deverson, 2013).

Average growth per day in pens from Farm 1, 2 and 3

	Treatment	Control	Difference	P value
Farm 1	0.662	0.644	+18g/day	Not significant
Farm 2	0.634	0.577	+57g/day	P > 0.01
Farm 3	0.573	0.534	+39g/day	P > 0.02

(taken from Bennet and Deverson, 2013)

The above table shows an increase in growth rate on two of the three farms involved in the trial. Coupled with this was an apparent increase in survival i.e. death rate in the treated calves 2:150, death rate in the untreated calves 10:150. No statistical significance was placed on this due to experimental design but if concurs with the general trend of increased "wellness".

"It is worth noting that Farm 3 was the only farm that kept the calves indoors during the entire trial. On the other two comments from those operating the trials at the farms included reports that treated calves coped with the stress of being turned out to pasture better than the control. That is to say that the calves on Farm 3 were raised absent a major stress event early in the calf's life, perhaps explaining the smaller effect noted on Farm 3. Data from Farm 3 also had a number of confounding factors ."(Prassinos, pers comm)

Anecdotal Evidence

Anecdotal evidence of the efficacy of BioBrew products abounds and the reader is referred to:
http://www.biobrew.net.nz/
https://www.facebook.com/EquiBrew and
https://www.facebook.com/biobrew
for further evidence.

BioBrew products:

Quality Assured consortia of probiotic microbes with high viability, high activity, and beneficial metabolites (including organic acids). Our products are acceptable

to animals, affordable for farmers, and conform to international regulatory frameworks.

References

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