

INHIBITORY EFFECTS OF LACTIC ACID BACTERIA ON SOME PATHOGENS INCLUDING MRSA

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ABSTRACT

Lactic acid bacteria is referred as a probiotic in scientific literature by Lilley and Stillwell (1965). It was redefined by Parker (1974) as organisms and substances which contribute to the intestinal microbial balance. The latest and the most accurate description of probiotics like lactic acid bacteria based food products was presented by Fuller (1989) as a live microbial feed supplement beneficial to the host by improving the microbial balance within its body. This balance is achieved by its inhibitory effect upon the harmful pathogenic microorganisms.

This paper presents some background on the scientific research and development of one such encapsulated product which has become a commercial reality. This product¹ is fermented by 12 strains of specially isolated and cultured lactic acid bacteria from the genera *Lactobacillus*, *Bifidobacteria*, *Streptococcus* and *Enterococcus*. The substrate consists of mountain fruits, plants, herbs and mountain spring water and the fermentation is done under natural conditions. The final capsule has a total count of 59 million colony forming units (cfu) of lactic acid bacteria. The high level of organic acids in the final fermented product further contributes to its antimicrobial property.

One of the strains used in the OMX capsule is *Enterococcus faecalis* TH10 isolated from the local food delicacy *tempeh*. This strain has shown inhibition on methicillin resistant *Staphylococcus aureus* (MRSA). The active inhibitory component was extracted with ethyl acetate at pH3 and did not show growth inhibition against the various lactic acid bacteria. Similar results were also obtained on *E.coli* 0157.

All strains of the lactic acid bacteria placed in the food supplement were found to be active against *Helicobacter pylori*. While more work is in progress, these findings establishes the effectiveness of the product as a functional food with significant antimicrobial properties

INTRODUCTION

Lactic acid bacteria are shown to play an important physiological role in the human gastrointestinal (GI) tract. Microbial interactions represent the main force in the homeostatis of the bacteria flora in the GI tract. Combined with the host this microflora form a unique ecosystem in which the complex interactions can either be synergistic or antagonistic depending on their strain identity, populations and metabolic activity. The healthy survival of the host is determined by these interactions. The ecosystem is destabilised as a result of gastrointestinal disorders and

* Branded under the label Ohhira Mountain fruits eXtract (OMX)

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other endogenous components such as enzymes, hormones and immunoglobulins can influence it.

The importance of maintaining a beneficial microbial balance in the GI tract is vital to the stability of its ecosystem and the optimal health of the host. The colon is also known to contain up to 3.5 pounds of bacteria of which some 700 types can give rise to alimentary toxemia while only 20 types are harmless. Toxemia results from harmful bacteria. A capsule of probiotics contain specially isolated lactic acid bacteria which can inhibit the activity of several pathogenic bacteria and thereby overcome this toxemia. Ultimately this can bring about a favourable balance of beneficial or synergistic bacteria over the harmful ones. This paper examines one such product developed through a period of intensive research and development and can serve as an effective functional food or health supplement to bring about this favourable colonic microbial balance. This paper presents the scientific rationale for one such product, its antimicrobial efficacy and how it consequently benefits the host.

METHOD OF PRODUCTION

The lactic acid bacteria are mass produced for encapsulation by a natural fermentation process. The substrate used consists of mountain fruits, plants, herbs and pure mountain spring water. Specially isolated and cultured strains of lactic acid bacteria are used. No other additives are used. The inoculum consists of four major groups comprising six strains of *Lactobacillus*, three strains of *Bifidobacteria*, one strain of *Streptococcus* and two strains of *Enterococcus*. The substrate is allowed to ferment naturally with the inoculum for five years after which the end product in paste form is extracted for live encapsulation. At the end of the fermentation the final product has a high level of four organic acids produced by the 12 strains in the inoculum:

LACTIC ACID
ACETIC ACID
FORMIC ACID
FUMARIC ACID

The shelf life of the bacteria in the capsule* has been monitored and found to be viable for a period of five years. An analysis of the metabolites in the final product has shown its efficacy by way of its functional ingredients as a functional food. Eighteen types of amino acids (Table 1),

TABLE 1: Amino acid contents in lactic acid bacteria capsule sample

<u>AMINO ACIDS</u>	<u>g / 100 g</u>
Arginine	0.10
Lysine	0.10
Histidine	0.08
Phenylalanine	0.26
Tyrosine	0.14
Leucine	0.41
Isoleucine	0.25
Methionine	0.05
Valine	0.28
Alanine	0.22
Glycine	0.19
Proline	0.42
Glutamic acid	1.11
Serine	0.22
Threonine	0.18
Aspartic acid	0.68
Tryptophan	0.04
Cystine	0.06

five minerals (Table 2) and nine vitamins were determined by qualitative analysis.

BIOSAFETY

For quality control the final product is subjected to qualitative analysis and quantitative estimation for the presence of any heavy metals (Table 3). Arsenic, lead, copper, tin, mercury were tested. The levels of these parameters were far below the accepted or permitted limit of international standards (Food Regulation Act, 1985). For instance, the lead level is 0.07 ppm compared to the allowed standard of 2 ppm. Copper present is 0.53 ppm compared to the standard of 300 ppm. It is also very reassuring to note that mercury, cadmium and antimony

TABLE 2: Level of essential minerals in samples of lactic acid bacteria capsules

<u>Mineral</u>	<u>Level (mg / 100g)</u>
Phosphorus	176
Iron	4.07
Calcium	123
Sodium	58.6
Potassium	899

are not detected. This ensured the biosafety of the fermented end product.

Antimicrobial properties of omx lactic acid bacteria

The most significant finding is the inhibitory activity of these specially isolated strains of lactic acid bacteria against three pathogenic bacteria - methicillin resistant *Staphylococcus aureus* (MRSA), *Helicobacter pylori* and *Escherichia coli* 0157. MRSA is a challenge to medical practitioners as it is involved in nosocomial infections worldwide. During an outbreak it has been shown to play havoc in hospitals. Antibiotics lead to only partial control during a widespread infection. The emergence of vancomycin resistant *Staphylococcus aureus* is also of great concern (New England Journal of Medicine, Feb 18th 1999 issue). One of the 12 strains used in the capsule - *Enterococcus faecalis* TH10 - demonstrated inhibition on the MRSA. This strain is used in the fermentation process and thrives well within the capsule. Originally isolated from our local Malaysian delicacy - *tempeh* - the strain was cultured and used as the inoculum for the production of the capsules. The strain was tested for its antagonism against MRSA. For qualitative test, a paper disc bioassay technique was used. Zones of inhibition were noted in comparison to the control. Further investigations revealed that the active component of TH10 extracted in ethyl acetate at pH3 retained its activity after treatment with various proteases. The bacteriocidal activity of the ethyl acetate extract was tested using MRSA and a variety of related lactic acid bacteria strains as the test organisms (Table 4). Contradictory to the common properties of bacteriocins, the extract did not show potent growth inhibition or activity against the various closely related lactic acid bacteria. However the growth inhibition against MRSA was evident while most of the lactic acid bacteria were not affected. *Streptococcus salivarius* and *Pediococcus acidilactici* were affected, but only when the dosage was twice the amount required for the inhibition of MRSA. In addition it was found that the active component retained the bacteriocidal activity against MRSA when it was treated with various proteases such as proteinase K, V8 protease, trypsin and achromopeptidase. These results suggest that the active component is not a peptide or protein. Further work is in progress to determine more details on its physical and biochemical characteristics which is beyond the present scope of this paper. But it is

important to note that the active component of TH10 did not show any hemolytic activity towards human and rabbit erythrocytes.

Inhibition of *helicobacter pylori*

The 12 strains of lactic acid bacteria in the test capsule showed antimicrobial activity against *H. pylori in vitro* as shown by loss of viability of the latter. A well diffusion

TABLE 3: Biosafety of lactic acid bacteria test capsule contents

N o.	Heavy metal	Level in OMX*	Permitted level (Food Regulation Act 1985)*
1.	Arsenic (As)	0.22	1
2.	Lead (Pb)	0.07	2
3.	Copper (Cu)	0.53	30
4.	Tin (Sn)	0.04	40
5.	Zinc (Zn)	3.33	40
6.	Mercury (Hg)	Not detected	0.05
7.	Cadmium (Cd)	Not detected	1
8.	Antimony (Sb)	Not detected	1
9.	Total plate count of lactic acid bacteria per capsule	59,000,000 cfu	

* parts per million (ppm), maximum

assay technique was used. One test capsule was dissolved and incubated in All-purpose medium with Tween (APT) broth with or without biotin for good recovery of the organisms from the capsule. The results obtained in Table 5 is the mean of duplicate tests. The colonies recovered from the capsule dissolved in water and then plated on APT agar and de Man, Rogosa and Sharpe (MRS) agar showed decrease in the number of colony forming units of the *H pylori* strains (NCTC11637 & NCTC11638). This preliminary results explains how viable lactic acid bacteria contained in live capsule based probiotic can bring about much relief to those suffering from peptic ulcers and gastritis by inhibiting the *H. pylori* infection.

Growth inhibition on *E.coli* 0157

The component from TH10 strain was extracted with ethyl acetate and then neutralised by regulating the pH. Then variable rates (0.75, 1.5, 3.0, 6.0, 12 and 24 mg) of the lactic acid bacteria extract were introduced on dipped paper discs into petri dishes containing cultured viable colonies of *E.coli* 0157 in several replicates. These petri dishes were incubated at 37 degrees centigrade. Growth inhibitions of the 0157 strain were monitored by observing the diameter of zones of inhibitions obtained around the

paper discs. Results indicated that inhibition of 0157 were obtained at all amounts of 3.0mg and above of the extract. Consistent results were obtained on repetition. Inhibitory effects were increased with more amount of the extract. The substance acting on the 0157 strain is initially made out to be of low molecular weight and further research is being carried out to specify its structure and mechanism of activity.

TABLE 4: Inhibition of ethyl acetate extract of *Enterococcus faecalis* th10

Test organism	Dosage (mg) ^a		
	20	10	5
Methicillin resistant <i>Staphylococcus aureus</i>	+	+	-
<i>Enterococcus faecalis</i> RIMD 3116001	-	-	
<i>Enterococcus faecium</i>	-	-	
<i>Streptococcus salivarius</i>	+	-	
<i>Pediococcus pentosaceus</i>	-	-	
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	-	-	
<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	-	-	
<i>Lactobacillus plantarum</i>	-	-	
<i>Lactobacillus delbrueckii</i>	-	-	

a

Dry weight of the ethyl acetate extract
+ Growth inhibition of test organism around paper disk
- No inhibition

MODE OF ACTION

At this stage the explanation that can be offered is the action of the different bacteriocins and the lowering of pH by the lactic acid bacteria test capsule inhibiting the proliferation of the antagonistic microorganisms. With the five years fermentation and low pH achieved it is found that the acidolin produced by the *Lactobacillus acidophilus* is particularly active against bacteria and viruses. This evidence is further supported by earlier research of Hamdan and Mikolajcik (1974). This makes the lactic acid bacteria test capsule highly effective in terms of its antimicrobial property as well as a functional food supplement.

TABLE 5: Inhibition of *helicobacter pylori* strains (nctc 11637 & 11638) after 24 hrs in WCACB broth with pre-dissolved omx capsule for 18 hrs

SAMPLES	TIME	
	0 hrs	24 hrs
<i>H. pylori</i> NCTC 11637	2 x 10 ⁷ cfu / ml	9 x 10 ⁹ cfu / ml
<i>H. pylori</i> NCTC 11637 + test capsule	2 x 10 ⁷ cfu / ml	3 x 10 ⁷ cfu / ml
<i>H. pylori</i> NCTC 11638	5 x 10 ⁷ cfu / ml	8 x 10 ⁹ cfu / ml
<i>H. pylori</i> NCTC 11638 + test capsule	5 x 10 ⁷ cfu / ml	8 x 10 ³ cfu / ml

REFERENCES

1. Lilley, D M and Stillwell, R H (1965). Probiotics: growth promoting factors produced by microorganisms. *Science* 147 : 747 - 748
2. Parker, R B (1974). Probiotics, the other half of the antibiotic story. *Anim. Nutr. Health* 29 : 4 - 8.
3. Fuller, R (1989). Probiotics in man and animals. *J. Appl. Bacteriol.* 66 : 365 - 378.
4. Hamdan, I Y and Mikolajcik, E M (1974). Acidolin: an antibiotic produced by *Lactobacillus acidophilus*. *Journal of Antibiotics* 8 : 631 - 636.