The Biorem Culture

Multi-strain, stress adapted Probiotic

OVERVIEW

The Biorem-culture is a multi-strain, stress adapted probiotic presented in both a liquid and powder form. Development on this product started in the early 1980's and a patent were registered in 1986 (Patent number 87/9287). The culture, containing 33 individual bacterial strains, used in both the animal and human health industries. Some of the characteristics of this culture still make it, after almost four decades, unique and very effective with an exceptional shelf life.

CHARACTERISTICS

1. The Biorem-culture contains 33 strains from 5 different species:

Pediococcus pentosaceus

Lacticaseibacillus paracasei

Lactiplantibacillus plantarum

Levilactobacillus brevis

Lacticaseibacillus rhamnosus

In some products Lactobcillus infantis, Lactobacillus acidophilus and Lactobacillus reuteri strains are also added, although it is not cultured in combination with the Biorem-culture strains.

2. Lucerne (Alfalfa) is used as part of the medium and carrier for the microbes and is included as part of the fermentation process during manufacturing. During the production of the liquid probiotic, it is used as part of the inoculum.

3. The Biorem-culture is stress adapted (stress buffered) and can also tolerate larger variations in pH and temperature than conventional probiotics, increasing survivability when passing through the stomach into the duodenum.

4. Immediate multiplication, post inoculation or dosing, occurs in patients, reducing the number of colony forming units (cfu's) required per gram or milliliter.

5. Shelf life has been tested over a period of 5 years, although 3 years can be guaranteed.

6. Antagonism against multiple strains of Escherichia coli, Salmonella, Clostridium and Campylobacter has been demonstrated *in-vitro*.

STRESS ADAPTATION

Most probiotics are manufactured by using simple sugars, such as dextrose, galactose and lactose, as energy sources for the microbes. During fermentation, simple sugars are more easily converted to ATP.

With the manufacturing of the Biorem-culture, more complex carbohydrates such as starches and non-digestible (for mammals) dietary carbohydrates are provided as an energy source. The Biorem-culture's bacterial strains harbor an abundance of genes encoding carbohydrate-active enzymes, allowing them to switch between various energy sources.

Multiple stressors are also added throughout the inoculation preparation process increasing the microbes' defenses and optimizes metabolic processes, further increasing its' ability to make use of complex and non-digestible carbohydrates.

Both these mechanisms add to the survivability and efficacy of the strains.

SHELF LIFE

Most manufacturers of probiotics use freeze drying and spray drying onto a carrier such as maltodextrin or dextrose as methods for preservation and concentration. The Biorem culture is not concentrated, but cultivated on its carrier, Alfalfa (lucerne), which also provides the energy source for the microbes, before it is dehydrated by air drying over a period of days. The organisms penetrate the growth medium and carrier during fermentation and is therefore not just present on the surface of the carrier.

The metabolic rate of the bacteria is slowed down significantly during the gradual drying period and enables the organisms to survive over a long period of time. Because the growth medium is also used as a carrier for the microbes and cannot be absorbed by the host immediately after consumption as is the case with simple sugar carriers, it provides an immediate and available source of energy to the microbes to multiply.

Shelf life and stability studies on the final product were performed to determine the survivability of the organisms.

STABILITY AND SHELF-LIFE STUDIES ON THE BIOREM-CULTURE

The results of a study on the viability of the micro population over a period of 4,5 months at 25^0 C, given in colonies per gram sample, grown on ROGOSA-agar, are presented in Table 1.1

WEEK	LAB counts (cfu/g) on Rogosa Agar
0	5.8×10^8
1	5.9×10^8
2	4.2×10^8
3	4.0×10^8
4	1.5×10^7
5	1.4×10^8
6	1.4×10^8
7	1.6×10^8
8	1.3×10^8

Table 1.1

Discussion

The decrease in the total population over the period of 18 months was insignificant, indicating the biological stability of the dry product.

The lactic acid bacterial population was represented as indicated in the composition of the culture.

The viability is greatly influenced by the relative moisture content varying between 6% to 12%. A drastic decrease in the viability occurs when the relative moisture count falls below 6%.

By extrapolation of the data a shelf life of 3 years with a minimum of 1.3×10^7 viable organisms per gram can be expected at room temperature.

This data was supported by a minor decrease in viable organisms in product tested after being stored for 3 years at room temperature:

An actual shelf-life study was done on the dry BIOREM-culture over several years (Table 1.2 to support the extrapolation of results in the study described in Table 1.1.

Batch no.	**Date Manufactured**	**Date Tested**	**Date Tested**
	12/10/1987	**10/08/1989**	**13/02/1991**
871012			
LAB Count (cfu/g)	6.1 X 10^8	5.0 X 10^8	
	04/02/1987		
870204			
LAB Count (cfu/g)	2.6 X 10^8	1.5 X 10^7	5.1 X 10^7

Table 1.2

An extended shelf life study was also performed, see Table 1.3 below, to confirm the previous findings (Table 1.1 and 1.2) and further study the duration of the shelf life of the Biorem-culture

Batch no.	**Date Manufactured**	**Date Tested**	**Date Tested**
	09/04/2006	**10/10/2008**	**20/04/2011**
060409			
LAB Count (cfu/g)	4.1 X 10^8	2.2 X 10^8	1.9 X 10^7
	12/02/2006		
060212			
LAB Count (cfu/g)	3.4 X 10^8	2.1 X 10^7	8.9 X 10^6

Table 1.3

Conclusion

These experiments as well as the continual testing of older samples confirmed extrapolations regarding the shelf life of the BIOREM-culture. For these tests, air dried product, with no special storing instructions other than that of keeping the lid of the container closed, store under cool, dry conditions, applied.

EFFICACY

The Biorem-culture has been used as an effective commercial probiotic in the market for animals since 1985 and for humans since 1987.

Over the period of its existence, several studies and trials have been conducted for several applications.

During stress and disease, a chain of processes and hormonal secretions, cortisol, corticosterone, aldosterone, and some other corticosteroids are most often induced. One of the functions of cortisol is to increase the absorption of readily available energy, including those found in sugars that form part of the mucosa in the small intestine and colon. These sugars also serves as the predominant energy source for the microbiome living in the digestive tract. Due to the sudden scarcity of energy sources, these organisms have to compete more intensely and this often results in a large portion dying off. This gives opportunistic pathogens such as E.coli species an opportunity to become dominant and leading to digestive disorders such as diarrhoea. Furthermore, the integrity of the mucosa is sometimes jeopardized, increasing the risk of further infections.

As stress are more often becoming a chronic occurrence, especially in modern society, it may result in further chronic digestive disorders such as irritable bowel syndrome (IBS), spastic colon and even cancers or tumors in the lower digestive tract.

The supplementation of conventional probiotics, using simple sugars and highly digestible starches as carrier, may prove to be ineffective, as these carriers also serves as an energy source for the supplemented organisms and is almost immediately absorbed through the small intestine into the bloodstream. The competition for available energy between the body, other microorganisms and the supplemented microbes leaves a low potential of survivability for the supplemented microbes.

With the different mechanisms implemented during manufacturing of the Biorem-culture, the probability of survival for the Biorem-culture improves significantly when compared to other probiotics. and may explain the following results obtained during 5 small human trials over a period of 8 months.

An efficacy study of the Biorem-culture was conducted in the following controlled trial.

Introduction

BIOREMferment is a Lactobacillus containing preparation and a probiotic by definition. Beneficial effects have been observed independently by several physicians in the treatment of either diarrhea or constipation. The increased incidence of colectomies over recent years is proof of the severe problem diarrhea and constipation still presents. This is also emphasized by the relatively large percentage of patients suffering from these conditions and the fact that conventional treatments are often ineffective. In a series of experiments the effect of BIOREMferment on patients suffering from either diarrhea or chronic constipation was assessed.

Material and Methods

BIOREMferment was used over an 8-month period to assess the effect on patients of both genders and of different backgrounds and ages. A total of 52 patients were involved and 5 different batches of BIOREMferment (prepared by professor. Steyn, Dept. of Microbiology, University of Pretoria) were used. In the first 2 trials only, the intact preparation was used and there were no controls to allow for placebo effects. In the third and fourth trials the intact preparation was compared to a control, representing a preparation in which the micro-organisms were inactivated by heat treatment. In the fifth trial a live preparation, a heat-inactivated preparation and a lactic acid suspension were compared. The patients were treated 3 times a day with 500 µl, mixed into a glass of water.

Patients suffering from the following disorders were treated: (a) diarrhea (n=8), (b) chronic constipation (n=42), (c) Crohn's disease (n=1), (d) chronic unresponsive diarrhea due to hemodialysis (n=1). Some of the patients who reacted favorably in earlier trials were included as controls in later ones.

Results

A summary of the results is given in Table 2.1. A good result in Table 2.1 indicated a clear improvement in a patient's stool habit and other associated problems such as flatulence and stomach cramps. The decision was usually based on whether the patient's situation changed to one in which the quality of life returned to normal. A satisfactory result was referred to as a noticeable improvement in stool habit, but where the quality of life was still not back to normal. (A variation of 4 stools a day or 1 stool every 4 days was used as the criteria.) An

unsatisfactory result indicated one where there was little or no improvement in stool habit and/or associated problems.

Trial #	Treatment	Number		Results		
				Good	Satisfactory	Unsatisfactory
1	Biorem-culture	20	20	16	2	2
2	Biorem-culture	10	10	8	1	1
3	Biorem-culture	12	6	5	1	0
	Inactivated Biorem-culture		6	0	2	4
4	Biorem-culture	15	8	3	4	1
	Inactivated Biorem-culture		7	0	3	4
5	Biorem-culture	30	10	3	4	3
	Inactivated Biorem-culture		10	0	2	8
	Lactic Acid		10	0	0	10

Table 2.1: The effect of 500 µl of BIOREMferment, inactivated BIOREMferment and a suspension of lactic acid, mixed into a glass of water, and taken over a day period. The results are of 5 different trials over an eight-month period.

Discussion

The patient with unresponsive diarrhea due to hemodialysis treatment showed a remarkable improvement when treated with BIOREMferment Treatment with the inactivated BIOREMferment had no effect and at the end of the trial the patient demanded continuation of the BIOREMferment treatment. BIOREMferment had a positive effect on patients with stress related chronic diarrhea. In patients with gastro-enteritis, where BIOREMferment was used as adjuvant to broad-spectrum antibiotics, the rapid return to a normal stool habit was observed.

The patient with Crohn's disease had a very positive response to BIOREMferment but due to pressure from the surgeon she agreed to a colectomy.

Results of the first three trials were most promising; inclusion of control patients in trial 3 proved that the beneficial action could only be attributed to BIOREMferment. Only in patients with a constipation history of longer than 10 years, combined with the abuse of purgatives the initial results were either good and turned less satisfactory after some time or were unsatisfactory from the onset. This can perhaps be attributed to permanent changes of damage to the intestinal mucous layer, preventing the normal colonization by micro-organisms, and may have partially influenced the outcome of trials 4 and 5.

Conclusions

The results during the initial trials with BIOREMferment were impressive and BIOREMferment could be an immense help in the treatment of chronic constipation and diarrhea, especially since treatment by colectomy for constipated patients have inherent dangers and should be avoided if possible.

It would appear that the misuse of purgatives over a long period inhibits the advantageous effect of BIOREMferment, although the result of the fourth and fifth trials had a marked effect on this conclusion.

In vitro studies have been conducted to determine the antagonism of the Biorem-culture against pathogenic and opportunistic pathogenic organisms.

Trial 3:

Experimental Results

The BIOREM-culture comprising the different strains exhibited antagonism in-vitro to pathogens as in Table 3.1. These confirmed Pathogens have been obtained from the University of Pretoria, Dept Microbiology.

Salmonella java
S. typhimurium
S. typhimurium 2005
S. typhimurium 1839
S anatum 840
S. loma-linda 2463
Escherichia coli 7917
E. coli 8082
E. coli 8025
E. coli 7821
E. coli 7734
E. coli 8386

Table 3.1

The Effect of Synergism

The *in-vitro* determination of antagonism of the different strains in the BIOREM-culture against pathogens have been done using the agar-disc technique and compared with a mixed culture of all the Lactic Acid Bacteria in the BIOREM-culture.

Lawns of the different BIOREM isolates were prepared on pour plates of MRS agar (Biolab) incubated for 48 hours at 26^0 C. Seven mm diameter agar-discs were cut with a sterile cork drill placed on Nutrient agar (Biolab) spray plates of pathogenic E. coli-culture obtained from the Department Medical Microbiology, University Pretoria.

Spray plates were prepared as follows: The pathogen was grown in STD I (Merck) broth and 0.1 ml culture was spread on STD I agar with a glass rod and left to dry for 1 hr. Three agar discs containing the Biorem Lactic Acid Bacteria were placed upon the spray plates with sterile MRS agar disc serving as control. These spray plates were then incubated for 48h at 37^0 C.

The diameter of clear zones around the discs indicating inhibition, were measured with a micrometer.

Results and Discussion

In-vitro determination of antagonism

Results are presented in Table 3.2

All isolates exhibited antagonism to E. coli to a greater or lesser degree. The area of the inhibition zone was taken as a measure of antagonism. Inhibition was found to be, in decreasing order, as follows:

Mixed culture: B28; B14; B15; B9.

It was clear that the mixed culture showed better inhibition than any single culture.

It was thus evident that the synergistic effect of the mixed culture enhanced antagonism.

Culture	Surface mm²	Culture	Surface mm²
Mixed	64.7	B28	62.5
B14	51.3	B32	51
B9	50.73	B18	36.48
B10	35	B31	37.4
B2	28.2	B22	15
B15	50.73	B1	20.3
B11	31.4	B41	40.1

Table 3.2: Antagonism of mixed and constituent cultures of Biorem against pathogenic Escherichia coli as determined by the surface, are of inhibition zones.

Trial 4: Biorem antimicrobial effect determination.

Procedure:

The following cultures were used to determine if Biorem will have an antimicrobial effect against these organisms:

Escherichia.coli

Salmonella serovar Typhimurium

Salmonella serovar Enteritidis

Campylobacter jejuni

All test isolates were grown overnight to ensure they were in the logarithmic growth phase.

The Biorem cultures “A” and “B” were also grown overnight in MRS broth. Biorem culture “C” was grown overnight in nutrient broth.

After incubation each test isolate was suspended to a cell density of 10^8cfu/ml in Tryptone Soy broth.

100µL of each suspension was spread onto the surface of a Plate count agar plate. Three holes of 9mm were punched into each agar plate.

100µL of each of the Biorem cultures were placed in a separate hole.

After inoculation the plates were incubated at 37°C for 24 hours.

Results:

All three cultures showed an antimicrobial effect against all the test organisms. Refer to the pictures below:

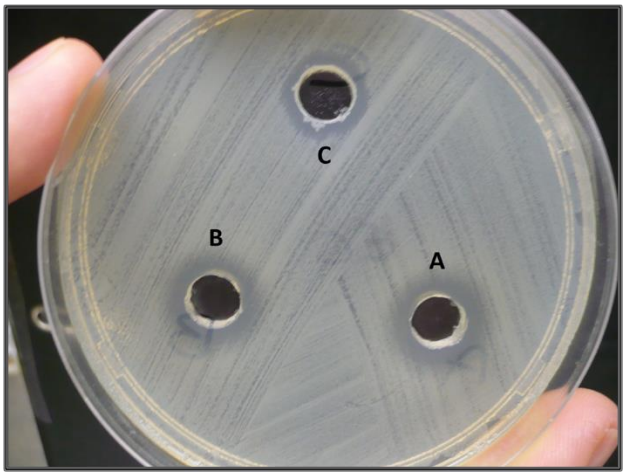

Figure 4.1 Escherichia coli

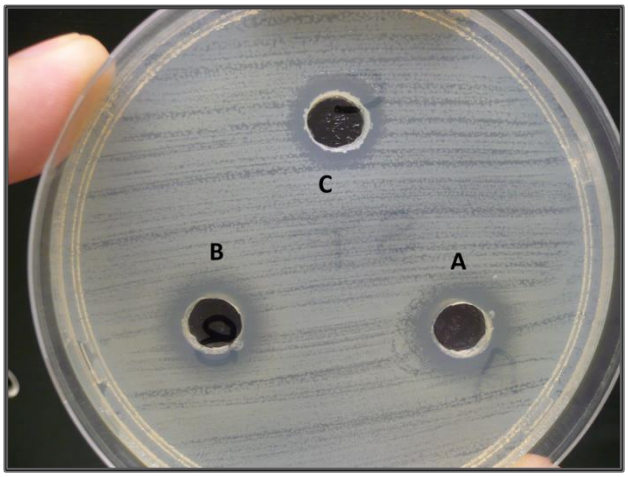

Figure 4.2 Salmonella typhimurium

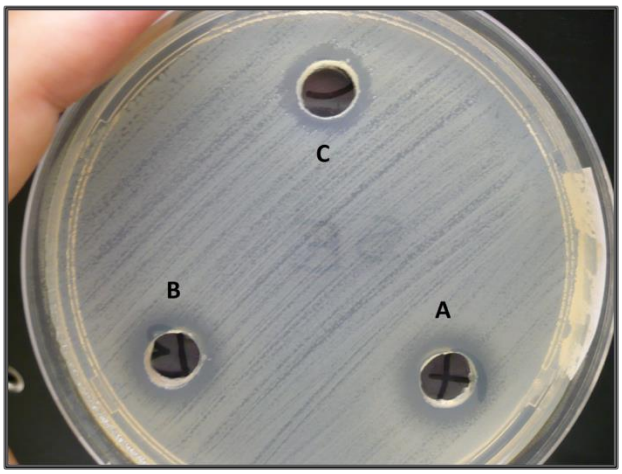

Figure 4.2 Salmonella enteritidis

Biorem Culture	A	B	C
Escherichia coli	15mm	15mm	14mm
Salmonella typhimurium	14mm	13mm	16mm
Salmonella enteritidis	16mm	14mm	14mm
Campylobacter jejuni	13mm	13mm	16mm

Table 4.1 Inhibition zone measurements

Conclusion

It was evident that all 3 preparations of the Biorem-cultures displayed antagonistic properties against each of the 4 pathogens.

APPLICATION

The Biorem-culture has been used in different applications.

As a finely milled powder, it can be incapsulated to be ingested orally.

With other supplements it can be blended to make a compound product.

It is used as a starter culture for numerous fermentations.

In a liquid form it can be consumed as is or blended with other liquids.

The milled powder is used by numerous feed manufacturers as a probiotic additive to animal feed.

Some of the Levilactobacillus brevis strains in the Biorem-culture has been shown to produce phenylethylamine (PEA), a mono amine alkaloid. PEA acts as a central nervous system stimulant in humans. It regulates monoamine neurotransmission by binding to the trace amine-associated receptor 1 (TAAR1) and inhibs the vesicular monoamine transporter 2 (VMAT2) in monoamine neurons. It also functions as a neurotransmitter in the human central nervous system. PEA increases the effects of dopamine and serotonin.

Certain Lacticaseibacillus paracasei strains in the Biorem-culture produce gamma-aminobutyric acid, an inhibitory neurotransmitter, that plays a role in calming neural activity.

This attribute may be the reason why the Biorem-culture is especially effective in alleviating autism, depression and anxiety.

More data of the Biorem-culture were obtained during trials, test and research not displayed in this document. More information can be provided on request. The strains and combination thereof used in the Biorem-culture are the property of Nandrea Health Products.

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