Old Herborn University Seminar Monograph 8:
Probiotics: prospects of use in opportunistic infections.
Editors: Roy Fuller, Peter J. Heidt, Volker Rusch, and Dirk van der Waaij.
Institute for Microbiology and Biochemistry, Herborn-Dill, Germany: 1-8 (1995).

PROBIOTICS: THEIR DEVELOPMENT AND USE

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SUMMARY

There is some confusion about the meaning of the word "probiotic" which can mean different things to different people. A new, more general definition is proposed which covers the use of live microbial supplements for animals, plants and foods. The basis of the probiotic concept is briefly reviewed. While the protective effect of the gut microflora is beyond question, the specific microorganisms responsible for the effect is less well defined. The results of field trials with probiotics often seem to be variable. The reasons which might account for this apparent lack of consistency are discussed.

INTRODUCTION

The habit of consuming fermented milks has a long history going back hundreds of years. PreChristian cave drawings show transfer of fermented milk to fresh milk with the intention of maintaining the ferment. These early producers were probably aware of the preservative effect that fermentation had on the milk which would otherwise have been wasted. In this way, it could be said to have had an indirect health benefit. What they were not aware of was the benefit derived from the ingestion of the microorganisms responsible for the fermentation.

It was not until the end of the last century that the consumption of fermented milk was related to health. This relationship was given a scientific basis by *Metchnikoff*, who attempted to reproduce the effect obtained with fermented milks by using specific cultures of bacteria isolated from those milks. The interest which his researches attracted led to a fashionable habit of consuming fermented milks which has persisted to this day and in recent years, has increased with the advent of the bioyoghurts which now grace the shelves of all our supermarkets.

In this paper, It will be discussed what we mean by probiotics, the way in which they have evolved and been developed and the reasons for the variations which bedevil the assessment of the effectiveness of probiotics as beneficial food supplements.

DEFINITION

The word "probiotic" has had several different meanings over the years and even at present it is used by different people to mean different things. It is, therefore, important at this stage, before we embark on any further discussions, to consider what it is we mean by probiotics.

The word was first used by *Lilley* and *Stillwell* in 1965 to describe substances secreted by one microorganism which stimulated the growth of another. It described, as the derivation of the word would demand, an agent which was the exact opposite of antibiotic. However, the word used in this sense was never widely adopted and in 1971 *Sperti* used it to describe tissue extracts which stimulated microbial growth.

The first use of the word to describe a microbial feed/food supplement was by *Parker* in 1974. He defined it as "organisms and substances which contribute to intestinal microbial balance". Later (*Fuller*, 1989) this was modified to read "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance". This modified definition removed the word "substances" which could have included antibiotics. It also stressed the viable nature of the preparations. There is now evidence that, in order to be effective, microorganisms should be viable.

It is five years since this definition was proposed and it may be that there is now a case for revising it. One criticism is that it is not wide enough and should embrace not only effects on the intestinal flora but other groups of the indigenous microflora, such as occur in the vagina and respiratory tract. *Havenaar* and *Huis in 't Veld* (1992) have attempted to extend the definition in such a way. They propose that probiotics should be defined as "a mono or mixed culture of live microorganisms which, applied to animal or man, affect beneficially the host by improving the properties of the indigenous microflora".

Two years later, even this definition may not be adequate. For example, should we include microbial stimulants such as the bifidogenic factors, and should we also extend it still further to include effects obtained in plants and even in food? With this in mind, the following definition was suggested for consideration and discussion during the seminar that formed the basis for this monograph: "A probiotic is a preparation consisting of live microorganisms or microbial stimulants which affects the indigenous microflora of the recipient animal, plant or food in a beneficial way".

All these definitions refer to microorganisms and would include bacteria, yeasts, fungi, viruses and bacteriophages. All these groups of organisms with the exception of the animal and plant viruses have been shown to have beneficial effects when administered to animals. The microorganisms included as probiotics are usually assumed to be non-pathogenic components of the normal microflora, such as the lactic acid bacteria. However, there is good evidence that non-pathogenic variants of pathogenic species can operate in much the same way as traditional probiotics. For example, avirulent mutants of *Escherichia coli*, *Clostridium difficile*, and *Salmonella typhimurium* can also protect against infection by the respective virulent parent strain. Are these what we understand as probiotics?

Perhaps the animal preparations should be separated from those applicable to plants and food. It might help to dispense with the word "probiotic" altogether, since it is becoming increasingly difficult to define. Some scientists already prefer to talk about "microbial feed supplements". Time will tell whether the term "probiotics" is too firmly ensconced to be dispensed with or whether a more easily defined term can be adopted to replace it.

At present, probiotics include the traditional fermented milks such as yoghurt which do not contain organisms which originate from the animal indigenous microflora. The justification is historic because fermented milk was what first stimulated interest in the possible beneficial effects of microbial supplements and led to the development of preparations which contained microorganisms derived from the animal gut with the potential to improve the nutrition and health of the consumer.

DEVELOPMENT

The scientific study of probiotics began with *Metchnikoff*. Towards the end of the last century there grew up a feeling that fermented milks were good for you. *Metchnikoff* generated the theory that under normal circumstances the microflora of the lower gut was having an adverse effect - the so-called autointoxication. He proposed that the ingestion of saccharolytic organisms in the form of fermented milk was reversing the effects of the proteolytic organisms which caused the autointoxication. His approach was encapsulated in his book "Essais optimistes" published in 1907. In it he says: "A reader who has little knowledge of such matters may be surprised by my recommendation to absorb large quantities of microbes, as a general belief is that microbes are harmful. This belief is erroneous. There are many useful microbes, amongst which the lactic bacilli have an honourable place".

Although this was said nearly ninety years ago, it would still surprise many non-microbiologists to learn that microorganisms are not necessarily bad for you.

Metchnikoff isolated microorganisms from fermented milk and used them in feeding trials. One isolate he called the Bulgarian bacillus. Although it is impossible to be certain, this was probably the same as the organism which later was classified as *Lactobacillus bulgaricus* and is now called *L. delbrueckii* subsp. *bulgaricus* which, together with *Streptococcus salivarius* subsp. *thermophilus,* is used to ferment milk to yoghurt.

After *Metchnikoff*'s death in 1916, the centre of activity moved from Europe to North America. Workers in the USA questioned the use of *L. delbrueckii* subsp. *bulgaricus*. They reasoned that since the effect was being manifested in the gut, it would be better to use an organism which originated from that site. At that time *L. acidophilus* was the lactic acid bacterium which was most commonly isolated from the gut. When this was used in human feeding trials it gave encouraging results in treatment of constipation. However, not all the trials with this organism gave positive results.

By the 1930's, treatment of infectious diseases was dominated by chemotherapy and by 1940 penicillin had been developed. Although this tended to suppress the interest in probiotics, it was responsible in an indirect way for increasing our understanding of the benefit that might be derived from the gut microflora. In the late 1940's, it was shown that low levels of antibiotics in the feed of animals improved their growth, implying that there was in the gut a population of microorganisms which were adversely affecting their development. It was important to know how this was operating and it stimulated research into the composition of the gut microflora. It soon became obvious that there were many species of lactic acid bacteria other than *L. acidophilus* present in the gut. As a result a variety of different species of the genera *Lactobacillus, Streptococcus* and *Bifidobacterium* were incorporated into probiotic preparations. These are still the three most commonly used probiotic organisms.

The current status of the probiotic effect and its scientific basis are reviewed in a recently published book (*Fuller*, 1992).

THE CONCEPT

The protective effect of the gut microflora is beyond question. It has been studied by many groups throughout the world and has been described as bacterial antagonism (*Freter*, 1956), bacterial interference (*Dubos*, 1963), barrier effect (*Ducluzeau* et al., 1970), colonisation resistance (*van der Waaij* et al., 1971) and competitive exclusion (*Lloyd* et al., 1977).

There is strong experimental evidence to support the belief that certain components of the gut microflora are involved in protection of the host against infectious disease.

If animals are reared in the complete absence of a microflora they become more susceptible to disease; the protection can be restored by colonising them with a gut microflora derived from the same animal species. The protective effect has also been studied by administering antibiotics by mouth to compromise the protective effect and get some indication of the types of microorganism responsible. The human studies have been confusing because the results have incriminated the anaerobes (*van der Waaij* et al., 1972), anaerobes and aerobes (*Wells* et al., 1988) and others have produced evidence to show that the anaerobes are not involved (*Gorbach* et al., 1988). Experimental studies in chickens (*Mead* and *Impey*, 1987) and rats (*Wilson* and *Freter*, 1986) suggest that a large number of strains are necessary for the full protective effect. A recent paper, however, was able to reproduce protection against *Campylobacter jejuni* by dosing chickens with only three species of bacteria - *Citrobacter diversus*, *Klebsiella pneumoniae* and *E.coli* (*Schoeni* and *Wong*, 1994).

However, we should regard this result with some caution until it has been tested against other strains of *C. jejuni.* Previous work (*Barrow* and *Tucker*, 1986) showed that protection could be obtained with three strains of *E. coli* but further work revealed that this was a specific effect which was only active against the strain of *Salmonella. typhimurium* used in the original trials.

One well documented condition in humans is the post antibiotic diarrhoea caused by *Clostridium difficile*. This condition is amenable to treatment with an enema produced from faeces of a healthy human adult (*Eiseman* et al., 1958).

There is, therefore, no doubt that the gut microflora is at least partly responsible for the resistance to intestinal infections. Various factors other than antibiotic therapy can affect the composition of the microflora. For example, high standards of hygiene, which are absolutely necessary and cannot be relaxed, may inhibit transfer of protective microorganisms from mother to offspring. In extreme cases, like children delivered by caesarian section into incubators, there is evidence that the flora can be deficient in lactobacilli (*Hall* et al., 1990). In such cases, early administration of a multi-strain probiotic might improve their resistance to disease.

FACTORS AFFECTING PROBIOTIC RESPONSE

The many claims that have been made for probiotics include suppression of diarrhoea, anti-tumour activity, stimulation of immunity, relief of lactose intolerance and growth stimulation of farm animals. Evidence in support of many of these claims will be presented in other papers in this monograph. It is not, therefore, intended to dwell on the details of the results obtained. Unfortunately, the results often appear inconsistent and variable and provide a stick with which to beat the probiotic concept. These differences are often more apparent than real. There are, in many cases, reasons for these apparent inconsistencies and these will now be considered in some detail. The result obtained in a probiotic trial could be affected by the following factors.

The type of organism in the probiotic

While it is perhaps obvious that the results obtained with two different species of microorganism cannot be compared, what is not always appreciated is that it is possible, for two different strains of the same species, to yield different results. Thus, although two probiotics may contain *L. acidophilus* the two strains used may differ in some apparently minor way which may still be significant in producing the probiotic effect. For example, the ability to adhere to the gut epithelium may not be related to any changes which affect the classification of the strain. It is well known that epithelial adhesion is a host specific effect and an *L. acidophilus* strain isolated from the chicken gut will not adhere to the epithelium of the pig. Other colonisation factors such as acid resistance and bile tolerance may vary within species; assuming these features are important in determining the probiotic response, their variability will be reflected in the effect obtained.

Method of production

Even if the two strains being used for production of the probiotic are identical, the way in which they are prepared can cause variation in the results. The effect of production methods on viability of the probiotic has been discussed at some length by *Lauland* (1994). Obvious differences in presentations such as whether the probiotic is a powder or a liquid suspension are readily appreciated. (*Ushe* and *Nagy*, 1985).

However, what is not so obvious are the changes which may be induced by the way in which the probiotic organism is grown and harvested. For example, the carbohydrate source in the growth medium can affect the ability to adhere to the gut epithelium of chickens and the adhesion capacity also changes during the growth cycle (*Fuller*, 1975).

Method of administration

The probiotic may be administered to the host animal in a variety of ways. It may be given as a powder, tablets, liquid suspension, capsule, paste or spray. Moreover, the amount and interval between doses may vary. Probiotics may be given only once or periodically at daily or weekly intervals. Little is known about the minimum dose required for the probiotic effect but trials in rats, humans and pigs indicate that the effect falls off after administration of the probiotic ceases (*Cole* and *Fuller*, 1984; *Goldin* and *Gorbach*, 1984). It therefore seems very likely that the effect obtained will be affected by the amount and frequency of dosing.

Viability of the preparation

Probiotic preparations cannot always be relied upon to contain the number of viable organisms stated on the label. In many of the published studies, the viability of the preparation used was not checked and negative results may be due to insufficient viable cells being present in the probiotic. In a survey of commercially available probiotic preparations, *Gilliland* (1981) found that the viable count varied greatly and three of the fifteen preparations tested had no viable lactobacilli. Sometimes lactobacilli other than the one listed on the label were present.

Condition of host

Pollman et al (l980) obtained a better probiotic response in starter than he did with growing-finishing pigs. It is widely thought that the earlier the probiotic supplement is introduced the more effective it will be. Certainly, during the early stages of life the gut microflora is in an unstable condition and organisms given by mouth are likely to find a niche which they can occupy. In mammals, the suckling period is different in many ways from the post-weaning period and administration of microbial supplements will survive or not depending on whether they find the environment suitable. Differences have also been observed in the response to fungal probiotics in lactating and non-lactating cows. While lactobacillus probiotics may be more effective in calves, they are of limited use in adult ruminants where fungal probiotics are more effective.

Condition of gut microflora

A probiotic will only be effective if the animal receiving it is subject to the adverse affect which the probiotic reverses. The most obvious example of this is an infectious disease but it is less apparent when probiotics are used to stimulate the growth of farm animals. If, like antibiotics, probiotics stimulate growth by antagonising a growth depressing organism present in the gut, then it will follow that if the organism is not present, no growth stimulation will occur. It has been shown that antibiotics are not effective in stimulating the growth of chickens if they are housed in clean surrounding.

It is not surprising that with all this scope for differences between experiments, that some inconsistency exits. Unfortunately, the information necessary to make judgements about the reasons for variability are often not given in the reports. When they are, they are frequently ignored and comparisons made between two sets of results that are not comparable. It may be that the conditions under which a probiotic will have its maximum effect are very strictly defined and that only if these conditions are met will it appear positive. There is no doubt that because of this the results with probiotics appear to be inconsistent but close scrutiny of the results leads one to conclude that with the right probiotic, administered in the right way at the right time we can expect to obtain a beneficial effect. More knowledge of how probiotics work and the optimal methods for administration will enable us to select more active strains and administer them in a fashion that will make the results more consistent and predictable.

It is expected that this monograph will contribute to the understanding of what these conditions are likely to be and will help to suggest ways in which future research endeavours may be aimed at establishing the basic facts about the probiotic effect that will lead to the development of new and more effective probiotic preparations.

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