Probiotics for animal use in Japan

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Summary: An overview is presented of recent studies on intestinal bacterial flora and the current state of probiotics (viable bacteria preparations) developed for use in domestic animals in Japan. They belong to two major categories, namely, veterinary drugs for treating gastro-intestinal disorders (such as diarrhoea), and feed probiotics to improve weight gain and feed efficiency, and also to maintain and promote normal health status of domestic animals and fowls. Feed probiotics are now used widely by the major mills as feed ingredients for piglets, calves, sows and fowls.

KEYWORDS: Bacillus - Bacteria - Farm animals - Feed additives - Growth promoters - Intestinal flora - Lactobacillaceae.

INTRODUCTION

The intestinal bacterial flora of domestic animals has an important role in digestion and absorption of feed ingested by the host. It takes part in the metabolism of dietary nutrients such as carbohydrates, protein, lipids and minerals and also in the synthesis of vitamins. Besides these physiological effects on nutrition, the bacterial flora of the intestine is generally thought to provide protection against certain diseases and infections by suppressing the growth of pathogenic organisms. Enteric bacteria are closely involved in the development of such conditions as colibacillosis, salmonellosis and clostridial enteritis of domestic animals and fowls, thus exerting a profound influence upon the health of the host.

The interrelationship between host and intestinal bacterial flora in domestic animals and fowls has been depicted by Mitsuoka as shown in Figure 1 (10, 12). Vitamin synthesis, aids to digestion and absorption of nutrients, resistance to infection and antagonism towards harmful micro-organisms can all be expected from the intestinal flora. These actions are beneficial for the host and serve to enhance livestock productivity. On the other hand, toxic products formed by harmful organisms in the intestine have effects such as putrefaction, gas formation and toxinogenesis in the alimentary tract which reduce growth, reproductive performance, feed efficiency and meat quality and also lessen the host's capacity to resist infection. When the normal balance in intestinal bacterial flora is upset as a result of stressful conditions, there is an abnormal proliferation of pathogenic organisms which otherwise occur very rarely, and this causes diarrhoea and enteritis. Such disease states give rise to overt infections with a deleterious effect on the host and a consequent decline in productivity.

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FIG. 1

Interrelationship between the intestinal flora and the host in domestic animals and fowls (10)
In light of the interrelationship between intestinal bacterial flora and animal host, maintenance of the normal balance of intestinal flora is essential for the health of domestic animals and fowls and also for the promotion and improvement of productivity, such as growth and feed efficiency, which is a problem of vital importance in the livestock industry.

In the livestock industry in recent years, animals and fowls have often been bred and maintained in densely populated colonies or flocks in order to attain a high economic efficiency. This worsening of the feeding environment, inadequate feeding and poor hygiene management due to a shortage of manpower, combine with many other stress factors to create an imbalance in the intestinal bacterial flora. Under these circumstances, antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents are currently used to suppress or eliminate harmful organisms in the intestine, thus improving the growth and feed efficiency of animals and preventing the decline of productivity.

In parts of Europe and America as well as in Japan, probiotics or viable bacteria preparations have for many years been used as drugs for intestinal regulation and for treatment of diarrhoea and enteritis in man. In view of their efficacy, trials of certain probiotics were conducted for the prevention and treatment of diarrhoea and enteritis of domestic animals. Several preparations originally intended for humans have been officially approved and become commercially available as veterinary drugs. To determine the potential usefulness of probiotics for improving animal growth and feed efficiency or for preventing decline of productivity when administered continuously in feed, like the antimicrobial feed additives, trials were initiated using probiotics for humans as feed additives (3, 16, 20). Subsequently, probiotics specifically for use in domestic animals and fowls were developed and are now commercially available (6, 7, 8). Their active ingredients are new bacterial strains selected by screening for the improvement of growth and feed efficiency. Probiotics prepared with enteric bacteria isolated from domestic animals or fowls are also on the market for veterinary use (5, 8).

Probiotics are now utilised for domestic animals and fowls in certain parts of Europe, the Americas and particularly in Japan, where probiotics are contained in one or more products supplied by practically every feed miller, including all the major formula feed manufacturers. Furthermore, the feed probiotic preparations developed in this country are generally considered to be among the best in the world, from the aspects of technology and product quality. This is the result not only of research and development activities of probiotic manufacturers, but also because veterinarians and zootechnical specialists in Japan are generally knowledgeable about enteric bacteria, thanks to the long pursued studies of Mitsuoka and other investigators, which have favoured the widespread understanding of probiotics by members of the livestock industry (11, 12, 13, 14, 15, 19).

In addition, the Japanese "Law concerning safety assurance and quality improvement of feed" of 1976 has greatly restricted the use of feed additives prepared from antimicrobial agents such as antibiotics and synthetic antimicrobials, which had previously been employed as growth promoters for animals and fowls. Probiotics thus began to attract the attention of formula feed manufacturers, as a substitute or compensation. Trials to evaluate their applicability and usefulness followed, demonstrating their efficacy. Probiotics have thus become extensively used as additives in formula feed over the last ten years.
This article will deal chiefly with those feed probiotics currently used widely in Japan.

**PROBIOTICS FOR ANIMAL USE IN JAPAN**

**Definition of probiotics**

The term “probiotic”, which essentially denotes a beneficial symbiotic association of any two types of living organisms (9), has become widely used as a general term for viable bacteria preparations.

Probiotics are referred to in Japanese as “seikin-zai” or “seikin-rui” which means viable bacterial preparations (1, 17) and are defined as “micro-organisms which exert beneficial effects in the gastro-intestinal tract of animals”. In a broad sense, probiotics would include micro-organisms used for fermentation and breakdown of feedstuff prior to use for feeding, such as silage additives and additives for fermented feed. It is common, however, to exclude these preparations from the category of “seikin-zai” since they are utilised for their microbial activity outside the animal’s body.

**Organisms used in probiotics for animals**

The organisms used in probiotics for animals in Japan are classified into four groups: aerobes, anaerobes, lactic acid bacteria and yeast, as follows.

A. Aerobes (Spore-forming bacteria of the genus *Bacillus*)

- *Bacillus cereus* (syn.: *B. toyoi*): A strain of spore-forming organism isolated from soil, developed as a probiotic for animal use. It is capable of growing under anaerobic conditions as well (1, 6, 7, 8, 12).

- *Bacillus coagulans* (syn.: *Lactobacillus sporogenes*): A spore-forming organism isolated from green malt. Because of its ability to produce lactic acid, the organism is also referred to as a sporulating, lactic acid-forming organism (1, 8, 12, 20).

- *Bacillus subtilis* (syn.: *B. natto*): The organism is spore-forming and distributed mostly on dry grass and hay. Strains isolated from “natto”, a conventional fermented food in Japan, are utilised (1, 3, 8, 12).

B. Anaerobes (Spore-forming bacteria of the genus *Clostridium*)

- *Clostridium butyricum*: Butyric acid-forming organisms isolated from the human intestine (1, 8, 12, 16).

C. Lactic acid bacteria (*Bifidobacteria*, *Lactobacilli* and *Enterococci*; non-spore-forming)

- *Bifidobacterium thermophilum*, *Bifid. pseudolongum*, etc.: These organisms isolated from pigs and cattle have been developed as probiotics for animal use. They are obligate anaerobes and produce lactic acid and acetic acid (1, 5, 8, 12).

- *Lactobacillus acidophilus*, *Lact. salivarius*, etc.: Most strains are isolated from man and fermented foods, while some are from pigs and cattle. These organisms are facultative anaerobes and form lactic acid (1, 8, 12).
Enterococcus faecalis (syn.: Streptococcus faecalis), Enterococcus faecium (syn.: Str. faecium), etc.: Strains isolated from man and animals are used. They are facultative anaerobes forming lactic and acetic acids (1, 8, 12).

D. Yeast, etc. (Yeast is currently used as a raw material to provide nutrition in feed, but practically none is utilised for probiotics in Japan.)

Among the organisms mentioned above, the most widely used at present as a probiotic for domestic animals and fowls is an avirulent strain of Bacillus cereus, the so-called B. toyoi. It has been developed as a probiotic for animal use (6, 7, 8). Other organisms used for this purpose include spore-forming bacteria such as C. butyricum and B. subtilis and lactic acid bacteria such as Bifidobacteria.

Transient and resident flora

Aerobes of the genus Bacillus are generally regarded as transient flora which do not settle down in the intestine, whereas anaerobes such as clostridia, bifidobacteria, lactobacilli and enterococci are considered as constituents of intestinal flora (11). In discussing the efficacy of probiotics, the fact that an organism either does or does not colonise the alimentary tract is often adduced as supporting evidence.

Those organisms regarded as transient flora are considered as hardly settling down in the intestinal tract. It has been pointed out that if indigenous, resident bacteria of the intestine such as lactobacilli are isolated and then orally administered to animals, they do not necessarily settle in the intestine. This may be due to a difference in animal species or, even when the recipient is of the same species, to elimination by antecedents (12).

At the same time, there have been many reports that certain organisms, whether they are transient (e.g. the genus Bacillus such as B. cereus and B. subtilis) or resident flora (e.g. the genera Bifidobacterium, Lactobacillus, Enterococcus), are effective for preventing and treating enteritis and/or promoting growth of domestic animals when applied as probiotics (1, 2, 3, 4, 5, 6, 7, 8, 12, 14, 15, 16, 18, 20).

In choosing a probiotic, it is important to select a bacterial species by its efficacy and safety in domestic animals or fowls. Whether the organism is transient or resident in the intestine does not necessarily matter. Furthermore, it is not essential for feed probiotics that the organism settle down in the intestine since feed probiotics are administered in feed continuously over a long period.

Aerobes and anaerobes

Bacteria are classified as aerobes, facultative anaerobes or obligate anaerobes according to whether or not oxygen is required for their growth. This growth characteristic is important for bacterial species used for probiotics. The upper portion of the alimentary tract has an aerobic environment because oxygen enters along with ingested food. This situation favours the proliferation of aerobic bacteria which consume the oxygen. The lower the portion of the intestinal tract, the less the concentration of oxygen dissolved in intestinal contents and, hence, the more advanced the anaerobic environment (11). It follows that the upper gastro-intestinal tract provides environmental conditions suitable for proliferation of aerobes, and the lower portion those suitable for proliferation of anaerobes.
Spore-forming and sporeless organisms

Another problem concerns spore-forming organisms versus non-sporulating organisms. Although much remains to be elucidated as to the mechanisms of probiotic activity, the basic view holds that probiotics exert their effects as viable microorganisms after they reach the appropriate site in the lumen of the alimentary tract of domestic animals and fowls (1).

Since probiotics are administered in feed for growth promotion and improved feed efficiency, the probiotic must not only produce the desired effects, but must also be reasonably stable in feed. It is also important for probiotics to be refractory to the influence of antimicrobial agents in feed as well as in the alimentary tract, for they are frequently used concomitantly with antimicrobial feed additives. In addition, they should be stable to gastric acid as they pass through the gastric lumen. Stability in feed matters less in cases of short-term administration, e.g. for treating diarrhoea. But feed probiotics which are incorporated into feed by millers need to be stable for at least two to three months (7, 8). It has been reported that probiotics consisting of non-sporulating organisms as active ingredients have inadequate stability in feed and premixes, when used alone or in combination with antibiotics (1).

For this reason, spore-forming organisms such as those of the genus Bacillus, which are resistant to changes in the external milieu, e.g. acids, alkalis and heat (1, 3, 6, 7, 8, 16, 20), are considered suitable for feed probiotics used by formula feed manufacturers. It must be noted, however, that probiotics with a low ratio of viable spores to viable bacterial cells in the product may not be sufficiently stable. Probiotics of spore-forming bacteria should preferably consist of viable bacteria which are virtually all in viable spore form (6, 7, 8).

For probiotics used in treating diarrhoea and other gastro-intestinal symptoms, however, such long-term stability in feed is not required since they are administered orally in high doses, or in feed for a brief period. Non-sporulating organisms such as bifidobacteria, lactobacilli and enterococci may therefore be used as well (4).

For probiotics, the number of viable organisms contained per unit quality is usually labelled. In order to guarantee the viable bacterial count in a given product, it would be necessary to establish assay procedures for counting the organisms in the probiotic preparation, and also in feed and, if possible, gastro-intestinal contents as well (6, 7, 8).

CHARACTERISTICS, ADVANTAGES AND EFFECTS OF PROBIOTICS FOR ANIMAL USE

The principal probiotics for animal use which are currently available in Japan are shown in Table I.

Restoration of a normal intestinal bacterial flora

Most probiotics for animal use exert the same basic effects. The probiotic is administered and remains viable in the gastro-intestinal tract, where it suppresses and eliminates harmful organisms proliferating there and, at the same time, facilitates
<table>
<thead>
<tr>
<th>Product and distributor</th>
<th>Organism</th>
<th>Sporulation</th>
<th>Growth</th>
<th>Animals</th>
<th>Use</th>
<th>Feed ingredients</th>
<th>Veterinary drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toyocerin (Toy Jozo)</td>
<td><em>Bacillus cereus</em> (syn.: <em>B. toyoi</em>)</td>
<td>+</td>
<td>Aerobic &amp; anaerobic</td>
<td>Swine, cattle, chicken</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lacris (Sankyo)</td>
<td><em>B. coagulans</em> (syn.: <em>Lactobacillus sporogenes</em>)</td>
<td>+</td>
<td>Aerobic</td>
<td>Swine</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Growgen (Eisai)</td>
<td><em>B. subtilis</em> (syn.: <em>B. natto</em>)</td>
<td>+</td>
<td>Aerobic</td>
<td>Swine, cattle, chicken</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Calsporin (Calpis)</td>
<td><em>B. subtilis</em></td>
<td>+</td>
<td>Aerobic</td>
<td>Swine</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Miyarisan (Nippon Kayaku)</td>
<td><em>Clostridium butyricum</em></td>
<td>+</td>
<td>Anaerobic</td>
<td>Swine, cattle, chicken</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Korolac B &amp; D (Nisshin F.M.)</td>
<td><em>B: Bifidobacterium thermophilum</em></td>
<td>-</td>
<td>Anaerobic</td>
<td>B: Swine, cattle</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>D: Bifid. pseudolongum</em></td>
<td>+</td>
<td>Aerobic &amp; anaerobic</td>
<td>Swine, cattle</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Biofermin (Takeda)</td>
<td><em>Enterococcus faecalis</em> (syn.: <em>Streptococcus faecalis</em>)</td>
<td>-</td>
<td>Aerobic &amp; anaerobic</td>
<td>Swine, cattle</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Balantol (Kohkin)</td>
<td><em>Enterococcus faecalis</em> (syn.: <em>Str. faecalis</em>)</td>
<td>-</td>
<td>Aerobic &amp; anaerobic</td>
<td>Swine, cattle, chicken</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lactfeed (Bio-seiyaku)</td>
<td><em>L. acidophilus</em> &amp; <em>Bifid. pseudolongum</em></td>
<td>+</td>
<td>Aerobic &amp; anaerobic</td>
<td>Swine, cattle, chicken</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

growth of beneficial bacteria such as lactobacilli, thus restoring and maintaining a normal (healthy) balance of intestinal bacterial flora (1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 20).

These normalising effects on the intestinal flora are of value in preventing and treating diarrhoea and in regulating the bowels, and they suppress the proliferation of such undesirable organisms as pathogenic *E. coli*, *Salmonella*, *C. perfringens* and beta-haemolytic bacteroides, and also increase beneficial bacteria.

A single dose of *B. cereus* probiotic produced a substantial reduction in the number of enterotoxigenic *E. coli* in the intestinal contents of piglets with diarrhoea (Fig. 2)
Dosage of Probiotic: $1 \times 10^8$ B. toyoi spores/head

**FIG. 2**

Changes in gastro-intestinal enterotoxigenic *E. coli* count following single administration of probiotic in piglets (18)

(6, 7, 18). Similarly, in weaned piglets, continuous administration of the viable spore preparation in feed resulted in a significant reduction of *E. coli*, with a significant increase of lactobacilli and an increasing count of the organism administered, accompanied by improved weight gain and feed efficiency (Fig. 3) (1, 7, 8). In newly weaned rabbits which continuously received the same spore preparation in feed, abnormal proliferation of *E. coli* was suppressed in the small intestine and caecum, with an effective prevention of diarrhoea, and improvement of weight gain and survival rate (2).

In cases of acute diarrhoea in dogs, manifested by decreased faecal counts of bifidobacteria and increased haemolytic *E. coli* and *C. perfringens*, treatment with *Bifid. pseudolongum* was effective (Fig. 4) (4).

The relationships of environmental stress factors of the hosts, and intestinal bacteria and the effect of probiotics are shown in Figure 5.

In chicks placed in a hot environment (heat stress), there was a drastic decrease of lactobacilli in the upper gastro-intestinal tract, and thus a marked disturbance in intestinal bacterial flora. Oral administration of a probiotic prevented the diminution of lactobacilli, and maintained normal intestinal flora despite exposure to heat (7).
Administrative Period
Dosage of Probiotic : $1 \times 10^6$ B. toyoi spores/g in feed

**FIG. 3**
Changes in intestinal bacterial flora in duodenum of weaned piglets following continuous administration of probiotic (7)

**FIG. 4**
Effect of probiotic administration on the faecal flora of dogs with diarrhoea (4)
Changes in intestinal lactobacilli following administration of probiotic in chickens under heat stress conditions (21)

Production of digestive enzymes and vitamin synthesis

Probiotics of the genus _Bacillus_ like _B. cereus_ and _B. subtilis_ or of the genus _Clostridium_ have been shown to produce various digestive enzymes such as amylase and proteases and to be capable of synthesis of B vitamins (3, 6, 8, 16).
Reduction of ammonia content

The concentration of ammonia in intestinal contents, faeces and portal blood was reduced following administration of a probiotic prepared with \textit{B. cereus} (Table II) (6, 7, 8).

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Control group ((\mu)g/ml)</th>
<th>Probiotic group ((\mu)g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>14.5 ± 1.56</td>
<td>10.2 ± 1.04*</td>
</tr>
</tbody>
</table>

*Significantly different from the control at the 5% level
Dosage of probiotic: \(6 \times 10^9 \textit{B. cereus (toyoi)}\) spores/head

Normalisation of ruminal function in cattle

Administered to cattle, a probiotic of \textit{B. cereus} increased the contents of volatile fatty acids (VFA), such as propionic acid, in ruminal juice, and maintained the pH of gastric juice within the normal range, thereby normalising ruminal function (Fig. 6) (6, 8).

Prevention of amine formation

Probiotics of \textit{Bifidobacteria} can prevent the formation of amines by intestinal bacteria (5).

Improvement of growth and feed efficiency of domestic animals and fowls, and prevention of decline in productivity

Through all of the effects described above, whether individual or combined, probiotics are of value from the economic viewpoint in affording improved weight gain, feed efficiency, maintenance and promotion of healthy performance in domestic animals and fowls. Furthermore, probiotics prevent a decline of reproductivity by improving survival rates of young animals (1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 19, 20).

Freedom from the undesirable problems of residues in livestock products and drug-resistant bacteria

In the United States, the EEC, Japan and many other countries, serious concern exists over potential residues of antibiotics and synthetic antimicrobial agents in meat, milk and eggs and increased resistance of organisms to such drugs. As a result, the use of antimicrobial agents in feed has been greatly restricted. Probiotics are generally thought to entail no such problem.

In Japan, the Ministry of Agriculture, Forestry and Fisheries is examining the question of whether probiotics should be designated and registered under the category of feed additives or an equivalent. Currently, however, probiotics are placed under...
Dosage of Probiotic: $2 \times 10^8$ Bacillus toyoi spores/kg B.W.

**FIG. 6**

Changes in total volatile fatty acid (VFA) and pH following administration of probiotic in cattle with ruminal hyperalkalinity (7)
the category of feed ingredients and therefore their use in feed is not subject to any particular legal restrictions.

**CURRENT TRENDS IN THE USE OF PROBIOTICS**

Probiotics as feed ingredients are chiefly used by formula feed manufacturers. Prestarter (artificial milk) and starter feed for piglets account for an overwhelming proportion of their use; one or more brands of feed on the market from virtually every manufacturer contains probiotics. Calf prestarter (milk replacer) and starter feed are second, followed by sow feed.

For chickens, probiotics have been utilised most frequently at poultry farms where feed is formulated. Recently, the use of probiotics in formula feed for layer replacement, broilers and breeders has become a general trend. Future applications will involve feed for beef and dairy cattle, feed for finishing pigs in a withdrawal period, and feed for fishes such as eels and yellow-tails which are cultured (7).

As veterinary drugs, probiotics are prescribed mainly for treating diarrhoea and other gastro-intestinal disorders. Their actual consumption for this purpose is small, since antibiotics and synthetic antimicrobial agents are preferred for treating diarrhoea in young animals.

The current nationwide market for probiotics in Japan is estimated at approximately five hundred million yen in terms of total annual sales (1). Use by formula feed manufacturers constitutes the largest proportion of the market. Among the feed probiotic products on the market, Toyocerin®, a viable spore preparation of bacteria of the genus *Bacillus*, is estimated to have the greatest market share at about 80%, followed by Miyarisan® and others.

**FUTURE DEVELOPMENTS**

The development and increasing use of probiotics for animals, particularly as feed ingredients, is largely a result of studies and technical progress made in this country. However, the market for these products is still much smaller than that for antibiotic and synthetic antimicrobial feed additives. There are some indications that probiotics will have to assume a role as substitutes for some feed antimicrobial agents and for hormones currently used as growth promoters.

Under these circumstances, continued development of probiotics is anticipated through exploitation of new uses and studies of their application, hopefully with further support from the feed and livestock industries.

**ACKNOWLEDGMENTS**

The valuable advice of Dr T. Mitsuoka, Professor at the University of Tokyo, in the preparation of this manuscript is gratefully acknowledged.
Résumé : L'auteur présente les études récemment conduites au Japon sur la flore bactérienne intestinale et la mise au point des probiotiques (préparations bactériennes viables) devant être utilisés chez les animaux domestiques de ce pays. Ces probiotiques appartiennent à deux grandes catégories : les médicaments vétérinaires pour le traitement des troubles gastro-intestinaux tels que la diarrhée, et les probiotiques alimentaires, destinés à améliorer le gain de poids et le taux de conversion alimentaire, ainsi qu'à maintenir et favoriser un état de santé normal chez les animaux et volailles domestiques. Les principales fabriques d'aliments pour animaux utilisent largement les probiotiques alimentaires comme ingrédients des aliments pour porcelets, veaux, truies et volailles.


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REFERENCES


