Pre and probiotics in food animal production

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Hotel Jen Manila, Roxas Blvd., Pasay City NAST Philippines

Soo-Ki Kim, Ph.D.

sookikim@konkuk.ac.kr Department of Animal Science and Technology Konkuk University



1) Philippines

2) Soilders of Philippines participated in Korea war



1950. 9. 19. -5 years-

3) Sister



ILKAM LAKE (一鑑湖)



Contents

- Probiotics
- Prebiotics
- Rumen fermentation by microbes
- Microbiological Ecology in Gut
- Application of probiotics
- Animal probiotics in Korea
- Future research

Introduction

Antibiotic concern

- Antibiotic resistance
- Residue in meet product
- Public concern
- Ban in EU (2003)
- Ban in Korea (2011)
- Requirement of efficiency alternative

Alternative development

- Enzymes
- Organic acid
- Minerals
- Amino acids
 - Phospholipids
 - Vitamins
- Probiotics

Selection criteria

- Safety
- Acid & bile tolerance
- Well growth ability
- Antimicrobial activity
- Antioxidant activity

Production

- Submerged liquid fermentation
- Solid state fermentation

Alternative antibiotics:

- Probiotics, prebiotics, essential oil compounds, organic acids
- Alternative antibiotics: Lactoferrin, lysozyme, bacteriocins and antimicrobial peptides



Animal probiotics



Synergistic effects

Probiotics + Prebiotics ≠ Synbiotics



 Probiotics: live microorganisms that are thought to have beneficial effects on the host





- Prebiotics: foods for probiotics, ingredients that stimulate the growth and/or function of beneficial intestinal microorganisms
- **Synbiotics:** blending of probiotics and prebiotics

Mechanisms of action





Current trends in human



- Anticarcinogenic: Colon, breast and others
- Antidiabetic
- > Anti-allergic
- > Anti-inflammatory Diseases: IBD, Ulcerative colitis, Crohn



disease, Pouchitis and Postoperative complications

- Genetically modified probiotics
- > Oral vaccine development



Influencing technological factors for functionality of probiotics



Int.J.Curr.Microbiol.App.Sci (2014) 3(3): 410-420

How to select or verify good probiotics ?



Prebiotics

Prebiotics

- In the intestine, prebiotics are fermented by beneficial bacteria to produce short chain fatty acids
- Short chain carbohydrates that are non-digestible by digestive enzymes
- Selectively enhance the activity of some groups of beneficial bacteria
- Prebiotics render many other health benefits in the large intestine such as reduction of inflammation
- Enhance the bioavailability and uptake of minerals, including calcium, magnesium, and possibly iron

Prebiotics

Fructooligosaccharides (FOS) Maltooligosaccharide (MOS) Xylooligosaccharides (XOS) Arabinoxylooligosachrides (AXOS) Galactooligosaccharides (GOS)

Polyols (xylitol, sorbitol, mannitol) Disaccharides (lactulose, lactilol) Oligosaccharides (raffinose, soybean) Oligofructose

Other non-digestible oligosaccharides (palatimose, isomaltose, lactosucrose) and polysaccharides (inulin, resistant starch)

Change for the concept of prebiotics

	Definition	Substantiation of prebiotic effect	Compounds
2010	A selectively* fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health ⁵²	Selectivity of effect on gut microbiota should be established <i>in vivo</i> using most up-to-date technology Health effects, or at least physiological effects, should be established in controlled trials and correlated with selective changes in gut microbiota composition or activity	Inulin FOS tGOS Lactulose
	*	•	
2015	A nondigestible compound that, through its metabolization by microorganisms in the gut, modulates composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host	The degree to which the effect of the prebiotic on composition and/or activity is "selective" is not a criterion The burden of proof for health claims does not change Definition places more focus on the causal link between the microbial metabolization of the compound, the resulting modulation of the gut microbiota, and the beneficial physiological effects	Inulin FOS tGOS Human milk oligosaccharides Candidate preblotics? • Resistant starch • Pectin • Arabinoxylan • Whole grains • Various dietary fibres • Noncarbohydrates that exert their action through a modulation of the gut microbiota

Figure 1 | Current and proposed definitions for the concept of prebiotics. *Selectivity was established by selective culture techniques and by targeted molecular methods (fluorescence *in situ* hybridization and quantitative PCR). ‡Prebiotic candidates, needs additional research. Abbreviations: FOS, fructo-oligosaccharides; tGOS, transgalacto-oligosaccharides.

Bindels, Laure B., et al. "Towards a more comprehensive concept for prebiotics." Nature Reviews Gastroenterology & Hepatology 12.5 (2015): 303-310.

Schematic overview of potential interactions between the gut microbiota and the intestinal mucosal immune system.



De Kivit, Sander, et al. "Regulation of intestinal immune responses through TLR activation: implications for proand prebiotics." *Frontiers in immunology* 5 (2014).

Rumen fermentation by microbes

Rumen digestion and fermentation



Utilization of carbohydrates





Bacteria attached



1

CH₂OH

но

OH



Dietary Additives to Manipulate Rumen Fermentation

- 1. Modulate ruminal pH and reduce lactate accumulation.
- 2. Reduce the risk of development of metabolic diseases like diarrhea in neonates and ruminal acidosis or bloat in older livestock.
- 3. Enhance rumen development in neonatal ruminants.
- 4. Improve the efficiency of ruminal energy utilization by reducing ruminal methanogenesis and decreasing the acetate to propionate ratio without reducing milk fat synthesis.
- 5. Improve the efficiency of ruminal nitrogen utilization by (i) reducing proteolysis, peptidolysis, and amino acid deamination, thus minimizing production and losses of NH₃ to the environment; (ii) inhibiting the activity of ruminal protozoa that phagocytize desirable bacteria, contribute to proteolysis and deamination, and serve as hosts for methanogens; (iii) enhancing the synthesis of microbial protein by facilitating coupling (synchrony) of ruminal energy and protein supply or by other means.
- 6. Increase ruminal organic matter & fiber digestibility.
- 7. Increase the level and efficiency of animal performance.
- 8. Be cost effective and approved by legislative authorities.

Effect of ARF

Anaerobic rumen fungi (ARF) based additives have been found to improve ruminant productivity consistently during feeding trials.

Administration of ARF during the few trials conducted, led to the increased weight gain, milk production, and total tract digestibility of feed components in ruminants.

ARF display very strong cell-wall degrading cellulolytic and xylanolytic activities through rhizoid development, resulting in the physical disruption of feed structure paving the way for bacterial action.

Puniya, Anil K., et al. "Role of live microbial feed supplements with reference to anaerobic fungi in ruminant productivity: A review." Journal of Integrative Agriculture 14.3 (2015): 550-560.



Microbial ecology in rumen and gut

Jami, Elie, Bryan A. White, and Itzhak Mizrahi. "Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency." *PloS one* 9.1 (2014): e85423.

Castillo-Lopez, E., et al. "Effect of feeding dried distillers grains with solubles on ruminal biohydrogenation, intestinal fatty acid profile, and gut microbial diversity evaluated through DNA pyrosequencing." *Journal of animal science* 92.2 (2014): 733-743.

Pitta, Dipti W., et al. "Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets." *Microbial ecology* 59.3 (2010): 511-522.

Looft, Torey, et al. "Bacteria, phages and pigs: the effects of in-feed antibiotics on the microbiome at different gut locations." *The ISME journal* 8.8 (2014): 1566-1576.

Stanley, Dragana, Robert J. Hughes, and Robert J. Moore. "Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease" *Applied microbiology and biotechnology* 98.10 (2014): 4301-4310.

Probiotics in feed, nutrition and environment for animal production

- Dersjant-Li, Yueming, et al. "A direct fed microbial containing a combination of three-strain Bacillus sp. can be used as an alternative to feed antibiotic growth promoters in broiler production." Journal of Applied Animal Nutrition 2 (2014): e11.
- Yeoman, Carl J., and Bryan A. White. "Gastrointestinal tract microbiota and probiotics in production animals." *Annu. Rev. Anim. Biosci.* 2.1 (2014): 469-486.
- Chowdhury, Piklu Roy, et al. "Genomic interplay in bacterial communities: implications for growth promoting practices in animal husbandry." *Frontiers in microbiology* 5 (2014).
- Song, J., et al. "Effect of a probiotic mixture on intestinal microflora, morphology, and barrier integrity of broilers subjected to heat stress." *Poultry science* 93.3 (2014): 581-588.
- Salim, H. M., et al. "Enhancement of microbial nitrification to reduce ammonia emission from poultry manure: a review." *World's Poultry Science Journal* 70.04 (2014): 839-856.

Others

Redondo, Leandro M., et al. "Perspectives in the use of tannins as alternative to antimicrobial growth promoter factors in poultry." Frontiers in microbiology 5 (2014).

Kritas, S. K., et al. "Reproductive performance of sows was improved by administration of a sporing bacillary probiotic (C-3102)." Journal of animal science 93.1 (2015): 405-413.

Mostafa, T. H., et al. "EFFECT OF USING SOME FEED ADDITIVES (TW-PROBIOTICS) IN DAIRY COW RATIONS ON PRODUCTION AND REPRODUCTIVE PERFORMANCE." Egyptian J. Anim. Prod 51.1 (2014): 1-11.

Sattler, V. A., et al. "Impact of a probiotic, inulin, or their combination on the piglets' microbiota at different intestinal locations." *Beneficial microbes* (2014): 1-11.

Carberry, Ciara A., et al. "Quantitative analysis of ruminal methanogenic microbial populations in beef cattle divergent in phenotypic residual feed intake (RFI) offered contrasting diets." *Journal of animal science and biotechnology* 5.1 (2014): 41.





Microbial Ecology in Gut







Gut microflora

- Creation of a synbiotic
- Combining probiotics with prebiotics could improve the survival of the bacteria crossing the upper part of the gastrointestinal tract
- Exerting enzymatic activities, increasing the passage rate of digestion and deconjugating bile salts and acids.
- Improved development of the gut and increased microvilli height which led to the enlargement of the microvillis' absorptive surface and enabled the optimal utilization of nutrients.

The **Immune response** is stimulated and the activity of host antibodies increased.

Competition for nutrients: probiotics compete with pathogens for important nutrients.

Competitive exclusion: probiotics block intestinal receptors, thereby excluding pathogens



pathogens and their

toxins adhere to the

mucous and the cell

receptors of the intes-

tine and damage it

Masking: where probiotics occupy intestinal receptors, enterotoxins are excluded.

Aggregation

by probiotics hampers the attachment and proliferation of pathogens





Modulation of gut microbiota









Three sections of a European registration dossier applying to probiotics

- Identity and quality: strain characteristics (taxonomy, metabolism, properties...), manufacturing process, stability (single or in mixture), method of analysis.
- 2. Safety: for the target animal species (harmless at 10 times the recommended dose), the handler, the consumer (lack of antibioresisance, genotoxicity and mutagenicity) and the environment.
- 3. Efficacy: to be demonstrated for the target species through at least three significant studies in two different places. The efficacy section describes the target species, the conditions (age, physiological stage, type of production), the usage doses, the claimed performances as well as the possible action mechanisms. The possible claims for probiotics can be effects on animal performance, production, welfare or environment.

AGRICULTURAL AND FOOD SCIENCE

Recent advances in silage microbiology

R. Muck (2013) 22: 3-15

Table 1. Microbial species recently isolated from silages

Species	Silage Type	Reference	
Lactic Acid Bacteria			
Enterococcus flavescens	Maize	Brusetti et al. (2006)	
Entercoccus mundti	Maize Stover	Pang et al. (2011b)	
Lactobacillus acetotolerans	Maize	Li and Nishino (2011b)	
Lactobacillus panis	Maize	Li and Nishino (2011b)	
Lactobacillus reuteri	Maize	Li and Nishino (2011b)	
Lactobacillus taiwanensis	Unknown	Wang et al. (2009)	
Lactobacillus zeae	Lucerne	Rossi and Dellaglio (2007)	
Leuconostoc lactis	Maize Stover	Pang et al. (2011b)	
Paralactobacillus selangorensis	Italian Ryegrass	Parvin et al. (2010)	
Pediococus dextrinicus	Italian Ryegrass	Parvin et al. (2010)	··· · ·
Pediococcus Iolii	Ryegrass	Doi et al. (2009)	Application to fTMR
Pediococcus parvulus	Maize	Li et al. (2011)	
Weissella cibaria	Maize, Maize Stover	Pang et al. (2011a,b)	
Weissella kimchii	Maize	Brusetti et al. (2006)	
Weissella paramesenteriodes	Maize	Li et al. (2011)	
Anaerobic Spore Formers			
Clostridium baratii	Maize	Rossi and Dellaglio (2007)	
Paenibacillus macerans	Maize	Rossi and Dellaglio (2007)	
Bacillus			
Bacillus megaterium	Maize	Brusetti et al. (2006)	
Enterobacteria			
Erwinia persicina	Italian Ryegrass	Li and Nishino (2011a)	
Pantoea agglomerans	Italian Ryegrass	Li and Nishino (2011a)	
Rahnella aquatilis	Italian Ryegrass	Li and Nishino (2011a)	
Acetic Acid Bacteria			
Acetobacter pasteurianus	Maize	Li and Nishino (2011b)	
Yeasts			
Candida apicola	Maize, Italian Ryegrass	Rossi and Dellaglio (2007)	
Candida intermedia	Maize	Li et al. (2011)	
Candida glabrata	Maize	Li et al. (2011)	
Candida magnolia	Maize	Li et al. (2011)	
Candida mesenterica	Maize	Rossi and Dellaglio (2007)	
Candida quercitrusa	Maize	Li et al. (2011)	
Saccharomyces martiniae	Maize	Li et al. (2011)	
Pichia deserticola	Maize	Li et al. (2011)	
Pichia fermentans	Maize	Rossi and Dellaglio (2007)	
Pichia kudriavzevii	Maize	Li et al. (2011)	

Value addition to agricultural and industrial wastes as feed

- Biotechnology, Fermentation, Microbes, Physiology (gut and gonadal morphometry)
- Vegetable residues (Yang et al., 2010), rice straw (Gao et al., 2008), tomato and apple pomace (Abdollahzadeh et al., 2010), and green tea grounds

Microbially fermented cassava peel (MFCP)

Lactobacillus coryneformis Lactobacillus delbrueckii Aspergillus fumigatus



Pig stomach

Advances in Microbiology, 2015, 5, 28-39

		Name of bacteria	
Name of Ingredients	Parameters	Bacillus sp. DDKRC1.	B. subtilis DDKRC5.
Rice bran	Optimum fermentation condition	60% moisture & 48 h incubation	60% moisture & 48 h incubation
	Increase in CP%	19.00 ± 0.12^{b}	10.46 ± 0.14^{a}
	Decrease in Cellulose%	6.60 ± 0.22 ^b	2.42 ± 0.15^{a}
	Decrease in NFE%	6.67 ± 0.11^{a}	9.86 ± 0.06^{b}
Sunflower cake	Optimum fermentation condition	50% moisture & 72 h incubation	60% moisture & 48 h incubation
	Increase in CP%	8.32 ± 0.05^{b}	5.70 ± 0.12^{a}
	Decrease in Cellulose%	14.65 ± 0.21 ^b	6.25 ± 0.08^{a}
	Decrease in NFE%	7.14 ± 0.05^{a}	10.31 ± 0.14^{b}

Table 5 Change of nutrient content of the feed ingredients fermented with potential gut microbes

Values bearing different superscripts in a column and row differ significantly P < 0.05.

De, Debasis, Tapas K. Ghoshal, and R. Ananda Raja. "Characterization of enzyme-producing bacteria isolated from the gut of Asian seabass, Lates calcarifer and milkfish, Chanos and their application for nutrient enrichment of feed ingredients." *Aquaculture Research* 45.9 (2014): 1573-1580.

Rev. sci. tech. Off. int. Epiz., 1989, **8** (2), 517-531. **Probiotics for animal use in Japan M. KOZASA** *



FIG. 5

Changes in intestinal lactobacilli following administration of probiotic in chickens under heat stress conditions (21)

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.

Research works in "Feed biotechnology lab"

Development of fermented phytobiotics

- Fermentation of plant extract juice
 - Hot pepper, Garlic, Onion, Chinese chives, Schisandraceae, Pine niddles etc.
- Isolation of beneficial probiotics
- Establishment of fermentation processing
- Establishment of formulation method
- Supplementation test to broiler, pig, cow
- Quenching materials on quorum sensing

In vitro test of Lactic acid bacteria under submerged liquid fermentation



NIU et al. In manusciript

Approaches to quorum sensing interference

Ramesh, K., et al. "Feasibility of Shrimp Gut Probionts with Anti-vibrio and Anti-QS in Penaeid Culture." (2014).



Plate 2: Anti-QS activity of shrimp gut isolates by parallel arrow streak method

Plate 1 to 12 indicates the isolates AVP01 to AVP12. The isolates AVP03 and AVP07 shows positive quorum quenching activities.

Probiotics in Korea

Legislation for probiotics

1) Subsidiary feeder : Simply report to city or county office

2) Non-medicine for animal : <u>Registration</u> to "Animal and plant quarantine agency" (<u>http://www.qia.go.kr</u>)



Probiotics registered in supplement feeds of Korea

Feed type	Microbes
	(1) Beneficial bacteria : Lactobacillus latics, Lactobacillus reuteri, Lactobacillus bulgaricus,
	Lactobacillus brevis, Lactobacillus salivarius, Lactobacillus acidophilus, Lactobacillus
	cottage, Lactobacillus curvatus, Lactobacillus fermentum, Lactobacillus plantarum,
	Lactobacillus helveticus, Lactobacillus nueric, Lactobacillus perolens, Lactobacillus
	paracasei, Lactobacillus crispatus, Rhodopseudomonas capsulata, Monascus purpureus,
	Bacillus lentus, Bacillus licheniformis, Bacillus subtilis, Bacillus cereus(only doyoi), Bacillus
	coagulans, Bacillus polyfermenticus, Bacillus pumilus, Bacillus clausii, Bifidobacterium
Probiotics	longum, Bifidobacterium Bifidum, bifidobacterium thermophilum, Bifidobacterium infantis,
	Enterococcus lactis, Enterococcus thermophilus, Enterococcus faecium, Clostridium
	butyricum, Pediococcus cerevisiae, Pediococcus acidilactici, Pediococcus pentosaceus
	(2) Beneficial fungi : Aspergillus niger, Aspergillus oryzae
	(3) Beneficial yeast: Beer's yeast, Torula yeast, Baker's yeast, Brewer's yeast, Irradiated
	dried yeast, Yeast culture
	(4) Bacteriophage : Salmonella gallinarum bacteriophage
	(5) (1) or (2), (3) of combination

Yearly production of probiotics and fermented feedstuffs



Number of domestic probiotics manufacturers and their production



Reference : Korea Feed Ingrediants Association, 2013

Number of domestic fermented feedstuffs manufacturers and their production



Domestic probiotics market

Species	Compound feeds (Ton)	Probiotics (Ton)	Probiotics(\$)
Poultry	3,900,000	7,800	23,400,000
Swine	5,660,000	11,320	34,000,000
Bovine	4,670,000	9,340	28,000,000
Etc	1,000,000	2,000	6,000,000
Total	15,230,000	30,460	91,400,000

Reference : Korea Feed Ingrediants Association, Bio-Feed Additives Research Center (2013)

Animal probiotics producing companies (64)

Company	Production(T)	Portion(%)
Amibio co.	18,799	60.24
Nonghyupfeed co.	2,445	7.83
Genebiotech	988	3.17
Synerbig	954	3.06
BBkorea co.	817	2.62
CTCbio co.	788	2.52
MKbio Science inc.	720	2.31
Nel company	608	1.95
Chungmi bio c o.	500	1.60
Sukwanggreen m	445	1.43
Easybio co.	442	1.42
Biotopia co.	410	1.31
Celltech co.	345	1.11
Fusionbio co.	341	1.09
Daeho co.	288	0.92
Bigbiogen co.,	258	0.83

Company	Production(T)	Portion(%)
Yeranggreentech	255	0.82
Dongwhamicorobe s co.	235	0.75
Handong co.	192	0.61
Byard co.	141	0.45
MStopia co.	138	0.44
Probionic co.	115	0.37
DOWbiomedica co.	111	0.36
Eunjinbio co.	108	0.35
Kofavet co.	96	0.31
Adbiotech co.	75	0.24
Daeduckbio co.	76	0.24
Vixxol co.	66	0.21
Microbiotech co.	49	0.16
ENT co.	51	0.16
Sesinbiotech	46	0.15
Paek kwang C&S c o.	45	0.14

Company	Production (T)	Portion(%)
Company	Froduction(1)	Fortion(%)
Shinil Biogen co.	38	0.12
Innobio co.	36	0.12
ShinghanBiochem co.	33	0.11
CheilBio co.	35	0.11
Miraesoo co.	30	0.10
Taerim Industry co. co.	22	0.07
Jinwoo co.	18	0.06
Koreassumbel co.	20	0.06
Hanpung co.	10	0.03
Hweedbest co.	7	0.02
Samyang Anipharm co.	7	0.02
Minwoo co.	4	0.01
Wooilbio F&M	0	0.00
Korin Korea co.	0	0.00
R&LNaturalLife co. co.	0	0.00
Yunwoongbiotech	0	0.00
Korea organic co.	0	0.00
Total(49)	31,207	100.00

Agriculture technology center in city or county area

-Production and supply of bacteria

Probiotics for animals Microbes for agricultural cultivation Free of charge



Liquid fermentation



Solid state fermentation









Representative feed additive manufacturer in the world

Company name	Country	Category	Main production
DSM	Netherlands	Enzyme feed additive	Roxozyme, Ronozyme
Danisco Animal Nutrition	Denmark	Enzyme feed additive	Avizyme, Grindazyme, Phyzyme
BASF Corporation	Germany	Enzyme feed additive	Natugrain, Natuphos
Alltech	U.S.A	Enzyme feed additive	Allzyme SF
Chr Hansen A/S	Denmark	Enzyme feed additive	Bioplus, Yieldcure, Lactiferm
Lohman Animal health & Co.	Germany	Probiotics	Microbisan®
LeSaffre	France	Probiotics	BioSaf®, Procreatin-7®
BioArmor Development SARL	France	Probiotics	Bioacton®
ADM	U.S.A	Probiotics	PremiDex
ALLtech	U.S.A	Probiotics	Gallipro

Reference : Ministry of Agriculture, Food and Rural Affairs(MAFRA), 2013

World's microbial feed additives market

- Banned of antibiotics caused fast growth of microbial feed additives market
- Develop enzyme feed additives, probiotics, prebiotics and supplied in market

Growth of the world's microbial feed additives market



The Ministry of Agriculture, Food and Rural Affairs, Korea (2013)

Bank of Microbial Resources in Korea

Microbial resources in Korea

Microbial resources are collected and management by government

(Ministry of Science, ICT and Future Planning, Ministry of Agriculture, Food and Rural Affairs, Ministry of trade, industry&energy etc.)

- Hold out more than 200,000 of bacteria, fungi, yeast, mushroom, virus, etc.

Providing service organization of microbial resources

http://www.genebank.go.kr/



Korean Culture Center of Microorganisms http://www.kccm.or.kr/



http://kctc.kribb.re.kr/kctc.aspx



http://www.knrrc.or.kr/index.jsp

- Korean Collection for Oral Microbiology http://kcom.knrrc.or.kr
- Culture Collection of Antimicrobial Resistant Microbes <u>http://www.ccarm.or.kr</u>
- Helicobacter pylori Korean Type Culture Collection <u>http://hpktcc.knrrc.or.kr</u>
- Korea Environmental Microorganisms Bank http://www.kbem.or.kr
- Extract Collection of Useful Microorganism http://www.ecum.or.kr

Almost no changes compared to 60 years before

?

Microbiological Process Report Production of Microbial Enzymes and Their Applicatio

L. A. UNDERKOFLER, R. R. BARTON, AND S. S. RENNERT

Takamine Laboratory, Division of Miles Laboratories, Inc., Clifton, New Jersey

Received for publication October 1, 1957

TABLE 1

Some commercial enzymes and source microorganisms

Source	Enzyme	Microorganism
Fungal	Amylases	Aspergillus oryzae
2	Glucosidases}	Aspergillus flavus
	Proteases	Aspergillus niger
	Pectinases	Aspergillus niger
	Glucose oxidase)	{Penicillium notatum
	Catalase	Aspergillus niger
Bacterial	Amylases)	
	Proteases	Bacillus subtilis
	Penicillinase	
Yeast	Invertase	Saccharomyces cerevisiae
	Lactase	Saccharomyces fragilis

Safety Efficacy Productivity Price

Enzymes

Tadele, Yilkal. "Effect of Exogenous Enzymes on Ruminal degradation of Feed and Animal Performance: A review." Advances in Life Science and Technology 28 (2015): 60-69.



Nanocoatings and nanofeed additives

Prasad, Ram, Vivek Kumar, and Kumar Suranjit Prasad. "Nanotechnology in sustainable agriculture: Present concerns and future aspects." *African Journal of Biotechnology* 13.6 (2014): 705-713.



Future research

- Probiotics+Prebiotics+Enzyme+Other supplements (for example, essential oil) : Synergistic effect
- Development of specific microbes for growth promotion
- Value added utilization of agro-industrial residues by fermentation
- Fusion sciences (Bio, Nano, IT, ET,...)
- Safety of animal probiotics and verification of efficacy of probiotics
- Gut microbiology and microbiota: Beneficial microbes

Further researches on probiotics and prebiotics will be required as the following:

- Improvement of productivity in animals by manipulating their microbial cohabitants
- Improvement of the quality of animal food products by supplementation of probiotics and prebiotics
- Decrease in the cost of prebiotics and probiotics
- New development of prebiotics and probiotics by fusion biotechnology in sustainable animal husbandry

