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Arazyme secreted by *Serratia proteamaculans*: current understanding in animal husbandry‡

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Abstract

Arazyme, an alkaline metalloprotease, is produced by *Serratia proteamaculans*, a symbiotic bacterium isolated from the intestinal ecosystem of *Nephila clavata*. Arazyme is known to play a crucial role in facilitating the digestion process in *N. clavata*. Recently, there has been increasing interest in exploring invertebrate-associated gut symbionts as a valuable source of novel and biologically active enzymes. Animal husbandry has shown significant interest in this spider-derived bioactive enzyme. This paper aims to provide a comprehensive review of the current understanding and knowledge of arazyme in the context of animal husbandry, offering valuable references for potential applications of this enzyme.

Introduction

Biologically active enzymes derived from invertebrate-associated gut symbionts represent a promising and underexplored resource. These enzymes play a crucial role in the efficient utilization of diverse nutrient resources by invertebrates (Jing *et al.*, 2020). Consequently, animal husbandry has shown considerable interest in harnessing these invertebrate-derived bioactive enzymes (Kannan *et al.*, 2019).

Arazyme, an alkaline metalloprotease, is secreted by *Serratia proteamaculans*, a Gram-negative aerobic bacterium belonging to the genus Serratia. It is a symbiotic bacterium isolated from the intestinal ecosystem of *Nephila clavata* (Fig. 1), a species of arachnid known as '棒络新妇' in Chinese and '무당거미' in Korean (Bersanetti *et al.*, 2005; Kwak *et al.*, 2007). Arazyme plays a pivotal role in facilitating the digestion process in *N. clavata*. With a size of 51.5 kDa, arazyme has been approved as a food additive (registration No. 2007-SA-0007) by the Ministry of Food and Drug Safety in the Republic of Korea. Notably, it exhibits remarkable enzymatic activity over a wide range of pH and temperature and displays resistance to detergents (Bersanetti *et al.*, 2005).

As a novel invertebrate-derived bioactive enzyme, arazyme holds broad application prospects in animal husbandry. This paper provided a review of the current knowledge and understanding of arazyme, aiming to offer valuable references for its potential applications.

Characteristics of arazyme

Arazyme, an enzyme with zinc-containing metalloprotease activity, relies on the presence of zinc for its function (Kwak *et al.*, 2007). Its deduced amino acid sequence exhibits significant homology to serralysins found in other enteric bacteria (Kwak *et al.*, 2007). Arazyme acts as a proteolytic enzyme, capable of hydrolysing substances such as albumin, casein, elastin, haemoglobin, keratin and collagen (Piao *et al.*, 2009). Notably, its proteolytic activity remains unaffected by inhibitors of aspartate, cysteine and serine proteases, as well as pepsin, trypsin and chymotrypsin. However, its activity is strongly inhibited by compounds like 1,10-phenanthroline and ethylenediaminetetraacetic acid (Bersanetti *et al.*, 2005; Kwak *et al.*, 2007). Furthermore, arazyme demonstrates optimal enzymatic activity at pH levels of 6.0 or higher and can function efficiently at temperatures up to 50°C (Bersanetti *et al.*, 2005).

Immunoregulatory property

Arazyme has been extensively studied for its immunoregulatory properties. Pereira *et al.* (2016) demonstrated that *in vivo* injection of both active and heat-inactivated arazyme exhibited significant effects in reducing tumour development, including primary and metastatic tumours. These effects were attributed to the activation of Toll-like receptor 4, leading to increased levels of interferon gamma and elevated counts of activated $CD8^+$ T lymphocytes. In a separate study, Pereira *et al.* (2014) found that intraperitoneal administration of arazyme

Figure 1. Image of *N. clavata* (source: https://commons.wikimedia.org/wiki/File:Nephila_clavata%28Female,Japan,2017.10.08%29.jpg).

exerted a dose-dependent cytostatic effect on human and murine tumour cells by reducing the expression of CD44 molecules on the tumour cell surface, thereby interfering with cell adhesion. Additionally, arazyme induced the production of protease-specific immunoglobulin G, which exhibited cross-reactivity with tumour matrix metalloproteinase-8. Ghadaksaz *et al.* (2022) developed a fusion protein, arazyme-linker-TGFαL3, with high affinity for the epidermal growth factor receptor. *In silico* immune simulation demonstrated that this chimaera protein has the potential to prevent cancer development by inducing an immune response and inhibiting cell proliferation. Similarly, Rahmani *et al.* (2023) designed a fusion protein, arazyme-linker-herceptin-HER2, which showed promising results as a candidate for breast cancer treatment. *In silico* immune simulation demonstrated that the predicted B-cell and T-cell epitopes of the fusion protein were capable of eliciting an immune response.

Overall, the mechanism of arazyme's immunoregulatory properties involves Toll-like receptor 4 activation, increased interferon gamma levels, activation of $CD8^+$ T lymphocytes, reduction of CD44 molecules and production of specific immunoglobulins. These studies collectively highlight the immunomodulatory potential of arazyme and its ability to impact immune responses, offering promising prospects for its application in immunotherapy.

Anti-inflammatory property

Bersanetti *et al.* (2005) observed that arazyme exhibited high hydrolytic activity on substance P, which is secreted by inflammatory cells, as well as peptides related to bradykinin, during *in vitro* co-culture. Additionally, Kim *et al.* (2015) conducted studies demonstrating that oral treatment of arazyme attenuated the development of atopic dermatitis-like lesions in mice induced by 2,4-dinitrochlorobenzene. This attenuation was achieved through the reduction of epidermal thickening, infiltration of inflammatory cells into the dermis and suppression of interleukin 4, interleukin 13 and immunoglobulin E levels. In another study by Kim *et al.* (2013), THP-1 human monocytic and EoL-1 human eosinophilic cells were treated *in vitro* with the *Dermatophagoides pteronyssinus* extract and administered with arazyme. The researchers observed that arazyme alleviated the severity of atopic dermatitis by regulating the expression of thymus and activation-regulated chemokine and skin barrier proteins in keratinocytes. They found that arazyme inhibited the production of monocyte chemoattractant protein 1, interleukin 6 and interleukin 8 in THP-1 and EoL-1 cells. Furthermore, arazyme suppressed the secretion of interleukin 6 and interleukin 8 in HMC-1 cells, reduced thymus and activation-regulated chemokine, monocyte chemoattractant protein 1, interleukin 6 and interleukin 8 levels in HaCaT cells, and upregulated the production of filaggrin, involucrin and loricrin in HaCaT cells. Additionally, Kim *et al.* (2014) observed that arazyme administration inhibited lipopolysaccharide-induced apoptosis in human umbilical vein endothelial cells *in vitro*. This inhibition was attributed to the suppression of monocyte chemoattractant protein 1 and interleukin 6 secretion as well as the downregulation of vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 expression, and reduction in reactive oxygen species production. Kim and Lee (2014) investigated the inhibitory effects of arazyme on neutrophil apoptosis in allergic diseases, specifically allergic rhinitis and asthma *in vitro*. They discovered that arazyme effectively inhibits neutrophil apoptosis through the PI3K/Akt/ERK/NF-κB pathway and the caspase 9/3 pathway. In the context of animal health, providing arazyme-containing diet has shown potential in reducing the occurrence of subclinical mastitis in dairy cows (Liu *et al.*, 2007). This reduction is manifested by a decrease in somatic cell count in milk.

In summary, the mechanism underlying arazyme's anti-inflammatory property involves multiple potential pathways. Arazyme may interact with protease-activated receptors and exhibit high hydrolytic activity on specific peptides. Additionally, it regulates the production of pro-inflammatory cytokines and chemokines and protects against apoptosis and oxidative stress. Although more research is needed to fully understand the precise molecular mechanisms, these findings collectively suggest that arazyme acts as an anti-inflammatory agent through various mechanisms, making it a promising candidate for inflammation regulation.

Anti-bacterial property

In a study conducted by Kim *et al.* (2021), it was found that arazyme at a concentration of 250 mg/ml exhibited remarkable anti-bacterial properties *in vitro*. The researchers observed that arazyme inhibited the growth of oral opportunistic pathogens such as *Candida albicans*, *Enterococcus faecalis*, *Staphylococcus epidermidis* and *Streptococcus mutans*. Notably, arazyme demonstrated over 80% inhibition against *C. albicans*, which is a major contributor to denture stomatitis. These findings highlight the potential of arazyme as an effective agent for controlling harmful bacterial growth and preventing oral infections associated with denture use.

However, it is important to note that while this study demonstrated the anti-bacterial properties of arazyme, further research is necessary to elucidate the underlying mechanisms by which arazyme exerts its effects on bacterial growth. Future experiments can help unravel the specific molecular interactions and pathways involved in the anti-bacterial activity of arazyme, providing a deeper understanding of its potential applications in controlling bacterial infections.

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Table 1. Application of arazyme in poultry husbandry

Animal and reference	Additive and effects
Layer (Kim *et al.*, 2009)	Supplementing 0.05, 0.075 or 0.10% arazyme to the diet
	Production performance: egg weight ↑ (observed in treatments containing 0.05 and 0.075% arazyme) *Internal environment of intestine*: viscosity of intestinal contents ↓ (observed in treatments containing 0.05 0.075 and 0.10% arazyme) *Excreta noxious gas emission*: NH_3 emission ↓ (observed in treatments containing 0.05 0.075 and 0.10% arazyme)
Layer (Kim, 2009)	Supplementing 0.05, 0.10 or 0.30% arazyme to the diet
	Production performance: egg weight ↑ (observed in treatment containing 0.10% arazyme); egg mass ↑ (observed in treatments containing 0.05, 0.10 and 0.30% arazyme); egg production ↑ (observed in treatments containing 0.05, 0.10 and 0.30% arazyme)
Broiler (Kim, 2009)	Supplementing 0.10% arazyme to the diet
	Growth performance: body weight, BWG and feed efficiency ↑ *Excreta noxious gas emission*: NH_3 emission ↓

NH_3, ammonia; BWG, body weight gain.

Organ protection property

In the study conducted by Park *et al.* (2008), the effects of oral arazyme on acute hepatic injury induced by CCl4 in mice were investigated. The findings revealed that arazyme plays a protective role by upregulating the expression of antioxidative proteins, inhibiting the TGF-*β*/Smad3 signalling pathway through downregulation of Smad3 and p-Smad3 expression levels, and preventing the decrease of SMP30 expression. These results suggest that arazyme has the potential to protect hepatocytes from chemokine-induced hepatic damage. In a separate study by Li *et al.* (2019), the hepatoprotective effects of oral arazyme in high-fat diet-induced non-alcoholic fatty liver disease-like mice were examined. The administration of arazyme demonstrated protective effects against hepatic steatosis and hepatitis, and inhibited the progression of non-alcoholic fatty liver disease. Arazyme exerted its hepatoprotective effects by inhibiting hepatic fatty acid and triglyceride synthesis, suppressing SREBP-1-mediated lipid accumulation and macrophage-mediated inflammation, and reducing palmitic acid-induced lipogenesis in HepG2 hepatocytes. Moreover, arazyme reduced macrophage recruitment by inhibiting the expression of inflammatory cytokines in the liver. Li *et al.* (2016) further investigated the hepatoprotective effects of oral arazyme in high-fat diet-induced non-alcoholic fatty liver disease-like mice. The supplementation of arazyme in the diet exhibited hepatoprotective effects by decreasing hepatic lipid accumulation and improving insulin resistance. Arazyme supplementation resulted in reduced plasma levels of alanine aminotransferase, thyroglobulin, non-esterified fatty acids, glucose and haemoglobin A1C. Additionally, arazyme improved hepatic steatosis and fibrosis, decreased hepatic triglyceride and total cholesterol contents, improved glucose tolerance and reduced pancreatic insulin contents and islet size. In cell studies, arazyme increased AKT phosphorylation in palmitic acid-induced HepG2 cells, improved insulin secretion and synthesis in pancreatic MIN6 *β*-cells and reduced hepatic lipid accumulation while reversing insulin resistance.

Overall, oral arazyme administration is able to upregulate antioxidative proteins, inhibit the TGF-*β*/Smad3 signalling pathway, prevent the decrease of SMP30 expression, inhibit hepatic fatty acid and triglyceride synthesis, enhance AKT phosphorylation and improve insulin resistance. These mechanisms collectively contribute to the hepatoprotective effects of arazyme, ultimately improving liver function in various liver injury and non-alcoholic fatty liver disease models.

Application of arazyme in feedstuff industry

The feed industry can extract several benefits from the use of arazyme. Firstly, arazyme, as a proteolytic enzyme, has the ability to hydrolyse proteins in feedstuff materials (Piao *et al.*, 2009). This enzymatic hydrolysis results in increased levels of digestible small molecular substances, particularly amino acids and peptides, present in the ingredient. Zi *et al.* (2011) conducted an enzymatic hydrolysis study on soybean meal using a combination of 0.5% phytase and 0.02% arazyme, which resulted in increased levels of free cysteine, aspartic acid, threonine, methionine and leucine in the hydrolysed soybean meal. Kim (2009) reported that when arazyme was applied to hydrolyse corn gluten meal, it led to elevated levels of free leucine. Similarly, hydrolysis of soybean meal with arazyme resulted in an increased level of free phenylalanine, valine, leucine and isoleucine (Kim, 2009). Furthermore, the hydrolysis of distiller's dried grains with solubles using arazyme increased the contents of free cysteine, valine and isoleucine (Kim, 2009). Fish meal hydrolysed with arazyme also exhibited increased level of free cysteine (Kim, 2009). Piao *et al.* (2009) note that when arazyme was applied to hydrolyse meat meal, fish meal, soybean meal, cottonseed meal, rapeseed meal, corn flour and corn gluten meal, there were notable increases in the contents of both essential and non-essential free amino acids, particularly lysine, threonine and tryptophan. Additionally, Liang and Piao (2010) found that arazyme hydrolysis of black rice bran resulted in increased free peptide content. By enhancing the free amino acid profiles of feedstuff materials, arazyme can improve the nutritional value of animal diets.

Additionally, the utilization of arazyme can help optimize feed formulations by providing a cost-effective alternative to expensive protein sources, such as plasma protein powder. Guan and Sun (2010) demonstrated that replacing 4% plasma protein powder in the diet with 0.1% arazyme and 3% fish meal effectively reduced feed costs without compromising growth performance.

Overall, the use of arazyme in the feed industry offers benefits such as improved feedstuff's free amino acid profiles and cost savings in feed formulation. These advantages contribute to the production of high-quality and cost-effective animal feeds, ultimately benefiting both feed manufacturers and livestock producers.

Application of arazyme in animal husbandry

In this paper, we conducted a review of scientific studies investigating the effects of dietary arazyme supplementation in various

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Table 2. Application of arazyme in swine husbandry

Animal and reference	Additive and effects
Growing-finishing pig (Ju *et al.*, 2010)	Supplementing 0.10% arazyme to the diet
	Nutrient digestibility: ATTD of dry matter, crude protein and energy ↑; AID of dry matter, crude protein, energy, EAAs and NEAAs ↑
Finishing pig (Wen, 2005)	Supplementing 0.05% arazyme to the diet
	Growth performance: final body weight ↑ (observed in treatment containing 0.05% arazyme); ADG and feed efficiency ↑ (observed in treatments containing 0.05 and 0.10% arazyme) *Nutrient digestibility*: ATTD of crude protein, crude fat, crude fibre, crude ash and phosphorus ↑ (observed in treatments containing 0.05 and 0.10% arazyme); ATTD of calcium ↑ (observed in treatment containing 0.05% arazyme) *Hematology*: serum alanine aminotransferase, amylase, albumin and glucose levels ↑ (observed in treatments containing 0.05 and 0.10% arazyme); serum urea nitrogen levels ↑ (observed in treatment containing 0.05% arazyme); serum alkaline phosphatase, lactate dehydrogenase, calcium and phosphorus levels ↑ (observed in treatment containing 0.10% arazyme) *Carcass trait*: carcass length and eye muscle area ↑ (observed in treatments containing 0.05 and 0.10% arazyme); carcass weight and slaughter rate ↑ (observed in treatment containing 0.10% arazyme); bone percentage ↓ (observed in treatment containing 0.10% arazyme) *Meat quality*: drip loss ↓ (observed in treatments containing 0.05 and 0.10% arazyme) *Nutritional value of pork*: SFA ↓ (observed in treatment containing 0.10% arazyme); UFA ↑ (observed in treatment containing 0.10% arazyme)
Gestating sow (Song *et al.*, 2022)	Supplementing 0.01% arazyme to the diet
	Offspring performance: number of weak offspring, diarrhoea rate and mortality rate ↓
Gestating sow (Qu *et al.*, 2009)	Supplementing 0.01% arazyme to the diet
	Offspring performance: body weight at weaning ↑; diarrhoea rate ↓
Gestating sow (Wang *et al.*, 2009)	Supplementing 0.01% arazyme to the diet
	Offspring performance: body weight at birth and weaning, average litter weight and ADG ↑; diarrhoea rate ↓ *Faecal condition*: faecal abnormal condition ↓
Weaning pig (Song *et al.*, 2022)	Supplementing 0.02% arazyme to the diet
	Growth performance: final body weight, ADG and feed efficiency ↑
Weaning pig (Xu *et al.*, 2009)	Supplementing 0.10% arazyme to the diet
	Growth performance: final body weight, ADG and feed efficiency ↑
Weaning pig (Wen, 2005)	Supplementing 0.05 or 0.10% arazyme to the diet
	Nutrient digestibility: ATTD of crude fat and phosphorus ↑ (observed in treatments containing 0.05 and 0.10% arazyme); ATTD of calcium ↑ (observed in treatment containing 0.05% arazyme); ATTD of crude protein and crude fibre ↑ (observed in treatment containing 0.10% arazyme)

ADG, average daily gain; EAA, essential amino acid; NEAA, non-essential amino acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid; ATTD, apparent total tract digestibility; AID, apparent ileal digestibility.

animal species, including poultry (Table 1), swine (Table 2), ruminant (Table 3) and aquatic animals (Table 4). The results of these studies consistently demonstrate the positive effect of incorporating arazyme into the diet of farming animals, leading to improvements in their growth and production performance.

Specifically, in poultry husbandry, the supplementation of arazyme to a diet has been shown to improve performance and reduce excreta noxious gas emissions in both broilers and layers (Kim, 2009; Kim *et al.*, 2009). Similarly, in swine husbandry, arazyme supplementation has been found to enhance the efficiency of nutrient utilization, leading to improved growth performance, carcass traits, meat quality, nutritional value of pork and offspring quality (Wen, 2005; Qu *et al.*, 2009; Wang *et al.*, 2009; Xu *et al.*, 2009; Ju *et al.*, 2010; Song *et al.*, 2022). In ruminant husbandry, the inclusion of arazyme has shown promising results in improving rumen fermentation, ultimately translating into improved milk quality, milk production and growth performance (Zhang and Sun, 2009; Wang, 2019). Furthermore, in the context of aquaculture, the addition of arazyme to fish and shrimp diets has demonstrated beneficial effects on nutrient digestibility, antioxidant capacity and growth performance (Wu *et al.*, 2007; Xie *et al.*, 2009).

The consistent positive outcomes observed across these studies highlight the potential of arazyme as a valuable tool in animal nutrition. The ability of arazyme to enhance growth and production performance in various species underscores its importance as a supplement in animal feed formulations. Further research and exploration of optimal dosage levels and other delivery methods are warranted to fully harness the benefits of arazyme in animal husbandry.

Conclusion

In conclusion, arazyme, a biologically active enzyme derived from invertebrate-associated gut symbionts, holds significant potential for various applications. It exhibits remarkable enzymatic activity over a wide range of pH and temperature, making it suitable for use under different conditions. Arazyme has been extensively studied for its immunoregulatory, anti-inflammatory, antibacterial and organ protection properties. In the feedstuff

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Table 4. Application of arazyme in aquaculture

Animal and reference	Additive and effects
Tilapia (Wu *et al*., 2007)	Supplementing 0.03, 0.05 or 0.10% arazyme to the diet
	Growth performance: body weight, BWG and feed efficiency ↑ (observed in treatment containing 0.10% arazyme) *Nutrient digestibility*: ATTD of protein ↑ (observed in treatment containing 0.10% arazyme); ATTD of dry matter ↑ (observed in treatments containing 0.05 and 0.10% arazyme) *Hematology*: serum SOD level ↑ (observed in treatment containing 0.10% arazyme)
Litopenaeus vannamei (Xie *et al*., 2009)	Supplementing 0.10 and 0.15% arazyme to the diet
	Hematology: serum lysozyme level ↑ (observed in treatment containing 0.10% arazyme); serum acid phosphatase level ↑ (observed in treatments containing 0.10 and 0.15% arazyme)

BWG, body weight gain; ATTD, apparent total tract digestibility; SOD, superoxide dismutase.

Table 3. Application of arazyme in ruminant husbandry

Animal and reference	Additive and effects
Dairy cow (Wang, 2019)	Daily supplementing 10, 15 or 20 g arazyme to the diet
	Milk quality: urea nitrogen contents ↓ *Hematology*: serum alanine aminotransferase content ↑ (observed in treatment daily containing 20 g arazyme); serum glucose content ↑ (observed in treatment daily containing 15 g arazyme); serum total cholesterol content ↑ (observed in treatments daily containing 15 and 20 g arazyme)
Dairy cow (Zhang and Sun, 2009)	Daily supplementing 10 g arazyme to the diet
	Production performance: milk yield ↑ *Milk quality*: butterfat percentage ↑
Large-tailed Han sheep (Wang, 2019)	Supplementing 0.05% arazyme to the diet
	Growth performance: ADG ↑ *Meat quality*: drip loss ↓

ADG, average daily gain.

industry, arazyme has been proven effective in hydrolysing proteins in feed materials, leading to enhanced free amino acid profiles and peptide contents. This offers opportunities to improve the nutritional value of feed formulations and reduce feed costs. In animal husbandry, arazyme has consistently shown positive effects on growth and production performance in poultry, swine, ruminants and aquatic animals. It improves nutrient utilization, intestinal health and meat quality, while reducing noxious gas emission. These findings emphasize the potential of arazyme as a valuable tool in animal nutrition.

Overall, the comprehensive review of arazyme presented in this paper provides valuable references for its potential applications. Further research is warranted to explore optimal dosage levels, delivery methods and underlying mechanisms of action.

Data. No new data were generated or analysed in support of this research.

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