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Review

Microbe-derived antimicrobial red pigments for color formation and microbial growth control in sausage: A mini-review

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Abstract Sausage is a popular processed meat product and its consumption has significantly increased over the past 20 years. Sodium nitrite is used to impart a distinct red color and cured flavor to sausage during its production. Moreover, sodium nitrite inhibits lipid oxidation and growth of pathogenic microorganisms in sausage. Despite these advantages, it is advisable to replace and reduce sodium nitrite use since sausages with sodium nitrite are classified as a Group 1 carcinogen. The replacement additives should not only impart red color but also control microbial growth because sausage color is an important factor in its marketing and the control of initial microbial concentration during sausage production process is critical for its safety, distribution, and storage. However, only few sodium nitrite alternatives can impart color while inhibiting microorganism growth. To address this issue, use of natural red pigments with antimicrobial activity is being considered. Interest in natural functional pigments, in particular, microbial pigments with physiological activities, has spiked due to their various advantages, such as sustainable supply in large amounts, high yield, and easy down-streaming processing, over those extracted from plants or insects. This review highlights the characteristics of microbe-derived antimicrobial red pigments and their potential application as alternatives to sodium nitrite in sausage processing.

Keywords antimicrobial activity, microbial pigment, red color, sausage, sodium nitrite

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1. Introduction

Consumption of meat and meat products, including sausage, has increased significantly over the past 20 years (Suurs et al., 2022). The consumption of processed meat such as meat derivatives, cold cults, and sausages remained unabated even during COVID-19 pandemic (De Nucci et al., 2022). Sausage is one of most popular meat products consumed worldwide (Zhang et al., 2022). Because of the high fat content in sausage, it is prone to undergo lipid oxidation, which can change flavor, odor, color, texture, and nutritional value, leading to quality deterioration of sausage products (Zhang et al., 2019). Furthermore, harmful compounds, such as peroxide and aldehyde, are generated during lipid oxidation

(Falowo et al., 2014). This type of oxidation is reportedly inhibited by sodium nitrite (Rivera et al., 2019; Thomas et al., 2013), a food additive used worldwide, including in the United States and Europe (Kalaycioglu and Erim, 2019; Samuel et al., 2018; Sojic et al., 2020). It acts as an antioxidant via reacting with oxygen getting oxidized to nitrate (Honikel, 2008). Moreover, sodium nitrite inhibits the growth of pathogenic microorganisms such as *Listeria monocytogenes*, *Bacillus cereus*, *Clostridium perfringens*, *Salmonella enterica* serovar Typhimurium, *Flavobacterium, Achromobacter*, *Aerobacter*, *Micrococcus* spp., and *Escherichia* (Krause et al., 2011; Lee et al., 2018; Rivera et al., 2019). Sodium nitrite has an excellent ability to inhibit growth of *Clostridium botulinum*, the causative agent of fatal botulinum toxin (Lee et al., 2018; Milkowski et al., 2010; Sindelar and Milkowski, 2012). Due to these qualities, sodium nitrite has been used in sausage production throughout history; rock salt containing sodium nitrite was reportedly used to produce cured meats, such as ham and sausage, in ancient Greece and Rome (Redondo-Solano, 2011). Furthermore, sodium nitrite is an effective color fixative and helps to maintain the red appearance of sausage (Choi et al., 2019; Rivera et al., 2019); therefore, it is generally used as an additive during sausage production (Grispoldi et al., 2022). Moreover, sodium nitrite reacts with myoglobin, which plays a major role in imparting color in sausage (Ning et al., 2019; Posthuma et al., 2018). Specifically, NO, the reduce form of sodium nitrite binds to the heme-iron of myoglobin, a sarcoplasmic heme protein, leading to development of a distinct red color (Posthuma et al., 2018; Rivera et al., 2019). Moreover, sodium nitrite contributes to the unique cured flavor of sausage (Ichimura et al., 2017; Mac Donald et al., 1980) and, sausage prepared without the addition of sodium nitrite lack this flavor (Sebranek and Bacus, 2007). While it remains unclear why sodium nitrite imparts the cured flavor, it is believed to be related to the presence of specific volatile compounds, such as 4-methyl-1,2-pentanone, 1,2,4-trimethyl-cyclohexane, 2,2,4-trimethyl-hexane, and 1,3-dimethylbenzene (Ichimura et al., 2017).

Presently, processed meats containing sodium nitrite are classified as Group 1 carcinogens due to sufficient scientific evidence that sodium nitrite produces the carcinogen nitrosamine (Table 1) (Ghaffari et al., 2019; Yang et al., 2019). The

Table 1. IARC[1] classifications of food-related carcinogenic agents (WHO, 2022)

Group	Description	Agent
Group 1	Carcinogenic to humans	Processed meat (consumption of) Salted fish, Chinese-style Alcoholic beverages
Group 2A	Probably carcinogenic to humans	Frying, emissions from high-temperature Red meat (consumption of) Very hot beverages at above 65°C (drinking)
Group 2B	Possibly carcinogenic to humans	Aloe vera, whole leaf extract Goldenseal root powder Pickled vegetables (traditional Asian) Carrageenan, degraded (Poligeenan) Citrus Red No. 2 Kava extract Ginkgo biloba extract

[1]IARC, International Agency for Research on Cancer.

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International Agency for Research on Cancer (IARC), a part of the World Health Organization (WHO), reported that consuming 50 g processed meat daily increased the risk of colorectal cancer by 18% (Bouvard et al., 2015). This report is based on 800 studies involving 22 expert groups from 10 countries (Infante et al., 2018). The lethal dose of sodium nitrite is 71 mg/kg, equivalent to 4.6 g in an adult weighing 65 kg (Alyoussef and Al-Gayyar, 2016). Excessive sodium nitrite intake can reduce the oxygen transport function of red blood cells, leading to methemoglobinemia and respiratory dysfunction (Katabami et al., 2016; Trasande et al., 2018). In infants and children, sodium nitrite can be fatal, causing blue baby syndrome (Knobeloch et al., 2000). In addition, the American Medical Association (AMA) reported a link between sodium nitrite ingestion and the risk of gastrointestinal or neural cancer (Trasande et al., 2018). As the harmful effects of sodium nitrite have been widely reported, it has become a major public health issue, with countries strictly regulating sodium nitrite levels in processed foods. Furthermore, consumer anxiety regarding cancer risk has affected sausage consumption to a certain extent. For these reasons, research studies have focused on reducing sodium nitrite levels or replacing it altogether in the sausage production. However, additives that can reduce the use of sodium nitrite while replacing its beneficial functions remain to be developed. This review highlights the potential application and characteristics of microbe-derived antimicrobial red pigments as an alternative to sodium nitrite in sausage production process.

2. Importance of color formation and microbial growth control in sausage

To address the growing consumer anxiety and distrust due to concerns over presence of sodium nitrite in processed meat, concerned production companies are actively developing new products that contain little or no sodium nitrite. It is expected that the preference for safer foods and healthier lifestyle among consumers will lead to the steady decrease in sodium nitrite use in the sausage industry. This combination of social trend and corporate efforts is highly desirable for development of safe food products. Several studies have attempted to develop additives that can replace sodium nitrite while maintaining its key advantageous features such as color fixation, microbial growth inhibition, flavor enhancement, and lipid oxidation inhibition (Alahakoon et al., 2015; Alirezalu et al., 2019; Berardo et al., 2016; Gassara et al., 2016; Lamas et al., 2016; Li et al., 2013). However, due to various factors—including unclear ingredients, prohibitive cost, and limited ability to perform the same functions as sodium nitrite—these substitutes have not reached the commercial stage (Liu et al., 2019). However, few sodium nitrite alternatives have been found to simultaneously produce color fixation and inhibit microbial growth—the two major requirements of sausage industry.

Sausage color is a critical factor in its marketing (Liu et al., 2019; Ning et al., 2019; Ruiz-Capillas et al., 2015). Red sausage is highly popular due to consumer perceptions; consumers associate grayish-brown color with old meat, while perceiving red as fresh meat. Recognizing this, butcher shops install lights with red filters to display their wares to make them more attractive to customers. However, the red color of fresh meat changes to grayish-brown in absence of sodium nitrite during the sausage production process. This color change significantly reduces the market value of sausage products.

Currently, insect-derived pigments, such as cochineal pigment or lac pigment, are added to sausages as sodium nitrate-alternative colorants (Chin and Choi, 2005; Nair et al., 2020; Ruiz-Capillas et al., 2015). The natural cochineal and lac pigments, derived from female cochineal insects (*Dactylopius coccus*) and the female *Coccus lacca* (*Laccifer lacca* Kerr), respectively, contain protein components (Kampmeier and Irwin, 2009; Wongwad et al., 2012) that may cause adverse effects including allergy, diarrhea, anaphylaxis, asthma, and hives (Greenhawt et al., 2009; Sugimoto et al., 2013). Thus, the use of insect-derived cochineal and lac pigments is not suitable as sausage pigments. Vegetable-derived paprika pigment is another type of red pigment used in sausage production (Sun et al., 2015). However, paprika pigment has its own distinct flavor that needs to be minimized before its use as a coloring agent (Kendrick, 2016). Indeed, the paprika flavor limits the widespread use of paprika pigment in sausages. As such, the goal is to develop natural pigments that can replace sodium nitrite by imparting red color but not any unwanted flavor.

Another important aspect is control of microbial growth in processed food products. Sodium nitrite inhibits growth of pathogenic microorganisms, including *C. botulinum* (Krause et al., 2011; Lee et al., 2018; Milkowski et al., 2010; Sindelar et al., 2012). Thus, additives that can effectively control microorganism growth in sausages should be developed to replace sodium nitrite. Currently, various natural preservatives—including nisin, ε-polylysine, chitosan, plant-derived compounds, and lysozymes—are being evaluated to this end (Alahakoon et al., 2015; Alirezalu et al., 2019; Nair et al., 2020). However, despite being effective in controlling the growth of microorganisms, none of them impart red color to sausage.

Keeping safety as the primary goal in sausage production is important to control the number of pathogenic microbes in processed meat. Under ideal conditions, bacteria divide every 20 minutes via binary fission (Table 2); this translates to exponentially fast growth rates. Given the fast rate of microbial growth, if the initial microbial concentration is high, sufficient inhibition of microbial growth may not be achieved. Moreover, special attention should be paid to microbial contamination in sausages because the meat used for sausage production can be easily contaminated during production and transport (Eisel et al., 1997; Ercolini et al., 2006). Generally, raw meat sold in markets harbors several microorganisms, and most countries have regulations that restrict the level of aerobic plate count in meat (<5×10^6 CFU/g or cm^2) (Kim et al., 2018). In other words, approximately 10^5–10^6 CFU/g or cm^2 microorganisms may already be present in the meat used for sausage production. Although sterilization kills most microorganisms during processing, it does not eliminate all. The higher the concentration of microorganisms persisting

Table 2. Bacterial generation time under optimal conditions (Mason, 1935)

Bacteria	Generation time (min)
Escherichia coli	12.5
Bacillus thermophilus	16
Vibrio cholerae	21.2
Salmonella paratyphi	23
Shigella dysenteriae	23
Streptococcus lactis	23.5
Staphylococcus aureus	27
Bacillus subtilis	26–32
Bacillus megaterium	31
Clostridium botulinum	35

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post-sterilization, the faster the decay of sausage products and the shorter their preservation period. Therefore, to increase safety and shelf-life of sausage products, the initial microbial contamination should be kept low. For instance, chicken with an initial microbial contamination of approximately 10^3 CFU/cm^2 that is stored at 4℃ is edible for approximately 12 days; however, if the initial microbial concentration is approximately 10^4 CFU/cm^2, slime forms within seven days and initiates meat decay (Rodriguez-Amaya, 2019). Therefore, reducing the initial microbial concentration is one of the key issues in the sausage industry. Lowering initial microbial concentration results in an extended expiration date, leading to increased profitability due to the greater sales period. Consequently, development of food additives that can lower the initial microbial concentration in sausage and offer sustained microbial control is important.

3. Natural functional pigments with physiological activities

The increasing awareness of the link among health, nutrition, and food safety is leading to the avoidance of hazardous food additives and a heightened interest in the health-promoting effects of foods worldwide. In particular, replacing commonly used synthetic pigments with natural pigments in processed foods has gained momentum (Oplatowska-Stachowiak and Elliott, 2017). Furthermore, the growing awareness about natural pigments as functional food ingredients has expanded their purpose beyond their basic role as coloring agents. The natural pigments in fruits and vegetables, such as include anthocyanins and curcumin, are attracting attention as physiologically active compounds (Brudzynska et al., 2021; Rodriguez-Amaya, 2019).

Anthocyanins are believed to be responsible for the “French paradox,” which describes the low incidence of coronary artery disease in French, who are known consume large quantities of wine (De Gaulejac et al., 1999). Anthocyanins exhibit antioxidant activity as well as exert suppressive effects in cancer and heart disease (Pojer et al., 2013; Speer et al., 2020). The bright yellow turmeric root, which is widely used as a spice and colorant in tropical Asia, has been recognized as a medicine in India and Southeast Asia since ancient times (Amalraj et al., 2017; Salehi et al., 2019; Vinod et al., 2019). In recent years, anti-inflammatory, antimicrobial, antimutagenic, tumor suppressive, and lipid regulatory effects of turmeric have been demonstrated (Amalraj et al., 2017; Salehi et al., 2019; Vinod et al., 2019).

4. Advantages of microbial pigments as sodium nitrite replacements in sausage industry

Natural pigments are obtained primarily from plants, insects, or microorganisms. Microorganisms are closely related to most of the foods that we encounter. They play an essential role in producing fermented foods that are beneficial to humans (Dimidi et al., 2019). In addition, microorganisms are used as sources of antibiotics, enzymes, organic acids, amino acids, and vitamins and they also produce mycoproteins that are considered a promising meat substitute (Humpenoder et al., 2022; Singh et al., 2017). Moreover, microorganisms are promising sources of food colorants because they produce various pigments such as carotenoid, quinine, flavin, violacein, melanin, and monascin (Behera et al., 2021; Dufosse et al., 2014; Ramesh et al., 2019).

Microbial pigments have several advantages over those extracted from plants or insects. First, large amounts of microbial pigments can be supplied sustainably (Kalra et al., 2020). Microorganisms grow much faster compared to plants and insects, without showing significant seasonal variations and no lack of raw materials (Panesar et al., 2015). Second, the production of microbial pigments and their down-streaming processing are easy (Tuli et al., 2015), which is essential for their mass production and cost efficiency. While several natural pigments have high production cost and low stability, microbial pigments have advantages of scalability and comparatively low production cost (Sen et al., 2019). Furthermore, unlike plants and insects, industrial application of microorganisms is relatively easy; moreover, they can be genetically or environmentally manipulated to produce higher pigment yields. In other words, metabolic engineering techniques, such as biosynthetic pathways, gene cloning, and recombinant DNA can help improve microbial pigment production (He et al., 2017; Lin et al., 2017). Moreover, microbial pigments can be used as color intensifiers, additives, and functional food ingredients in addition to their health-promoting functionalities, such as anti- cancer, antioxidant, and antimicrobial activity.

Considering these advantages of microbial pigments, devising a strategy to replace sodium nitrite in sausage production with such pigments is desirable. To replace the carcinogenic sodium nitrite currently used as a colorant and preservative in sausage, microbial pigments should be able to impart stable red color as well as confer significant antimicrobial activity (Fig. 1). However, few studies have examined and reported color imparting and antimicrobial efficacy of microbial pigments as additives in sausage. Therefore, there is a need for further research studies to support the application of microbe-derived antimicrobial red pigments to sausage processing.

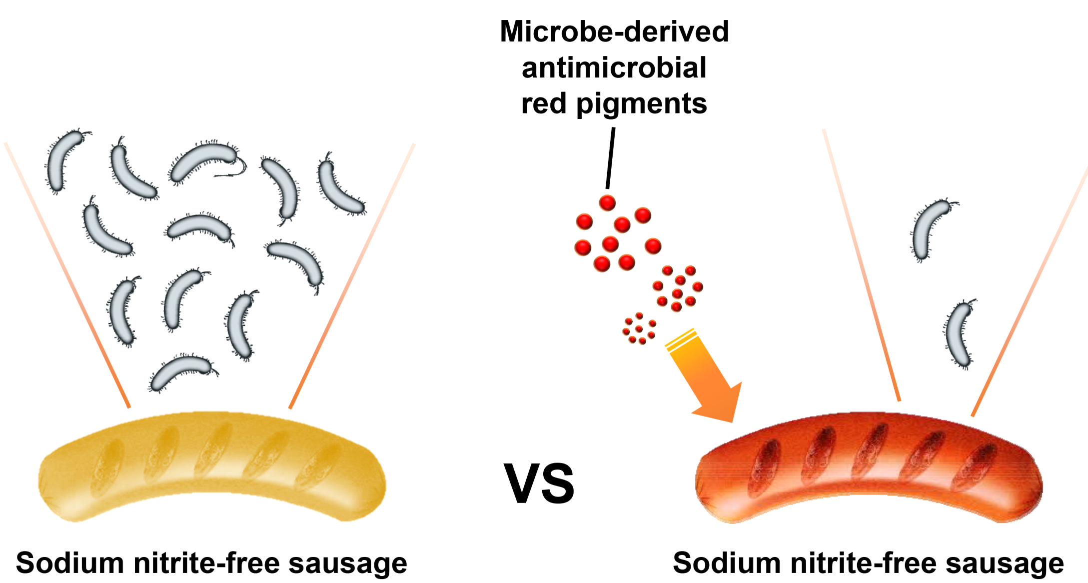

Fig. 1. Schematic diagram proposing the use of microbe-derived antimicrobial red pigments as suitable sodium nitrite substitutes in sausage production, in terms of color formation and microbial growth regulation.

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5. Microbe-derived antimicrobial red pigments

Food additives, including microbe-derived antimicrobial red pigments, as sodium nitrite replacements in sausage processing must impart the desired red color and inhibit microbial growth. Therefore, information on microbe-derived antimicrobial red pigments with potential as sodium nitrite alternatives has significant implications. Here, we discuss carotenoids, prodigiosin, and *Monascus* pigments as potential alternatives (Fig. 2).

5.1. Carotenoids

Carotenoids are the most widely distributed natural pigments and are responsible for colors ranging from red through orange to yellow. The structures of approximately 600 types of carotenoids

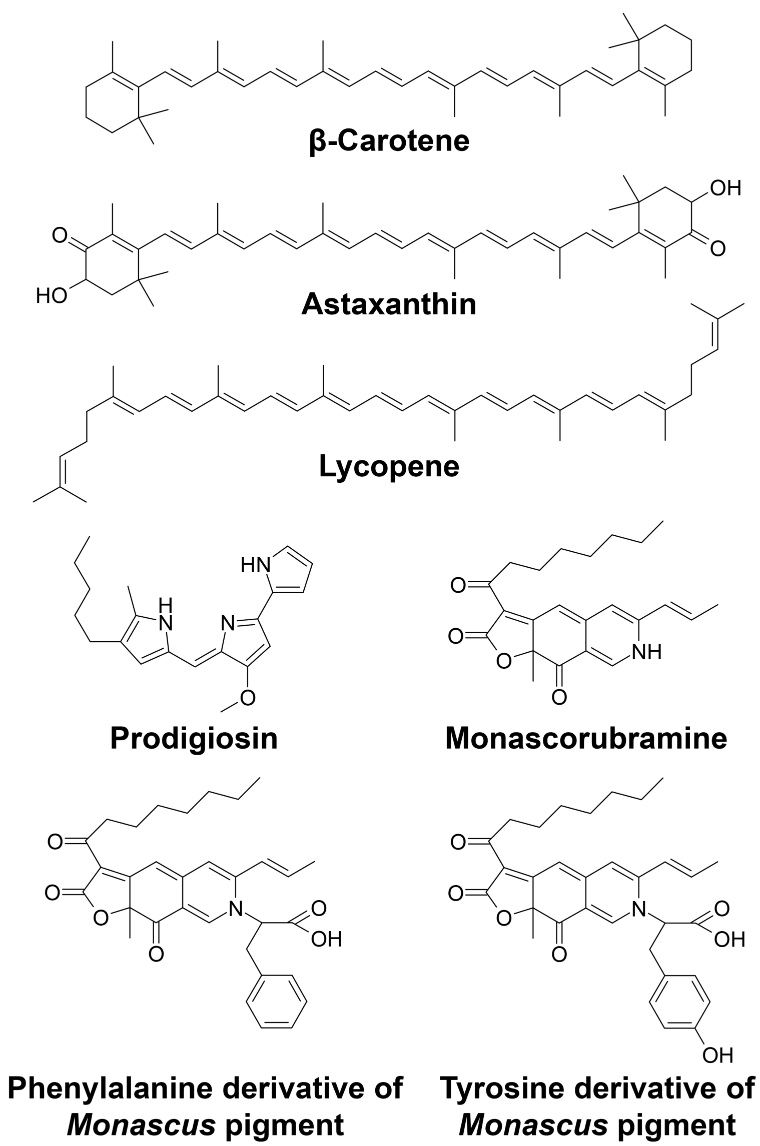

Fig. 2. Chemical structures of carotenoids, prodigiosin, monascorubramine, and *Monascus* pigment derivatives.

have been identified and these pigments have been classified as xanthophylls and carotenes according to their chemical composition (Jackson et al., 2008; Kean et al., 2008). Although carotenoids are present mainly in vegetables and fruits, their occurrence is not necessarily limited to plants. Cyanobacteria, algae, non-photosynthetic bacteria, yeast, and fungi can also synthesize carotenoids (Alagoz et al., 2018; Foong et al., 2021). Carotenoids are lipid-soluble pigments that serve as precursors of vitamin A, which is an essential dietary component for animals (Britton and Khachik, 2009; Maoka, 2020; Wade et al., 2017). In addition, carotenoids quench free radicals, hinder lipid peroxidation, and mitigate damage caused by reactive oxidant species, thereby protecting cells from oxidative damage (Pradhan et al., 2022). Recently, several carotenoids have received increased attention owing to their role in reducing the risk of cancer and eye diseases, and are being used commercially as nutraceuticals (Sachdeva et al., 2020).

Some red carotenoids exhibit antimicrobial activity. *Rhodotorula mucilaginosa* AY-01, isolated from the soil, produces red carotenoid pigments (Yoo et al., 2016). The pigments were extracted and partially purified by immobilized metal affinity chromatography. These red carotenoids showed significant antimicrobial activity against two antibiotic-resistant bacteria named as PWG and F1 isolated from the porcine semen. Similarly, the UV-C resistant *Micrococcus roseus*, isolated from the soil samples, produces a red carotenoid pigment that exhibited UV-protective, antioxidant, and antimicrobial activities (Mohana et al., 2013). Specifically, the red carotenoid pigment exhibited inhibitory activity against Gram-positive bacteria with zone of inhibition, minimum inhibitory concentration, and minimal bactericidal concentration ranging between 6.5-15.0 mm, 0.25-2.0 mg/mL, and 6.0-10.0 mg/mL, respectively.

5.2. Prodigiosin

Prodigiosin, a natural red pigment with pyrrolylpyrromethane skeleton, is a secondary alkaloid metabolite produced by a variety of bacteria (Williamson et al., 2006; Yip et al., 2021). Prodigiosin was first characterized from the human pathogen, *Serratia marcescens*, and was later found to be synthesized by Gram-negative bacteria such as *Vibrio psychroerythrus*, *Vibrio gazogenes*, *Rugamonas rubra*, *Hahella chejuensis*, *Serratia rubidaea*, and *Pseudomonas magneslorubra* (Sanchez-Munoz et al., 2020). In addition, Gram-positive actinomycetes, including *Streptoverticilli um rubrireticuli* and *Streptomyces longisporusruber*, synthesize prodigiosin and its derivatives (Khanafari et al., 2006). This pigment exhibits several pro-health characteristics such as antimalarial, anticancer, antineoplastic, immunosuppressive, insecticidal, and UV-resistant properties, and is a potential therapeutic molecule (Behera et al., 2021; Darshan and Manonmani, 2015; Ren et al., 2017). Notably, prodigiosin exhibits significant antimicrobial activity as well. Prodigiosin inhibited the growth of three clinical pathogenic bacteria (methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Enterococcus faecalis*) and *Escherichia coli* when used at the concentrations of 250 and 500 μg/μL, as demonstrated using disc- diffusion assays (Yip et al., 2021). The mechanism underlying its antimicrobial activity probably involves the inhibition of bacterial protease production and biofilm formation. In addition, prodigiosin produced by *Serratia marcescens* IBRL USM 84, isolated from the marine sponge *Xestospongia testudinaria*, inhibited 13 bacteria, including *B. subtilis*, *Enterococcus avium*,

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S. aureus, *Staphylococcus saprophyticus*, and *Streptococcus pyogenes* (Ibrahim et al., 2014). Interestingly, Gram-positive bacteria tended to be more susceptible than Gram-negative bacteria to prodigiosin-mediated inhibition. Significantly, prodigiosin is not genotoxic and exerts little or no toxicity on normal cells (Guryanov et al., 2013; Guryanov et al., 2020), which makes it a potential candidate for use in food industry.

5.3. Monascorubramine and Monascus pigment derivatives

Monascorubramine is a red pigment produced by molds of the genus *Monascus*, which are considered edible microorganisms (Feng et al., 2012; Kim and Ku, 2018; Mostafa and Abbady, 2014). It has been used in food preparations in Asia since ancient times. The *Monascus* pigments, including monascorubramine, have been approved as food additives and are widely used in Asian countries, such as Korea, Japan, and China (Choe et al., 2020a; Manan et al., 2017). Although, red yeast rice containing the *Monascus* pigments has been approved by the United States Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) as a new dietary ingredient and a food supplement, respectively, the *Monascus* pigments have not yet been used as food additives in the United States (Choe et al., 2020b; Manan et al., 2017). This is due to the production of citrinin, a mycotoxin, during the culture of *Monascus* species that is harmful to humans (Manan et al., 2017). However, some *Monascus* species do not produce citrinin and some of citrinin can also be effectively removed during the separation and purification processes used to isolate the *Monascus* pigments (Kang et al., 2014; Zhen et al., 2019). Indeed, the use of *Monascus* pigments in food is permitted and safely managed in several countries following citrinin detection tests (Saithong et al., 2019; Tangni et al., 2021). Thus, the use of *Monascus* pigments in food may be encouraged to replace currently used harmful pigments.

Among various *Monascus* pigments, a red pigment purified from culture broth of *Monascus perpureus* inhibits the growth of Gram-positive *Bacillus* species (Mukherjee and Singh, 2011). Similarly, monascorubramine, a red pigment produced by *Monascus perpureus* and purified by column and thin layer chromatography, exhibited antimicrobial activity against Gram-positive bacteria (Chatterjee et al., 2009). Moreover, some amino acid derivatives of *Monascus* pigments exhibit stable red color and significant antimicrobial activity (Choe et al., 2020c; Jung et al., 2003; Kim et al., 2006a; Kim et al., 2006b). Specifically, these red *Monascus* pigment derivatives structurally contain amino acids, such as phenylalanine (Phe) and tyrosine (Tyr) and exhibited high antimicrobial activities against Gram-positive and Gram-negative bacteria as well as filamentous fungi, with minimum inhibitory concentrations ranging 4-16 μg/mL. This antimicrobial activity is mainly attributed to the hydrophobicity and absorption of the Phe and Tyr derivatives.

6. Conclusions

Food additives that are to be considered as alternatives to sodium nitrite, a commonly used colorant and preservative in sausage production, should impart stable red color and exhibit significant antimicrobial activity. Antimicrobial red pigments can fulfil these two roles. Microbe-derived pigments have enormous potential as alternatives to sodium nitrite owing to their advantages in terms of sustainable supply, high yield, and easy downstream processing. Promising microbe-derived red pigments with the antimicrobial activity include carotenoids, prodigiosin, and *Monascus* pigment derivatives. Nevertheless, for industrial use of these pigments, it is necessary to increase their production in a cost-effective manner via metabolomics, metabolic engineering, and biotechnology. In addition, their toxicity should be evaluated systematically to ensure their safety as food additives.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization; Writing - original draft; Writing - review & editing: Choe D.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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