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Biomedical Applications of Nisin

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Abstract

Nisin is a bacteriocin produced by a group of Gram-positive bacteria that belongs to *Lactococcus* and *Streptococcus* species. Nisin is classified as a Type A (I) lantibiotic that is synthesized from mRNA and the translated peptide contains several unusual amino acids due to post-translational modifications. Over the past few decades, nisin has been used widely as a food biopreservative. Since then, many natural and genetically modified variants of nisin have been identified and studied for their unique antimicrobial properties. Nisin is an FDA approved and GRAS (generally regarded as safe) peptide with recognized potential for clinical use. Over the past two decades the application of nisin has been extended to biomedical fields. Studies have reported that nisin can prevent the growth of drug-resistant bacterial strains, such as methicillin resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, Enterococci and *Clostridium difficile*. Nisin has now been shown to have antimicrobial activity against both Gram-positive and Gram-negative disease-associated pathogens. Nisin has been reported to have anti-biofilm properties and can work synergistically in combination with conventional therapeutic drugs. In addition, like host defense peptides, nisin may activate the adaptive immune response and have an immunomodulatory role. Increasing evidence indicates that nisin can influence the growth of tumors and exhibit selective cytotoxicity towards cancer cells. Collectively, the application of nisin has advanced beyond its role as a food biopreservative. Thus, this review will describe and compare studies on nisin and provide insight into its future biomedical applications.

Keywords

Nisin; Lantibiotic; Antimicrobial peptide; Infectious Disease; Oral Disease; Cancer; Biofilm

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Nisin: A Bacterially-Derived Antimicrobial

Nisin is an antimicrobial peptide produced by certain Gram-positive bacteria that include *Lactococcus* and *Streptococcus* species (Lubelski *et al.*, 2008; de Arauz *et al.*, 2009). Nisin was first identified in 1928 in fermented milk cultures and commercially marketed in England in 1953 as an antimicrobial agent (Rogers and Whittier, 1928; Delves-Broughton *et al.*, 1996). In 1969, nisin was approved by the Joint Food and Agriculture Organization/ World Health Organization (FAO/WHO) as a safe food additive. Currently, nisin is licensed in over 50 countries, and it has made a significant impact in the food industry as a natural biopreservative for different types of foods (de Arauz *et al.*, 2009). In the United States (US), nisin was approved by the Food and Drug Administration in 1988 and was given a generally regarded as safe (GRAS) designation for use in processed cheeses (Cotter *et al.*, 2005).

The originally described variant of nisin, known as nisin A, is composed of 34 amino acids and is produced by *Lactococcus lactis* (Gross and Morell, 1971). Nisin belongs to a group of cationic peptide antimicrobials collectively called Type A (I) lantibiotics (Smith and Hillman, 2008). Nisin and other lantibiotics have gained considerable attention due to their potent and broad spectrum activity, low likelihood of promoting the development of bacterial resistance, and low cellular cytotoxicity at antimicrobial concentrations (Asaduzzaman and Sonomoto, 2009; Van Heel *et al.*, 2011; Cotter *et al.*, 2013; Shin *et al.*, 2015). Similar to other lantibiotics, nisin contains several unusual amino acids as a result of enzymatic post-translational modifications (Sahl *et al.*, 1995). Nisin contains dehydrated amino acid residues (serine and threonine) and thioether amino acids that form five lanthionine rings, which are characteristic of nisin and lantibiotics (Karakas *et al.*, 1999; Wiedemann *et al.*, 2001). As a food biopreservative, nisin serves as a broad-spectrum bacteriocin against mostly Gram-positive foodborne bacteria (Delves-Broughton *et al.*, 1996; Severina *et al.*, 1998; Cleveland *et al.*, 2001). However, research has now shown that the antimicrobial action of nisin can extend to a range of non-food related bacteria (Blay *et al.*, 2007; Shin *et al.*, 2015). Studies have demonstrated that purified nisin and nisin in combination with other antibiotics can be effective against Gram-negative pathogens and that certain bioengineered nisin variants can enhance the activity against both Gram-positive and Gram-negative pathogens (Kuwano *et al.*, 2005; Naghmouchi *et al.*, 2010; Field *et al.*, 2012). In addition, with recent improvements in biotechnology, researchers from interdisciplinary fields have bioengineered newer forms of nisin variants that have therapeutic potential for human diseases (Piper *et al.*, 2011; Field *et al.*, 2012; Rouse *et al.*, 2012; Balciunas *et al.*, 2013; Field *et al.*, 2015).

Since its discovery, nisin has garnered significant influence in the food industry as an alternative biopreservative. However, with demonstrated safety over the past 40 years, the use of nisin has begun to expand to include a diverse array of unrelated applications, such as those related to the biomedical industry (Fig. 1). Many lantibiotics (and more broadly, other bacteriocins) have been reported to possess additional biological activities beyond their antimicrobial activities (Asaduzzaman and Sonomoto, 2009; Benmechernene *et al.*, 2013; Kamarajan *et al.*, 2015). For example, nisin has beneficial properties in the context of biomedical applications, including bacterial infections, cancer, oral diseases and more. This review will provide a comprehensive overview of the latest findings by focusing on the

advances in nisin bioengineering and the new discoveries in biomedical applications of nisin.

Natural and Bioengineered Variants of Nisin

Several other naturally-occurring variants of nisin have been reported. These variants have been identified from a range of taxonomically distinct organisms isolated from a broad range of environments. Nisin A was first discovered in *L. lactis,* an organism that is commonly found in dairy products and is the most widely studied nisin variant (Fig. 2) (Gross and Morell, 1971). Nisin Z, the closest variant of nisin A, was isolated from *L. lactis* NIZO22186 (Mulders *et al.*, 1991). Nisin Z differs from nisin A by a single amino acid residue at position 27, asparagine instead of histidine (Fig. 2; Table 1) (Mulders *et al.*, 1991). Nisin A and Z share similar properties as antimicrobials, but nisin Z has a superior rate of diffusion and solubility under neutral pH conditions (De Vos *et al.*, 1993). Nisin F was isolated from *L. lactis* F10 in the feces of a freshwater catfish in South Africa and differs from nisin A by two amino acid residues (De Kwaadsteniet *et al.*, 2008). Nisin F has two amino acid substitutions at position 27 and 30 (Fig. 2; Table 1). Nisin Q was isolated from *L. lactis* 61-14 that was cultured from a river in Japan (Zendo *et al.*, 2003). Nisin Q contains four substitutions at position 15, 21, 27 and 30 (Fig. 2; Table 1). Nisin A, Z, F and Q have antimicrobial activity against a range of *Staphylococcus aureus* targets (Piper *et al.*, 2011). Nisin U and U2 are more distantly related variants that were isolated from *Streptococcus uberis*, an organism that commonly inhabits the lips, skin, and udder tissues of cows and is found in raw milk (Wirawan *et al.*, 2006). Nisin U and U2 contain nine and ten amino acid substitutions respectively, compared to nisin A (Table 1). Recently, nisin H was isolated from a *Streptococcus hyointestinalis* strain derived from porcine intestine (O'Connor *et al.*, 2015). The amino acid sequence of nisin H has similarities to nisin peptides produced by both *lactococcal* and *streptococcal* strains (Table 1). Nisin H maintains the terminal amino acids found in nisin A, Z, F, and Q, while harnessing features of nisin U and U2, including a dehydroaminobutyric acid substitution at position 18 (Table 1). Furthermore, nisin P was identified by genome mining techniques in *Streptococcus gallolyticus subsp. pasteurianus*, an organism found in the alimentary tract of ruminants (Zhang *et al.*, 2012). The protein sequence of nisin P closely resembles that of nisin U2 but differs from it by two substitutions at position 20 and 21 (Fig. 2; Table 1). Thus far, based on published reports, there are at least eight nisin variants that have been isolated, identified and sequenced for cross-analysis.

The potential for utilizing genetic tools to modify the activity of bacteriocins has been recognized for several decades (Gillor *et al.*, 2005). In addition to the naturally occurring nisin variants, there are bioengineered forms of nisin that have been developed in attempts to enhance the efficacy and stability of nisin under different physiologic conditions, and to enhance its pharmacokinetic properties for a variety of biological applications (Field *et al.*, 2015). Here, we describe several bioengineered nisin variants that have been recently identified. Nisin Z N20K and M21K were derived from the genetic modification of *L. lactis* NZ9800 and first reported by Yuan and colleagues. These genetically modified nisin variants exhibited enhanced activity against pathogenic Gram-negative bacteria, such as *Shigella*, *Pseudomonas* and *Salmonella* species (Yuan *et al.*, 2004). Nisin Z N20K and M21K contain

substitutions in the flexible hinge-region of the peptide backbone structure of nisin Z (Table 1). Furthermore, these variants displayed greater thermal stability at higher temperatures and solubility at neutral or alkaline pH (Yuan *et al.*, 2004). The hinge region of nisin, which consists of three amino acids, asparagine-methionine-lysine, is located between the first three and the last two lanthionine-constricted rings of nisin. Modifications in the hinge region have been studied extensively because this region is important for the insertion of nisin into the bacterial membrane (Hasper *et al.*, 2004; Lubelski *et al.*, 2009; Ross and Vederas, 2011). Healy and coworkers demonstrated that mutants of the hinge region exhibited enhanced activity against specific indicator strains such as *L. lactis* HP, *Streptococcus agalactiae* ATCC 13813, *Mycobacterium smegmatis* MC2155 and *S. aureus* RF122 (Healy *et al.*, 2013). In addition, Zhou and colleagues demonstrated that by altering the length of the hinge region of nisin, the efficacy of nisin against a panel of test microorganisms can be altered in a temperature and matrix dependent manner (Zhou *et al.*, 2015). Recently, a wide range of bioengineered nisin peptides with greater activity and enhanced therapeutic properties against foodborne and clinical pathogens began surfacing in the literature. The newly bioengineered variants include nisin A K22T, A N20P, A M21V, A K22S, S29A, S29D, S29E and S29G (Table 1) (Field *et al.*, 2008; Field *et al.*, 2012). Field and colleagues applied site-directed and site-saturation mutagenesis to the hinge region residues of nisin A to successfully identify variants that displayed enhanced bioactivity and specificity against a range of Gram-positive drug-resistant, clinical veterinary and food-borne pathogens (Field *et al.*, 2012). Thus, based on emerging reports, bioengineered variants of nisin appear to be promising candidates for future applications in health care.

Nisin and Treatment of Infectious Diseases

Certain human infectious diseases, such as antibiotic-resistant skin and soft tissue infections and especially biofilm-associated infections can be difficult to prevent and/or treat (Mah and O'Toole, 2001; Gilbert *et al.*, 2002; Fauci and Morens, 2012). While conventional medical treatments that are based on antibiotics and antivirals have been used for bacterial and viral infections, the emergence of drug resistance has led to the search for alternative or adjunctive methods to treat these drug resistant diseases (Zetola *et al.*, 2005). With decades of safe usage in the food industry, investigators have started exploring nisin as a potential alternative agent for infectious diseases, including drug-resistant infections, thereby also decreasing the use of antibiotics (Table 2) (Balciunas *et al.*, 2013).

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *enterococci* (VRE) have become major medical problems in hospitals around the world. The difficulty in treating these infections has been extensively documented (Huycke *et al.*, 1998; Chambers, 2001; Köck *et al.*, 2010; Ahire and Dicks, 2015). MRSA and VRE are leading causes of bacterial nosocomial infections, urinary tract infections, and are known to be resistant to many standard therapies. For example, MRSA infections account for up to 70% of the *S. aureus* infections in intensive care units (Sahm *et al.*, 1999; Diekema *et al.*, 2001). Both MRSA and VRE infections can manifest as skin infections and in medical settings as bacteremias, pneumonia, and post-surgical infections (Huycke *et al.*, 1998; Center for Disease Control and Prevention, MRSA Infections, 2015). Numerous studies have been published regarding the efficacy of nisin as an antimicrobial therapeutic (Piper *et al.*, 2009;

Dosler and Gerceker, 2011; Okuda *et al.*, 2013; Singh *et al.*, 2013; Ahire and Dicks, 2015). Piper and coworkers reported that nisin was especially effective against antibiotic resistant *staphylococci,* and that further research into nisin and other lantibiotic compounds could result in promising antimicrobial alternatives (Piper *et al.*, 2009). Dosler and colleagues investigated the *in vitro* effects of nisin against MRSA strains, and concluded that nisin was a good candidate for further research by itself or in combination with conventional antibiotics, such as vancomycin or ciprofloxacin (Dosler and Gerceker, 2011). Other studies have shown that nisin in combination with conventional antibiotics can promote synergistic effects (Brumfitt *et al.*, 2002; Singh *et al.*, 2013). An earlier study by Severina and colleagues demonstrated that nisin exhibited bactericidal effects against a large panel of Gram-positive bacteria, including MRSA, VRE and *S. pneumoniae* (Severina *et al.*, 1998). In addition, a nisin producing *L. lactis* strain was shown to reduce the intestinal colonization of VRE in a mouse infection model (Millette *et al.*, 2008).

Bacteria that adhere to implanted medical devices or damaged tissue can form a biofilm and cause chronic infection (Stewart and Costerton, 2001). Biofilms are surface associated communities of microorganism that can be up to 1,000-times more resistant to antimicrobials. Treatment of these biofilms accounts for over a billion dollars in healthcare costs each year in the US (Mah and O'Toole, 2001; Gilbert *et al.*, 2002). Okuda and colleagues investigated the antibiofilm effects of nisin against MRSA biofilms on medical devices and reported that nisin A compared to two other bacteriocins (lacticin Q and nukacin ISK-1) was most effective in the prevention of biofilm formation (Okuda *et al.*, 2013). Recently, Ahire and Dicks demonstrated that a combination therapy of 2,3-dihydroxybenzoic acid, an antibiotic extracted from *Flacourtia inermis* fruit, and nisin resulted in an increase in iron concentrations that reduced biofilm formation of the MRSA Xen 31 strain (Ahire and Dicks, 2015).

The potential for using nisin to treat local site-specific infections has also been explored. For example, the antimicrobial effects of nisin against mastitis, respiratory, gastrointestinal, and skin infections has been reported (Table 2). In respiratory tract infections, although viral etiologies are common, these can progress to bacterial infections that further compromise the health status (Hament *et al.*, 1999). The upper and lower respiratory tract is primarily infected by *S. aureus* (Micek *et al.*, 2007; Weber *et al.*, 2007; Bosch *et al.*, 2013). De Kwaadsteniet and colleagues reported that nisin F safely inhibited the growth of *S. aureus* in the respiratory tract of immunocompromised rats (De Kwaadsteniet *et al.*, 2009). Furthermore, studies have shown that nisin can exert synergistic effects when combined with lysozyme and lactoferrin, which are both antimicrobial proteins and normally secreted in the human respiratory tract (Nattress *et al.*, 2001; Murdock *et al.*, 2007; De Kwaadsteniet *et al.*, 2009). It was proposed that while nisin deters cell growth by binding to the lipid II precursor of the cell wall, lysozyme and lactoferrin can further damage the glycosidic bonds in the peptidoglycan wall, and sequester iron necessary for cellular respiration, respectively (Arnold and Cole, 1977; Ganz, 2004; De Kwaadsteniet *et al.*, 2009).

Superficial and invasive skin and soft tissue infections are commonly caused by *S. aureus* (Fridkin *et al.*, 2005; Daum, 2007). MRSA skin infections are relatively uneventful but failure to treat effectively can result in death (Dakota, 1999). Heunis and coworkers

investigated the efficacy of nisin using an electrospun nanofiber wound dressing containing nisin, which diffused active nisin onto skin wounds (Heunis *et al.*, 2013). In a murine excisional skin infection model, the nisin-containing wound dressing significantly reduced the *S. aureus* colonization as analyzed by bioluminescence. In addition, the wound showed signs of accelerated healing (Heunis *et al.*, 2013). Mastitis is a common inflammatory disease in lactating women that causes breastfeeding cessation (Foxman *et al.*, 2002). *S. aureus* and *S. epidermidis* are two common etiologic agents that cause mastitis-associated infections (Foxman *et al.*, 2002). Considering the potent antimicrobial properties of nisin against *staphylococcal* strains, investigators have explored using nisin as a clinical therapeutic for mastitis. Cao and colleagues reported that a nisin-based formulation was effective in the treatment of clinical mastitis in lactating dairy cows caused by several different mastitis pathogens (Cao *et al.*, 2007). In addition, Wu and coworkers demonstrated that nisin Z was effective in treatment of subclinical mastitis caused by multiple mastitis pathogens in lactating dairy cows (Wu *et al.*, 2007). Recently, Fernandez and others reported that topical nisin treatment alleviated clinical signs of mastitis and significantly reduced the *staphylococcal* count in breast milk of nisin treated women (Fernandez *et al.*, 2008). Overall, as an alternative to conventional antibiotics, the latest research suggests that nisin has potential as a therapeutic against certain infectious pathogens and disease conditions.

Nisin and Oral Health

The pervasiveness of oral diseases, such as caries and periodontal diseases, remains high in developed and developing countries (Marcenes *et al.*, 2013). Oral diseases are considered a major public health burden due to their high prevalence and incidence (Petersen, 2003). Therefore, research on new strategies to prevent and treat oral diseases are a focus of industry and many academic, and government institutions (Centers for Disease Control and Prevention, Chronic Disease Prevention and Health Promotion, 2015). Oral biofilms, including dental plaque, play a key role in the etiology and the progression of biofilm-associated oral diseases (Marsh, 2010; Zijnge *et al.*, 2010). Enhanced antimicrobial resistance is associated with the accumulation of pathogens that cause dental caries and periodontal disease (Marsh, 2003; Aas *et al.*, 2005). Nisin's potential as an oral antimicrobial was first described by Johnson and colleagues, who demonstrated that there were fewer numbers of *streptococci* in the dental plaque of monkeys that received nisin in their foods (Johnson *et al.*, 1978). Later, Howell and coworkers demonstrated that a nisin-based antimicrobial mouthrinse exhibited promising clinical results in prevention of plaque build-up and gingival inflammation in beagle dogs (Howell *et al.*, 1993). Thus, the idea of using nisin to improve oral health has been around for some time.

Emerging evidence continues to support the antimicrobial properties of nisin against oral pathogenic bacteria relevant to caries and periodontal diseases. Tong and colleagues demonstrated that nisin A can inhibit the growth of cariogenic bacteria, including *Streptococcus mutans* (Tong *et al.*, 2010). Scanning electron microscopy confirmed that nisin exerted bactericidal activity by forming small pores on the surface of cells (Tong *et al.*, 2010). Furthermore, investigators have reported that nisin in combination with poly-lysine and sodium fluoride displayed synergistic properties in inhibiting planktonic and biofilm forms of *S. mutans* (Najjar *et al.*, 2009; Tong *et al.*, 2011). Nisin A has been shown to inhibit

the growth of Gram-positive oral bacteria such as *Streptococcus sanguinis, Streptococcus sobrinus and Streptococcus gordonii* (Tong *et al.*, 2010). In addition, Shin and coworkers demonstrated that high purity nisin Z can inhibit the growth of Gram-negative oral colonizing pathogens, including *Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans and Treponema denticola* (Shin *et al.*, 2015). Shin and colleagues also reported that nisin exerted anti-biofilm effects on saliva derived multi-species biofilms without causing cytotoxicity to human oral cells (Fig. 3) (Shin *et al.*, 2015). As a cationic bacteriocin, nisin's mode of action may include inhibition of coaggregation of oral colonizers. Indeed, cationic antimicrobials can selectively inhibit coaggregation interactions of oral biofilm species (Smith *et al.*, 1991).

In addition to dental caries and periodontal disease, nisin's potential application to other oral diseases has been explored. Investigators have reported that nisin can inhibit *Enterococcus faecalis*, which is an opportunistic Gram-positive pathogen frequently recovered from infected root canals of teeth (Stuart *et al.*, 2006). In an *ex vivo* root canal system, nisin successfully eradicated the colonization of *E. faecalis* (Turner *et al.*, 2004). Nisin, when paired with MTAD (a common intracanal irrigant, consisting of 3% doxycycline, 4.5% citric acid, and 0.5% polysorbate 80 detergent), improved the post-antibiotic sub-MIC effects of MTAD against *E. faecalis,* and made it less resistant to alkaline environments (Tong *et al.*, 2014). Another potential oral application of nisin was demonstrated in the treatment of oral candidiasis. *Candida albicans* is one of the most prevalent pathogens that cause mucosal fungal infections (Pfaller *et al.*, 2002; Trick *et al.*, 2002). The invasion of candida species into oral epithelial cells is a signature of oropharyngeal candidiasis (Eversole *et al.*, 1997; Drago *et al.*, 2000; Farah *et al.*, 2000). Le Lay and colleagues reported that nisin Z can significantly reduce the growth and transition of *C. albicans* (Le Lay *et al.*, 2008). In addition, nisin Z has the potential to work synergistically with oral gingival cells to provide greater resistance against *C. albicans* infections (Akerey *et al.*, 2009). Thus, with recent reports highlighting the therapeutic potential of nisin in oral diseases, future studies will be essential to further evaluate the potential clinical role of nisin.

Bacteriocins and Cancer: Nisin as a Cancer Therapeutic

The potential for using bacterially-derived compounds to control infectious disease also extends to controlling cancers (Frankel *et al.*, 2002; Lundin and Checkoway, 2009; Nobili *et al.*, 2009). For example, antimicrobial peptides have been indicated to exhibit cytotoxic effects on cancer cells and thus may have therapeutic potential (Meyer and Harder, 2007; Boohaker *et al.*, 2012). Specifically, purified bacteriocins, including pyocin, colicin, pediocin, microcin, and nisin have shown inhibitory properties against neoplastic cell lines and in xenograft mouse models (Cornut *et al.*, 2008; Lagos *et al.*, 2009; Yates *et al.*, 2012; Shaikh *et al.*, 2012; Yang *et al.*, 2014). This is relevant because current treatment strategies have yet to reduce cancer-related deaths below a half million per year in the USA alone (Centers for Disease Control and Prevention, Leading Causes of Death, 2015). Cancer is a complex disease characterized by the dysregulated growth of abnormal cells. Significant progress has been made in the treatment of cancers, however the majority of treatments involve surgery and chemo- and radiation therapy, which are detrimental to normal cells and tissues and cause further morbidity (Patel *et al.*, 2014; DeSantis *et al.*, 2014).

Recently, Joo and colleagues explored the cytotoxic and antitumor properties of nisin A and discovered that it blocks head and neck squamous cell carcinoma (HNSCC) tumorigenesis (Joo *et al.*, 2012). Nisin mediated these effects by inducing preferential apoptosis, cell cycle arrest, and reducing cell proliferation in HNSCC cells compared to primary oral keratinocytes. Nisin also reduced HNSCC tumorigenesis *in vivo* in a mouse model (Joo *et al.*, 2012). Mechanistically, nisin exerted these effects on HNSCC, in part, through cation transport regulator homolog 1 (CHAC1), a proapoptotic cation transport regulator and through a concomitant CHAC1-independent influx of extracellular calcium (Mungrue *et al.*, 2009; Joo *et al.*, 2012). Nisin can interact with the negatively charged phospholipid heads of the cell membrane, thereby mediating its reorganization and forming pores that allow an influx of ions (Giffard *et al.*, 1996; Moll *et al.*, 1997). Since HNSCC cells and primary oral keratinocytes differ in their lipid membrane composition and function, and response toward calcium fluxes, the ability of nisin to differentially alter the transmembrane potential and membrane composition of HNSCC cells may explain its predominant effects on these cells (Ponec *et al.*, 1984; Ponec *et al.*, 1987; Tertoolen *et al.*, 1988; Eckert, 1989; Gasparoni *et al.*, 2004; Tripathi *et al.*, 2012). Indeed, recent reports support this premise as the basis for the nisin-mediated differential apoptotic cell death and reduced proliferation of HNSCC cells compared to primary keratinocytes (Schweizer, 2009).

Recently, Kamarajan and coworkers focused on investigating the translational potential of a high purity form of nisin Z for the treatment of HNSCC (Kamarajan *et al.*, 2015). The data support the role of nisin as an alternative therapeutic for HNSCC, since nisin promoted HNSCC cell apoptosis, suppression of HNSCC cell proliferation, inhibition of angiogenesis, inhibition of HNSCC orasphere formation, inhibition of tumorigenesis *in vivo*, and it prolonged survival *in vivo* (Fig. 4) (Kamarajan *et al.*, 2015). Considering that the FDA has approved 83.25 mg/kg in humans as the no-observed-effect-level (NOEL) for nisin (66.7 mg/kg was used in mice as a cancer therapeutic dose), this study demonstrated the promising potential for nisin as an anti-cancer agent. In addition, Preet and colleagues demonstrated that combining doxorubicin, a conventional cancer drug, with nisin can potentiate the effectiveness of the treatment in terms of decreasing tumor severity in skin carcinogenesis (Preet *et al.*, 2015). Kamarajan and colleagues also showed that high purity forms of nisin A and Z synergize with cisplatin to induce apoptosis in HNSCC cells that are highly resistant to ionizing radiation and cisplatin (Global Medical Discovery, 2015). Therapeutic strategies for utilizing nisin alone or in combination with other conventional drugs to treat cancer are still at an early stage. However, the few studies that have been reported demonstrate the significant anti-cancer potential of using nisin as a promising alternative or adjunctive therapeutic. Furthermore, increasing evidence suggests an etiologic linkage between the microbiome and cancers (Wroblewski *et al.*, 2010; Bultman, 2014). Recent studies indicate that certain bacteria (i.e. oral bacteria) may promote carcinogenesis in humans (Ahn *et al.*, 2012; Michaud and Izard, 2014). In these scenarios, it is possible that nisin may have dual benefits by altering or disrupting the microbiome and inhibiting the growth of cancer cells. Thus, nisin may be a useful therapeutic since it exerts both antimicrobial/biofilm and anti-cancer properties.

Immunomodulatory Role of Nisin

Host-defense peptides (HDPs) are ubiquitous in nature. HDPs are small amphiphilic cationic peptides, which play an essential role in the innate immune response (Sahl and Bierbaum, 2008). Almost all living organisms use antimicrobial peptides or HDPs as an innate defense mechanism. Interestingly, despite differences in size and native structure, HDPs and bacterially secreted bacteriocins share similar physicochemical properties (Hancock and Sahl, 2006). Nisin is both a cationic and amphiphilic peptide, and thereby mediates diverse effects on membrane processes similar to HDPs (Cotter *et al.*, 2005). Pablo and colleagues demonstrated that short-term dietary administration of nisin (as Nisaplin, containing 2.5% nisin A, 77.5% NaCl and non-fat dried milk) resulted in an increase in CD4 and CD8 T-lymphocytes, while decreasing the B-lymphocyte levels (Pablo *et al.*, 1999). In addition, prolonged administration of nisin resulted in a return to normal levels of both B- and T-lymphocytes (Pablo *et al.*, 1999). This study provided the first evidence of nisin's influence on the immune system of mice. Recently, Begde and coworkers reported that nisin was able to activate neutrophils, and suggested that nisin may be influencing multiple subsets of host immune cells (Begde *et al.*, 2011). Considering that nisin appears to behave similar to HDPs, it is possible that the immunomodulatory properties associated with HDPs may also apply to nisin (Kindrachuck *et al.*, 2013). Bacteriocins were once thought to have a very limited role in disrupting bacterial membranes and exerting bactericidal activity. However, Kindrachuck and colleagues demonstrated that purified nisin Z was capable of modulating the innate immune response by inducing chemokine synthesis and suppressing LPS-induced pro-inflammatory cytokines in human peripheral blood mononuclear cells (Kindrachuck *et al.*, 2013). Furthermore, nisin Z promoted immunomodulatory responses within both *ex vivo* and *in vivo* model systems (Kindrachuck *et al.*, 2013). These reports underscore nisin's significant potential for use in a variety of human diseases that are mediated by the host immune response and pathogenic biofilms, like periodontal disease. Given that the periodontal lesion is characterized by an initial burst of neutrophils that is followed by a B- and T-cell mediated immune response in its later stages, nisin could play a significant therapeutic role in modifying both the immune and biofilm signature of this lesion (Page and Schroeder, 1976). The ability of nisin to alter the host immune response provides yet another opportunity for its potential use within health care settings. Since information regarding the role of nisin in modulating the host immune response is limited, this area merits further examination.

Resistance to Nisin

Bacteriocins have different modes of action when compared to antibiotics (Cleveland *et al.*, 2001; Cotter *et al.*, 2005). Specifically, lantibiotic bacteriocins, such as nisin, require a docking molecule (lipid II), through which they target cells by forming pores in the membrane. This depletes the transmembrane potential and/or the pH gradient and results in the leakage of cellular materials (Peschel and Sahl, 2006). Although in binding to lipid II nisin is similar to other antibiotics, such as vancomycin, nisin is unique in that it can span the entire membrane by using the pyrophosphate cage as the anchoring point (Hsu *et al.*, 2004). Some evidence suggests that resistance against nisin can arise from mutations that induce changes in the membrane and cell wall composition (thickening of the cell wall to

prevent the nisin binding to lipid II), reducing the acidity of the extracellular medium to stimulate the binding of nisin to the cell wall and induce its degradation, prevent the insertion of nisin into the membrane, and transport or extrude nisin out across the membrane (Mantovani and Russell, 2001; Kramer *et al.*, 2006; Kramer *et al.*, 2008). These changes may occur independently or together and have been described as physiological adaptations (Sun *et al.*, 2009). The cellular mechanisms of resistance to nisin are, however, still not well understood. One key reason for this stems from the fact that only a few examples of nisin resistance have emerged and only under laboratory conditions.

Lipid II plays an essential role in bacterial cell wall biosynthesis and growth, and nisin initiates its mode of action by binding to lipid II with high affinity (Breukink *et al.*, 1999; Wiedemann *et al.*, 2001). Kramer and colleagues tested whether nisin resistance could result from differences in the lipid II levels of Gram-positive bacteria (Kramer *et al.*, 2004). Those studies suggested that there was no direct role for lipid II in nisin resistance as there was no correlation with the amount of lipid II present and increase in resistance (Kramer *et al.*, 2004). It was recently reported that lack of antibiotic resistance to a newly described antibiotic was due to its targeting the highly conserved lipid II component of bacteria; nisin may be working in the same way (Ling *et al.*, 2015).

Nisinase is a dehydropeptide reductase that can inactivate nisin through an enzymatic reaction (de Freire Bastos *et al.*, 2014; Draper *et al.*, 2015). Nisinase activity has been detected in *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Clostridium botulinum*, *L. lactis subsp. cremoris*, *E. faecalis* and *S. aureus* (Kooy, 1952; Carlson and Bauer, 1957; Alifax and Chevalier, 1962; Rayman *et al.*, 1983). However, despite all of the reports suggesting the presence of nisinase in several different species, there has not been a conclusive study indicating the presence of nisinase in *L. lactis* (Pongtharangku and Demirci, 2007). In addition, Sun and coworkers reported that nisin resistance protein (NSR) is a nisin degrading protease that non-nisin producing bacteria can produce as a novel mechanism for nisin resistance (Sun *et al.*, 2009). NSR was capable of proteolytically cleaving the C-terminal tail of nisin, thereby inactivating and reducing nisin's antimicrobial activity by a 100-fold (Sun *et al.*, 2009).

Currently, the majority of studies on the mechanisms of nisin resistance have been focused on single foodborne pathogens, such as *Listeria monocytogenes* (Crandall and Montville, 1998). A number of mechanisms are now known to contribute to and affect nisin resistance, including environmental stress and specific genetic components (Mantovani and Russell, 2001; Gravesen *et al.*, 2001; Thedieck *et al.*, 2006; Begley *et al.*, 2010). As the applications of nisin expand even further into the biomedical field, it will be critical to study and monitor the development of nisin resistance in pathogenic organisms and cells relevant to disease processes. Antibiotic resistance is not an uncommon phenomenon, however, bacteriocins such as nisin are distinctly different from conventional antibiotics in both their synthesis and mode of action (Cleveland *et al.*, 2001). Thus, characterization of specific genetic or protein components that may contribute to nisin resistance will be important to better understand any potential resistance issues that may arise in clinical settings.

Concluding Remarks: Outlook

In recent years, nisin research has shown its potential use in a broad range of fields, including food biopreservation and biomedical applications. Among different classes of lantibiotics, nisin is the most well-known and best-studied lantibiotic (Benmechernene *et al.*, 2013). Considering that variants of nisin are now available in high purity forms from numerous commercial vendors, it is projected that more studies on different applications of nisin will be published. In addition, the mode of action of nisin in the context of human systems and disease will be better understood for newer biomedical applications. Currently, antibiotic resistance is a major concern in the food and biomedical industries. Until now, nisin has shown promising laboratory and clinical results as a useful therapeutic agent. Furthermore, different variants and forms of nisin may be combined with conventional drug(s) to promote synergistic outcomes. Further validation of nisin's usefulness in biomedical fields will require *in vivo* studies to evaluate its efficacy. Although nisin has been associated with the development of minimal resistance, it will be critical to continue surveying for potential novel mechanisms of nisin resistance *in vitro* and *in vivo*. There is still much knowledge to be gained, however current findings support the incorporation of nisin and/or other bacteriocins into a variety of disease therapies.

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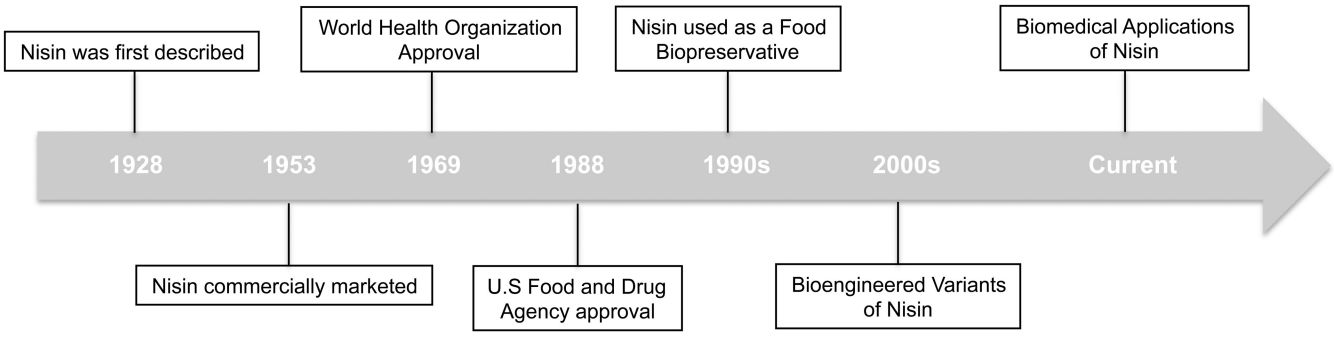

Figure 1. Timeline of Nisin Development

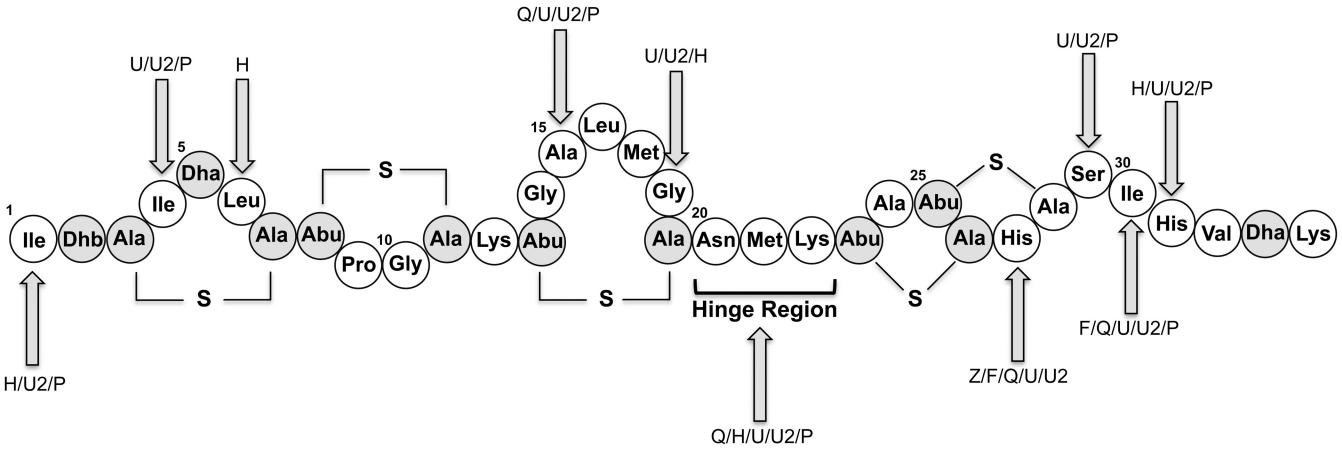

Figure 2. Peptide Structure of Nisin

Modified amino acids are colored gray with black letters. Dha, dehydroalanine (from Alanine); Dhb, dehydrobutyrine (from Threonine); Ala-S-Ala, lanthionine; Abu-S-Ala; β-methyllanthionine. The hinge region is composed of Asparagine-Methionine-Lysine. Arrows indicate the sites of amino acid substitutions for natural variants.

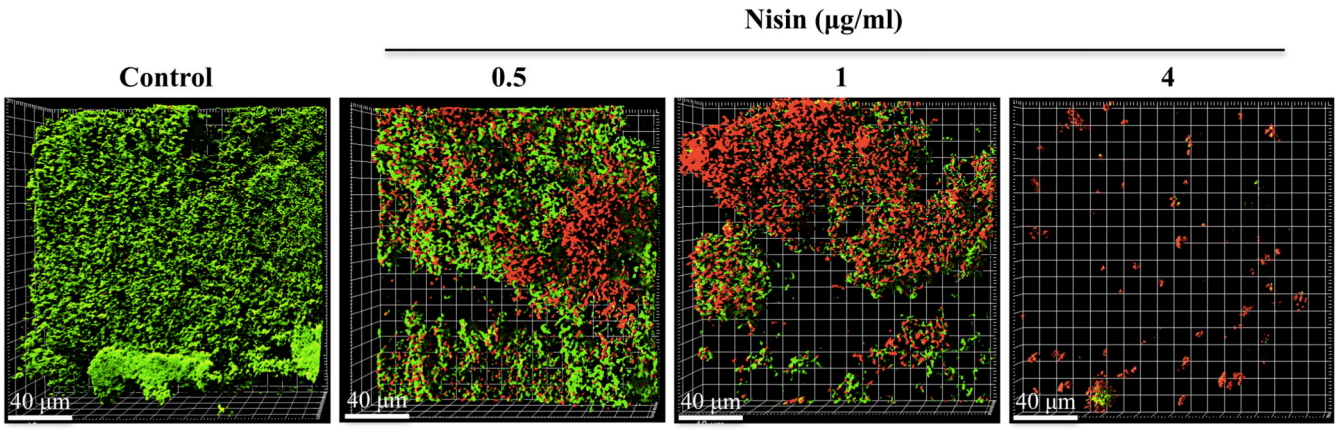

Figure 3. Nisin inhibits the formation of multi-species biofilms in a Bioflux controlled flow microfluidic model system

Cell containing saliva was added, then fed filter sterilized cell free saliva for 20–22 h at 37°C with or without nisin. Confocal microscopy images are represented in the x–y plane. A green signal indicates viable live cells (Syto 9) and a red signal indicates damaged/dead cells (propidium iodide). These images were previously published (Shin *et al.*, 2015).

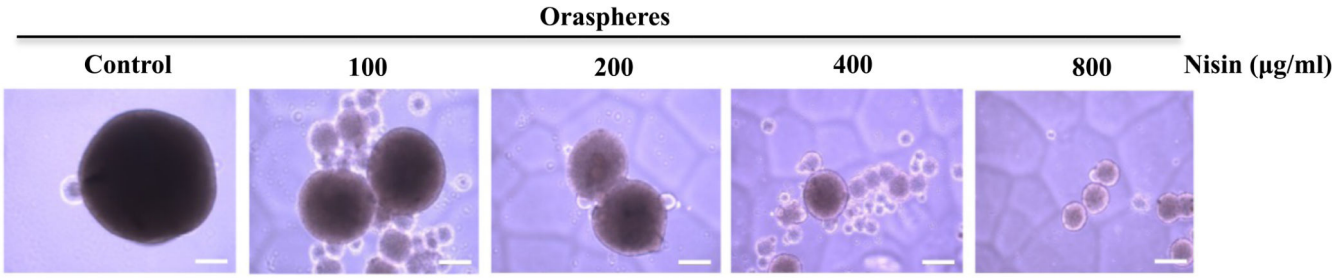

Figure 4. Nisin Z inhibits orasphere formation in HNSCC cells

Phase contrast images of oraspheres in HNSCC cells (UM-SCC-17B) cultured under suspension conditions and treated with control media or media containing nisin Z (100 to 800 μg/ml) for 36 h. These images were previously published (Kamarajan *et al.*, 2015).

Table 1
Natural and Bioengineered variants of nisin

Amino acids in blue letters indicate the flexible hinge region. Yellow highlights indicate amino acid substitutions compared to nisin A. Please note that this table does not contain all variants that have been reported to date.

Natural Variants	Unmodified Amino Acid Sequences	Origin
Nisin A	ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK	*Lactococcus lactis* strains (Gross *et al.*, 1971)
Nisin Z	ITSISLCTPGCKTGALMGCNMKTATCNCSIHVSK	*Lactococcus lactis* NIZO 22186 (Mulders *et al.*, 1991)
Nisin F	ITSISLCTPGCKTGALMGCNMKTATCNCSVHVSK	*Lactococcus lactis subsp. lactis* F10 (de Kwaadsteniet *et al.*, 2008)
Nisin Q	ITSISLCTPGCKTGVLMGCNLKTATCNCSVHVSK	*Lactococcus lactis* 61-14 (Zendo *et al.*, 2003)
Nisin H	FTSISMCTPGCKTGALMTCNYKTATCHCSIKVSK	*Streptococcus hyointestinalis* (O'Connor *et al.*, 2015)
Nisin U	ITSKSLCTPGCKTGILMT CPLKTATCGCHFG	*Streptococcus uberis* (Wirawan *et al.*, 2006)
Nisin U2	VTSKSLCTPGCKTGILMT CPLKTATCGCHFG	*Streptococcus uberis* (Wirawan *et al.*, 2006)
Nisin P	VTSKSLCTPGCKTGILMT CAIKTATCGCHFG	*Streptococcus galloyticus subsp. pasteurianus* (Zhang *et al.*, 2012)
Bioengineered Variants		
Nisin A S29A	ITSISLCTPGCKTGALMGCNMKTATCHCAIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2012)
Nisin A S29D	ITSISLCTPGCKTGALMGCNMKTATCHCDIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2012)
Nisin A S29E	ITSISLCTPGCKTGALMGCNMKTATCHCEIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2012)
Nisin A S29G	ITSISLCTPGCKTGALMGCNMKTATCHCGIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2008)
Nisin A K22T	ITSISLCTPGCKTGALMGCNMTTATCHCSIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2008)
Nisin A N20P	ITSISLCTPGCKTGALMGCPMKTATCHCSIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2008)
Nisin A M21V	ITSISLCTPGCKTGALMGCNVKTATCHCSIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2008)
Nisin A K22S	ITSISLCTPGCKTGALMGCNMSTATCHCSIHVSK	*L. lactis* NZ9800 (Field *et al.*, 2008)
Nisin Z N20K	ITSISLCTPGCKTGALMGCKMKTATCNCSIHVSK	*L. lactis* NZ9800 (Yuan *et al.*, 2004)
Nisin Z M21K	ITSISLCTPGCKTGALMGCNKKTATCNCSIHVSK	*L. lactis* NZ9800 (Yuan *et al.*, 2004)

Table 2
Overview of Biomedical Applications of Nisin

Disease	Nisin	Model	Results	References
Infections associated with drug resistant pathogens				
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin exhibited bactericidal effect against a large panel of Gram-positive bacteria including MRSA, *S. pneumoniae* and *enterococci*	Severina *et al.*, 1998
	Nisaplin (2.5% w/w purity)	*In vitro*	Nisin exhibited bactericidal effects against clinical isolates of *S. pneumoniae*, including penicillin- and other drug-resistant strains	Goldstein *et al.*, 1998
	Nisin A (> 95% purity)	*In vitro*	Nisin was active and highly bactericidal against *C. difficile*. Nisin was not absorbed by the gastrointestinal tract and did not have indiscriminate activity against all bowel flora or all anaerobes	Bartoloni *et al.*, 2004
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin was active against drug resistant *S. aureus*	Piper *et al.*, 2009
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin exhibited bactericidal effect against both MSSA and MRSA strains. In addition, it enhanced the activity of ciprofloxacin and vancomycin when used in combination	Dosler *et al.*, 2011
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin exhibited bactericidal activity against both MRSA and other *staphylococcal* biofilms grown on medical devices	Okuda *et al.*, 2013
	Nisaplin (2.5% w/w purity)	*In vitro*	Nisin incorporated with 2,3-dihydroxybenzoic acid in nanofibers inhibited formation of MRSA biofilms	Ahire and Dicks, 2015
Gastrointestinal Infections				
	Nisin A (> 95% purity)	*In vitro*	Nisin did not disrupt the intestinal epithelial integrity, suggesting that it may be suitable for the treatment of gastrointestinal tract infections	Maher and McClean, 2006
	Nisin A and Z (> 95% purity)	*In vitro*	Nisin A and Z exhibited similar inhibition effect against a broad range of intestinal Gram-positive bacteria	Blay *et al.*, 2007
	Nisaplin (2.5% w/w purity)	*In vitro*	Nisin was tableted with a pectin/HPMC mixture to form an enzymatically controlled delivery system for potential colonic drug delivery	Ugurlu *et al.*, 2007
	Nisin Z	*In vitro* and Mice	Nisin producing strain *L. lactis* modulated the intestinal microbiota and reduced the intestinal colonization of vancomycin-resistant *enterococci* in infected mice.	Millette *et al.*, 2008
	Nisin A and Z (unknown purity)	*Ex vivo* using jejunal chyme from fistulated dogs	Nisin was insensitive to degradation by the components of the jejunal chyme	Reunanen and Saris, 2009
	Nisin F(Purity in arbitrary units)	*In vitro* and Mice	Nisin may have a stabilizing effect on the bacterial population of the gastro intestinal tract	van Staden *et al.*, 2011

Disease	Nisin	Model	Results	References
Respiratory Infections				
	Nisin F (Purity in arbitrary units)	*In vitro* and Rats	Nisin was used to control intranasal *S. aureus* infection	De Kwaadsteniet *et al.*, 2009
	Nisaplin (2.5% w/w purity)	*In vitro* and Mice	Low blood and tissue levels of nisin were sufficient to prevent the death of mice infected with *S. pneumoniae*	Goldstein *et al.*, 1998
Skin and Soft Tissue Infections				
	Nisaplin (2.5% w/w purity)	*In vitro* and Mice	Nisin-containing nanofiber wound dressings significantly reduced *S. aureus* induced skin infections	Heunis *et al.*, 2013
Mastitis				
	Nisin Z (18000 IU/mg)	Cows	Intramammary administration of nisin was effective in the treatment of mastitis in lactating dairy cows	Cao *et al.*, 2007 Wu *et al.*, 2007
	Nisin A (Approximately 6 μg/ml)	*In vitro* and Human	Topical treatment of nisin was effective in the treatment of *staphylococcal* mastitis	Fermandez *et al.*, 2008
Cancer				
	Nisin A (2.5% w/w purity)	*In vitro* and Mice	Nisin reduced HNSCC tumorigenesis by inducing preferential apoptosis, cell cycle arrest, and reducing cell proliferation in HNSCC cells	Joo *et al.*, 2012
	Nisin A (2.5% w/w purity)	*In vitro* and Mice	Combination of nisin and doxorubicin decreased tumor severity in skin carcinogenesis	Preet *et al.*, 2015
	Nisin AP and ZP (* P stands for pure; 95% purity)	*In vitro* and Mice	Nisin promoted HNSCC cell apoptosis, suppression of HNSCC cell proliferation, inhibition of angiogenesis and cancer orasphere formation. Nisin inhibited tumorigenesis and prolonged survival of mice	Kamarajan *et al.*, 2015
	Nisin AP and ZP (* P stands for pure; 95% purity)	*In vitro*	Nisin synergizes with cisplatin to induce apoptosis in HNSCC cells that are highly resistant to ionizing radiation and cisplatin	Global Medical Discovery, 2015
Oral Health				
	Nisaplin (2.5% w/w purity)	Monkeys	Nisin reduced the numbers of *streptococci* in the dental plaque of monkeys that received nisin in their foods	Johnson *et al.*, 1978
	Nisin (Ambicin N) (unknown purity)	Dogs	Nisin based mouthrinse prevented plaque build-up and gingival inflammation in beagle dogs	Howell *et al.*, 1993
	Nisin A (2.5% w/w purity)	*Ex vivo* using the root canals of human teeth	Nisin eradicated the colonization of *E. faecalis*	Turner *et al.*, 2004
	Nisin Z (unknown purity)	*In vitro*	Nisin significantly reduced the growth and transition of *C. albicans*	Le Lay *et al.*, 2008
	Nisin Z (unknown purity)	*In vitro*	Nisin may work synergistically with oral gingival cells to provide greater resistance against *C. albicans* infections	Akerey *et al.*, 2009

Disease	Nisin	Model	Results	References
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin inhibited the growth of cariogenic bacteria, including *S. mutans*	Tong *et al.*, 2010
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin in combination with poly-lysine and sodium fluoride displayed synergistic effects in inhibiting planktonic and biofilm forms of *S. mutans*	Najjar *et al.*, 2009; Tong *et al.*, 2011
	Nisin A (2.5% w/w purity)	*In vitro*	Nisin paired with MTAD improved post-antibiotic sub-MIC effects of MTAD against *E. faecalis*	Tong *et al.*, 2014
	Nisin ZP (* P stands for pure; 95% purity)	*In vitro*	Nisin inhibited growth of Gram-positive and Gram-negative oral pathogens and saliva derived multi-species biofilms without cytotoxicity to human oral cells	Shin *et al.*, 2015