Consortium for Educational Communication

Module

on

Sanitizers And Antibiotics Used In The Food

By

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1. Introduction

Disinfection and hygiene are concepts that have been applied by humans for thousands of years. Chemical disinfection of a sort was practiced at the time of Persian imperial expansion. 450BC. Practical procedures involving chemical agents were also applied in the field of food preservation. As the science of chemistry developed, newer and purer chemical disinfectants began to be used.

There are numerous commercial products available for disinfecting and sanitizing surfaces in food premises, such as restaurants or processing plants. Various criteria have been set for choosing food-contact surface sanitizers and numerous active ingredients have been described that have been proven effective for disinfecting and sanitizing food contact surfaces.

Antibiotics are drugs used to treat bacterial infections. They work by killing or stopping the growth of harmful bacteria. Since the 1940s, antibiotics have been given to farm animals like cows, pigs and poultry in order to treat infections or prevent an illness from spreading. Low doses of antibiotics are also added to animal feed to promote growth. This means a greater production of meat or milk in shorter periods of time. These low doses may also reduce animal death rates and improve reproduction. For these reasons, antibiotic use has become widespread in agriculture. In 2011, 80% of all antibiotics sold in the US were for use in food-producing animals.

2. Need of Sanitizers and Antibiotics

One of the major advances in human history was the ability to preserve food. It was the prerequisite to man settling down in one place, instead of moving from place to place in the never ending hunt for fresh food. The history of the development of food preservation methods may be divided into a number of stages or eras, depending upon the techniques used to maintain the foods in an edible state. Animals are treated routinely with antibiotics to prevent, treat, or control disease. Even under the best conditions of agricultural management, crowding and stress can lead to disease

The objective of cleaning and sanitizing food contact surfaces is to remove food (nutrients) that bacteria need to grow, and to kill those bacteria that are present. It is important that the clean, sanitized equipment and surfaces drain dry and are stored dry so as to prevent bacteria growth.

Similarly, the need for a simple inexpensive method of reducing losses of flesh foods due to bacterial spoilage prompted an investigation of the value of antibiotics. Antibiotics and other antimicrobial agents are used widely against pathogens for protection of human and animal health. Production of sufficient safe and nutritious animal-sourced foods for human consumption is necessary for the security of food supply. To ensure this production and animal wellbeing, proper management of livestock includes appropriate use of vaccines, parasiticides, good handling and housing practices, and appropriate nutrition. These practices can reduce, but not eliminate, disease incidence. Prudent use of antimicrobial agents remains necessary for prevention, control, and treatment of infectious disease in food animals.

3. Sanitizers used in the food

3.1 Sanitization and Sanitizers

Sanitization is defined as the process of reducing microbial load from the surfaces to the levels considered safe as far as public health is concerned. *Sanitizers* reduce, but do not completely eliminate, microorganisms from the inanimate environment. There are two types of sanitizers

1. Non-food contact sanitizers:

These must demonstrate a 99.9% reduction against Gram-positive (*S. aureus*) and Gram-negative bacteria (*Klebsiella pneumonia* or *Enterobacter aerogenes*) over a parallel untreated control in ≤5 min. EPA policy does not allow veridical, tuberculocidal or fungicidal sanitizing claims.

2. Food contact sanitizers:

These must demonstrate a 99.999% reduction against Gram-positive (*S. aureus*) and Gram-negative bacteria (*Escherichia coli*) over a parallel control in 30 s. Though the EPA requires effectiveness to be demonstrated in 30 s, existing policy limits the contact time stated on the label to 60 s. EPA policy does not allow veridical, tuberculocidal or fungicidal sanitizing claims.

3.2 Sanitization in Food Processing

Sanitizing (the reduction in numbers of microorganisms) is required in food plant operations in which wet surfaces provide favourable conditions for the growth of organisms. In many situations, thorough cleaning will provide adequate microbial control simply by physically removing the microorganisms, or by removing nutrients which they require for growth. On the other hand, the rapid growth rates of bacteria in some foods require that equipment surfaces be almost sterile during operation in order to produce a wholesome finished product.

3.3 Properties of Sanitizers in Food Processing

Goldenberg and Reif (1967) state that a sanitizer or disinfectant:

a) must be efficient under the conditions of use;

b) must be safe for use by the operatives applying it

c) must not influence flavour or odours of foods processed by equipment cleaned by its use

d) must be easily rinsed leaving no toxic residue

e) should be easy to use.

3.4 Types of Chemical Sanitizers

Four basic types of chemical sanitizers are approved for use in the food industry. These are:

- quaternary ammonium compounds (quats)
- iodophors
- chlorine-based surfactants

- acid-ionic surfactants

Advantages and disadvantages of these sanitizers are summarised below:

Type of Sanitizer	Advantages	Disadvantages
Quats	noncorrosive non-irritating no flavour/odour	not effective against gram negative bacteria film formation
Iodophor	noncorrosive, easy to use, non-irritating, effective against a broad spectrum of microorganisms	flavour/odour moderately expensive
Hypochlorites	effective against a broad spectrum of microorganisms, inexpensive, easy to use	corrosive, discolouration, may oxidize lipids, affected by organic matter, flavour/odour, may irritate skin

3.4.1 Chlorine based sanitizers

Chlorine was first used in Germany to treat water supplies in 1894. In the early 1930s, the use of chlorine solutions to wash and rinse food processing equipment was begun, and by 1946 it was recognized that the use of chlorinated sprays at selected points in food processing lines resulted in lower bacterial counts in the finished product, reduced build-up of bacterial slimes, and reduced odours.

The major sources of chlorine are:

- Sodium hypochlorite
- Granular calcium hypochlorite
- Granular chlorocyanurates
- Gaseous chlorine

- Chloramine T

- Generation from sodium chloride

Of these, sodium hypochlorite is the easiest to use and least expensive.

The moiety producing antibacterial activity is hypochlorous acid. This can be formed by any of the following hydrolytic reactions:

$$Cl_2 + H_2O \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} HOCl + H^+ + Cl^-$$

$$NaOCl + H_2O \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} HOCl + Na^+ + OH^-$$

$$NH_2Cl + H_2O \underset{OH^-}{\overset{H^+}{\rightleftharpoons}} HOCl + NH_4^+ + OH^-$$

Hydrogenion concentration is obviously a very critical factor in determining the hypochlorous acid content and the antibacterial activity that can be obtained from chlorine-containing compounds. pH levels in the 5 -6 range and below result in the greatest percentages of hypochlorite as undissociated hypochlorous acid, with the strongest influence of pH occurring between 6 and 8.

There is currently little confirmed information about the manner in which chlorine is able to exert its effect on microorganisms. Several proposed mechanisms are:

- Destruction of protective bacterial capsules

- Oxidation of cell protoplasm

- Formation of nascent oxygen

- Formation of toxic chloramines

- Alteration of cell permeability
- Precipitation of bacterial protein
- Prevention of enzyme regeneration

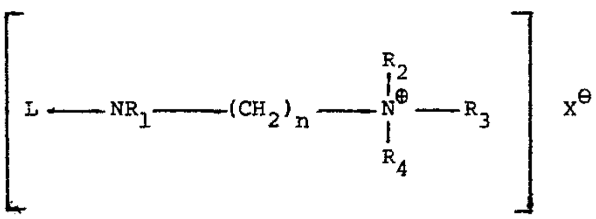
Chlorine-based sanitizers normally are employed at concentrations which provide 100 or 200 ppm available chlorine when used as surface sanitizers. In-plant treatment of process water usually is aimed at producing a concentration of from 0 to 0.5 ppm available chlorine, although this varies with the product. Drinking water is usually chlorinated to a level of 0.025-2.0 ppm, depending on the anticipated contamination of the water and the level of residual organic matter. Contact time and concentration of chlorine are critical factors. At 4 ppm, a 90% reduction in total count is obtained within 1 min, whereas with only 1 ppm hypochlorite the same level of reduction requires 10 min.

The benefits of chlorination are many, however, it should be emphasized that chlorination does not replace good sanitation and careful management practices nor does it improve a raw material or finished product of questionable quality.

3.4.2 Iodophors

Iodophors refer to the iodine-based sanitizers. If iodine is complexed with a surface active agent such as alkylphenoxypolyglycolether, a relatively high concentration (25-30%) is solubilized for use as a sanitizer. Such a compound is called an iodophor.

The general structure of an iodophor is



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Wherein L represents an acyl radical derived from lanolin fatty acid, R, is hydrogen or a C_{1-4} **alkyl group, R_2 and R_3***each represent a C_{1-4}* **alkyl group, R_4 is an alkyl, aralkyl, hydroxyalkyl, or unsaturated aliphatic hydrocarbon radical, X is a compatible anion**

Like chlorine-type germicides, iodophors are active against both gram positive and gram negative bacteria, as well as yeasts and moulds. Most iodophors maintain their activity over a fairly broad pH range, which permits them to be mixed with acidic materials such as phosphoric acid. As a result, iodophors possess not only surfactant and disinfectant properties, but also are capable of removing or preventing the build-up of scale. This property has made these compounds especially useful in the dairy industry, where the accumulation of milk stone (calcium and magnesium phosphates) is a problem. In this context, iodophors may be used in clean-in-place systems.

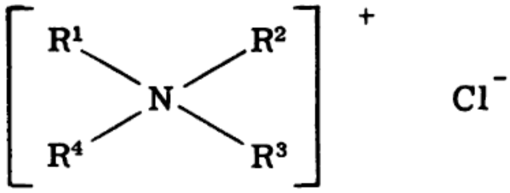
Iodophors are more costly than chlorine-based sanitizers; however, they normally are used at much lower concentrations (12.5—25 ppm) than the latter. In addition to cost, other disadvantages are corrosiveness (especially on galvanized iron), release of free iodine at temperatures greater than 43**°C, and high chemical oxygen** demand (COD) in plant wastewaters. Additionally, certain types of plants which process starch-containing products are unable to utilize iodophors because of the tendency of iodine to form a purple complex with starches.

Despite these disadvantages, iodophors remain highly regarded as germicides because they can penetrate organic soil, are unaffected by hard water, and are relatively non-irritating and therefore useful as a hand dip for food plant workers.

3.4.3 Quaternary Ammonium Sanitizers

These compounds, also called quats, have wetting properties; however, their principle use in the dairy and food industries is as a sanitizing agent.

Their chemical structure can be represented as:



where R^1, R^2, R^3, and R^4 are organic groups such as alkyl, methyl, benzyl, and cetyl-benzyl.

Quaternary Ammonium compounds (or Combinations Thereof) Approved for Use on Food-Contact Surfaces in Food Plants—Aqueous Solutions (April, 1980)[a]

1. *n*-alkyl (C_{12-18}) benzyldimethylammonium chloride
2. *n*-alkyl (C_{12-18}) benzyldimethylammonium chloride plus *n*-alkyl (C_{12-18}) dimethylethylbenzylammonium chloride
3. Same as above plus tetrasodium ethylenediaminetetraacetate and/or α-(*p*-nonylphenol)-ω-hydroxypoly(oxyethylene)
4. di-*n*-alkyl (C_{8-10}) dimethylammonium chlorides plus isopropyl alcohol (average molecular weight, 332–361).
5. *n*-alkyl (C_{12-18}) benzyldimethylammonium chloride plus sodium metaborate, α-terpineol and α [*p*-(1,1,3,3 tetramethylbutyl)phenyl]-ω-hydroxypoly(oxyethylene).
6. di-*n*-alkyl (C_{8-10}) dimethylammonium chloride (average molecular weight, 332–361) plus *n*-alkyl (C_{12-18}) benzyldimethylammonium chloride (average molecular weight, 351–380) plus ethyl alcohol. Ratio of first two compounds to be 60:40.

It is thought that quat molecules actually adsorb to the surfaces of bacteria and so are more efficient on a mole-for-mole basis than most other sanitizers used by the food industry. Their principal action is bactericidal; that is, bacteria are actually killed. However, mostly gram positive bacteria are destroyed, and gram negative organisms, many of them significant in foods, may not be appreciably affected. Usually the growth rate of gram negative bacteria in quat solutions is relatively slow and defused within a day or two following dilution from the concentrated quat, there should be no problem with these materials.

Quats are substantive and so provide residual antibacterial activity on treated surfaces. The most common use-concentrations are 500-1000 ppm. These compounds are stable in the presence of organic soil; they are odourless, colourless, noncorrosive, and non-irritating. Greatest effectiveness is achieved at a pH level of about 10, and at high temperatures.

3.4.4 Combinations

The combination of a detergent and a sanitizer into one formulation has the advantage that both cleaning and sanitization procedures can be accomplished simultaneously. In addition, as is the case with quats and non-ionic detergents, combinations of the two may be more effective than either alone. Proprietary mixtures of non-ionic detergents and quats as well as anionics plus chlorine-releasing compounds are available from commercial sources.

Sequences of detergents and sanitizers may be used to exploit a specific biological circumstance. For example, Wilson and Nelson (1979) studied detergent-sanitizer combinations and found that a sequence of an alkaline cleaner to which 50 ppm available chlorine had been added, followed by a rinse and an acidic detergent treatment, seemed to sensitize the spores of an anaerobic organism to a subsequent treatment with an iodophor solution containing 25 ppm available iodine. This combination has produced virtual sterility of food storage tanks used to store tomato products.

4. Antibiotics used in the food

Antibiotics are secondary metabolites produced by microorganisms that inhibit or kill a wide spectrum of other microorganisms. Most of the useful ones are produced by moulds and bacteria of the genus *Streptomyces.* Some antibiotic like substances are produced by *Bacillus* spp. and by some strains of *Lactococcus lactis.*

Three antibiotics have been investigated extensively as heat adjuncts for canned foods: subtilin, tylosin, and nisin. Nisin, however, is used most widely in cheeses. Chlortetracycline and oxytetracycline were widely studied for their application to fresh foods, whereas natamycin is employed as a food fungistat.

In general the use of chemical preservatives in foods is not popular among many consumers; the idea of employing antibiotics is even less popular. Some risks may be anticipated from the use of any food additive, but the risks should not outweigh the benefits overall.

15 considerations on the use of antibiotics as food preservatives were noted by Ingram et al.,

and several of the key ones are summarized as follows:

- The antibiotic agent should kill, not inhibit, the flora and should ideally decompose into innocuous products or be destroyed on cooking for products that require cooking.
- The antibiotic should not be inactivated by food components or products of microbial metabolism.
- The antibiotic should not readily stimulate the appearance of resistant strains.
- The antibiotic should not be used in foods if used therapeutically or as an animal feed additive.

A system of classifying bacteriocins that places them into one of four classes has been presented. The Klaenhammer system is based primarily on the genetics and biochemistry of these compounds.

I. Class I includes the lantibiotics such as nisin

II. Class II are small heat-stable peptides such as lactacin F

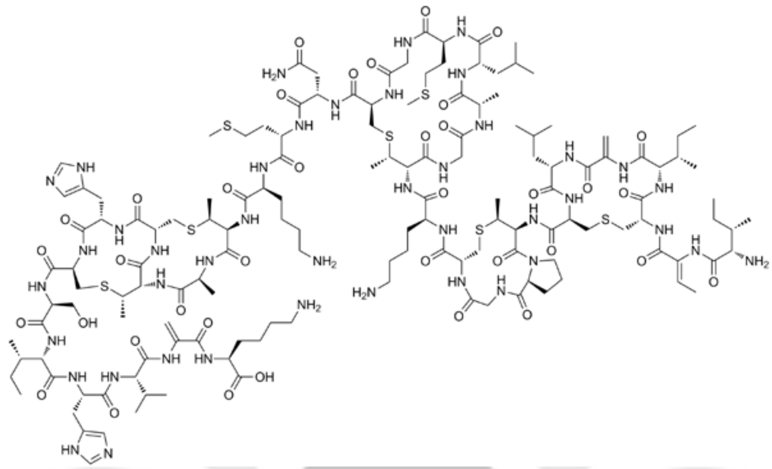
III. Class III are large heatlabile proteins such as helveticin J

IV. Class IV are proteins that form a complex with other factors.

Unlike antibiotics, bacteriocins inhibit only closely related species and strains of gram-positive bacteria. They consist of small proteins, and most are plasmid mediated. It appears that some species and strains of all genera of lactic acid bacteria possess the capacity to produce bacteriocins or bacteriocin-like compounds. Although early attention was focused on the lactics associated with dairy products, producing species and strains have been recovered from meats and other non-dairy fermented products.

4.1 Nisin and Other Bacteriocins

This is a polypeptide agent that is structurally related to subtilin, but unlike subtilin, it does not contain tryptophan residues.



The first food use of nisin was by Hurst51 to prevent the spoilage of Swiss cheese by *Clostridium butyricum*. This is the most widely used of these compounds for food preservation.

It is a hydrophobic compound, and it can be degraded by metabisulfite, titanium oxide, and certain proteolytic enzymes. The compound is effective against gram-positive bacteria, primarily spore formers, and is ineffective against fungi and gram-negative bacteria. Among some of its desirable properties as a food preservative are the following:

- It is nontoxic.
- It is produced naturally by *Lactococcus lactis* strains.
- It is heat stable and has excellent storage stability.
- It is destroyed by digestive enzymes.
- It does not contribute to off-flavours or off-odours.

- It has a narrow spectrum of antimicrobial activity.

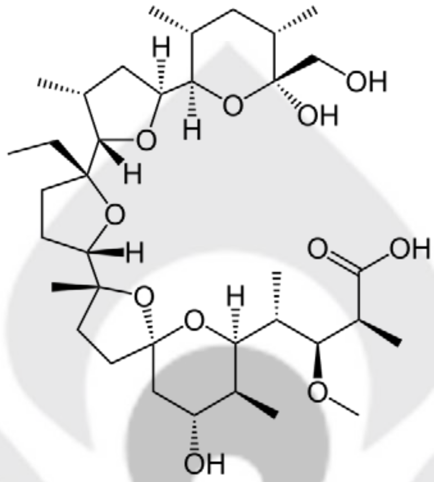
Typical usable levels for nisin are in the range of about 2.5-100 ppm. Nisin has been combined with low heat to destroy *L. monocytogenes* in cold-pack lobster meat. In addition to its use in certain canned foods, nisin is most often employed in dairy products—processed cheeses, condensed milk, pasteurized milk, etc. Its use is also permitted in processed tomato products and canned fruits and vegetables. It is most stable in acidic foods.

Because of the effectiveness of nisin in preventing the outgrowth of germinating endospores of *C. botulinum* and the search to find safe substances that might replace nitrites in processed meats, this agent has been studied as a possible replacement for nitrite. Employed in culture media without added nitrite, the quantity of nisin required for 50% inhibition of *C. botulinumtype* E spores was 1-2 ppm, 10-20 ppm for type B, and 20-40 ppm for type A.

With respect to mode of action, nisin and subtilin appear to be identical. The structural genes appear to be the same for nisin, subtilin, and other antibiotics. The cell target for these agents is the cytoplasmic membrane, where they depolarize energized bacterial membranes (reduce transmembrane potential) and form voltage-dependent multistate pores. The result of a pore formation is the loss of accumulated amino acids and the inhibition of amino acid transport. A nisin-resistant mutant of *L. monocytogenes* has been shown to contain significantly less phospholipids in its membrane. This has led to the assumption that the membrane targets for nisin are phospholipids which implies that fewer phospholipids would make membranes less susceptible to pore formation.

4.2Monensin

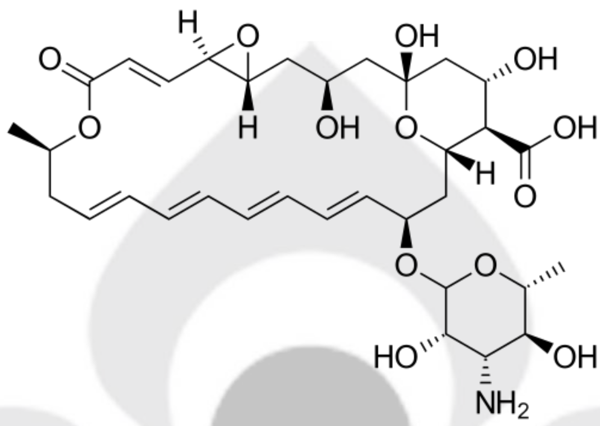
This antibiotic was approved by the FDA as a cattle feed additive in the 1970s, and it is used primarily to improve feed efficiency in ruminants. Its amino acid-sparing action has been demonstrated in fistulated cows.



It inhibits gram-positive bacteria, and thus its long-term use has the potential of shifting the gastrointestinal tract bacterial biota from one that is normally gram positive to one that is more gram negative. Like nisin, monensin is an ionophore (destroys selective permeability of cell membranes), and the two agents compare favourably as feed additives.

4.3 Natamycin

This antibiotic (also known as pimaricin, tennecetin, and myprozine) is a polyene that is quite effective against yeasts and moulds but not bacteria. Natamycin is the international non-proprietary name, as it was isolated from *Streptomycesnatalensis.*



In granting the acceptance of natamycin as a food preservative, the joint Food and Agriculture Organization/the World Health Organization (FAO/WHO) Expert Committee took the following into consideration:

- It does not affect bacteria
- it stimulates an unusually low level of resistance among fungi
- it is rarely involved in cross-resistance among other antifungal polyenes
- DNA transfer between fungi does not occur to the extent that it does with some bacteria.

The use of natamycin is limited as a clinical agent, and it is not used as a feed additive. It has been shown by numerous investigators to be effective against both yeasts and moulds.

It has been studies that 1 to 25 ppm of natamycin were effective against 16 different fungi (mostly moulds). To control fungi on strawberries and raspberries, 50 ppm of nystatin are required for effectiveness. In controlling fungi on salami, the spraying of fresh salami with a

0.25% solution has found to be effective.

Natamycin appears to act in the same manner as other polyene antibiotics. It binds to membrane sterols and induces distortion of selective membrane permeability. Because bacteria do not possess membrane sterols, their lack of sensitivity to this agent is thus explained.

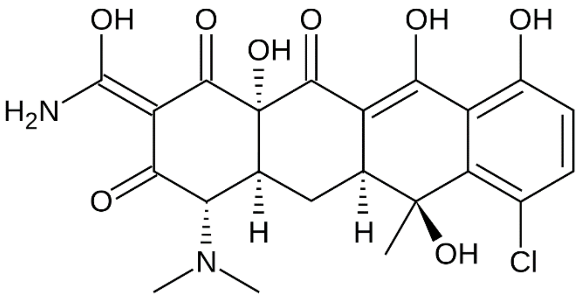
4.4 Tetracyclines

The efficacy of this group of antibiotics in extending the shelf life of refrigerated foods was first established in fish.

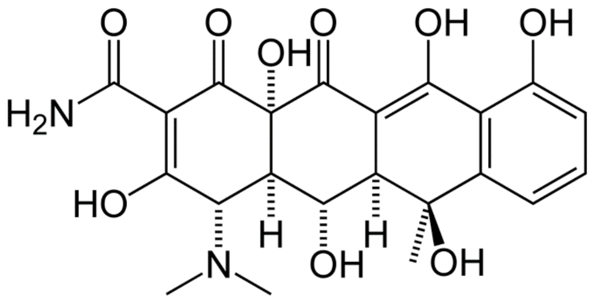
[redacted]

The tetracyclines are both heat sensitive and storage labile in foods, and these factors were important in their initial acceptance for food use. They are used to treat diseases in humans and animals and are used also in feed supplements. The risks associated with their use as food preservatives in developed countries seem clearly to outweigh the benefits.

Subsequently, the effectiveness of chlortetracycline (CTC) and oxytetracycline (OTC) in delaying bacterial spoilage of not only fish and seafood but poultry, red meats, vegetables, raw milk, and other foods. CTC is generally more effective than OTC.



Chlorotetracycline

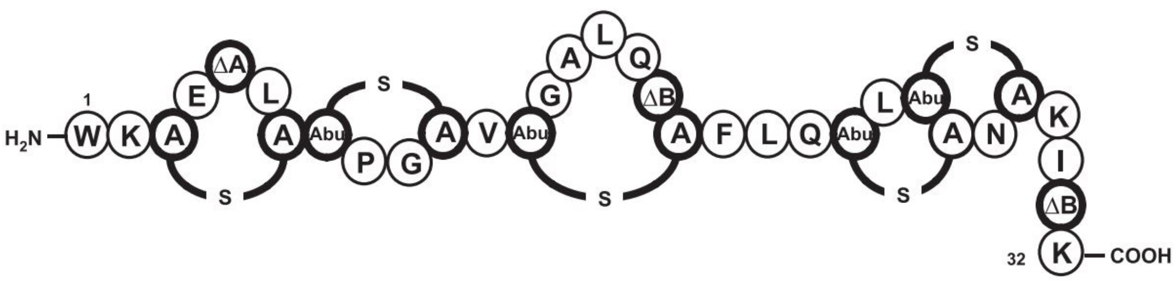


Oxytetracycline

The surface treatment of refrigerated meats with 7-10 ppm typically results in shelf-life extensions of at least 3-5 days and a shift in ultimate spoilage flora from gram-negative bacteria to yeasts and moulds. When CTC is combined with sorbate to delay the spoilage of fish, the combination has been shown to be effective for up to 14 days. Rockfish fillets dipped in a solution of 5 ppm of CTC and 1% sorbate had significantly lower aerobic plate counts (APCs) after vacuum-package storage at 2°C after 14 days.

4.5 Subtilin

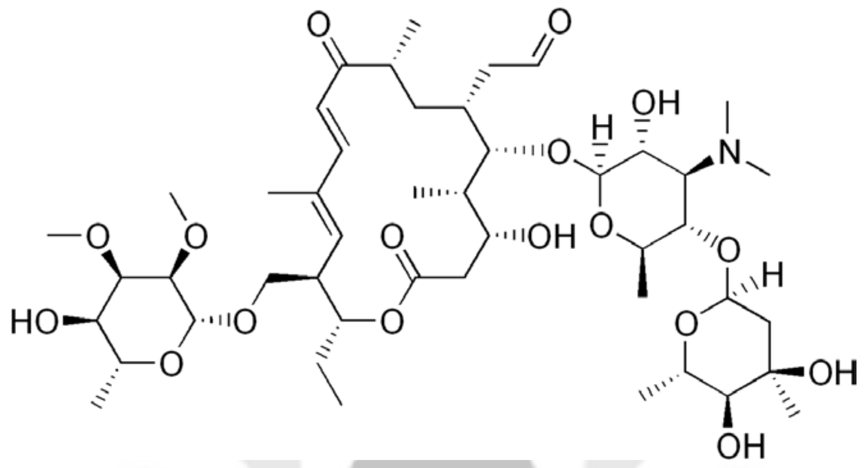
This antibiotic was discovered and developed by scientists at the Western Regional Laboratory of the USDA. It is structurally similar to nisin, although it is produced by some strains of *Bacillus subtilis.*



It is effective against gram-positive bacteria, is stable to acid, and possesses enough heat resistance to withstand destruction at 121°C for 30-60 minutes. Subtilin is effective in canned foods at levels of 5-20 ppm in preventing the outgrowth of germinating endospores. This antibiotic may be just as effective as nisin. Its mode of action is similar to that of nisin.

4.6 Tylosin

This antibiotic is a nonpolyene macrolide, as are the clinically useful antibiotics erythromycin, oleandomycin, and others.



It is more inhibitory than nisin or subtilin. It has been found to be effective for canned food. When 1 ppm was added to cream-style corn containing flat-sour spores and canned, no spoilage of product occurred after 30 days with incubation at 54°C. Tylosin also finds its usage in animal feeds and for treatment of some diseases of poultry.

As a macrolide, it is most effective against gram-positive bacteria. It inhibits protein synthesis by associating with the 50S ribosomal subunit and shows at least partial cross-resistance with erythromycin.