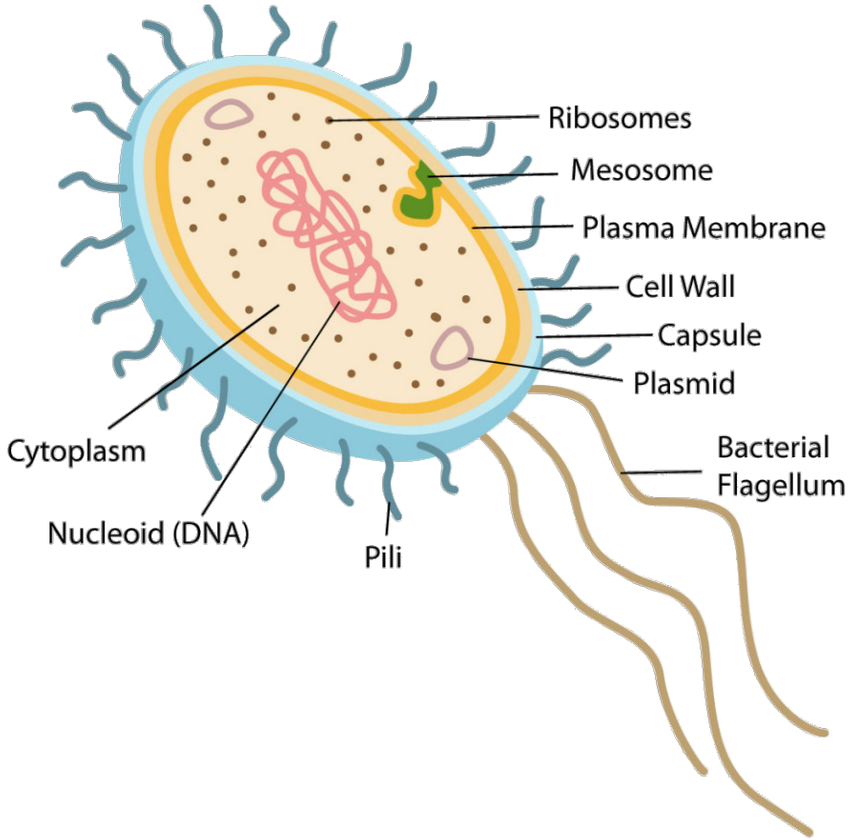
KlenCu+

High Concentration Nano Copper Aqueous Solution

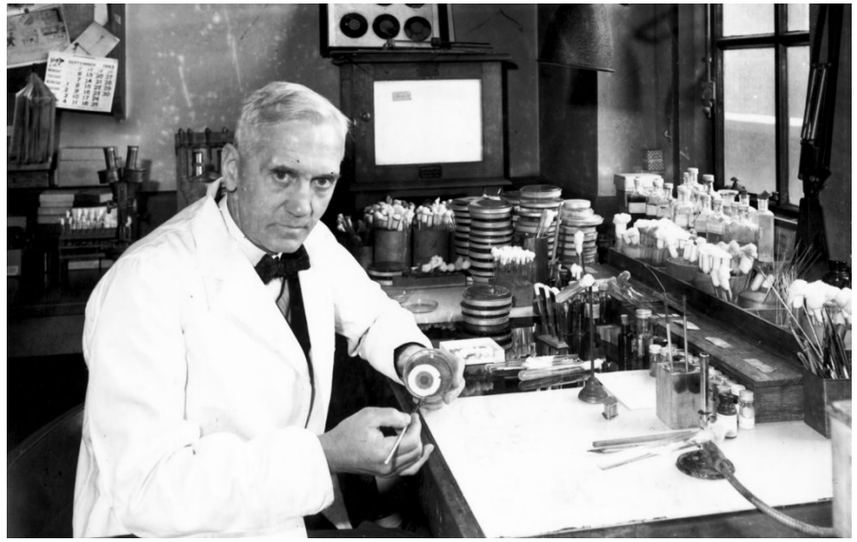
SPIREGENE BIOTECH CO., LTD.
TAIWAN BIOTECH GROUP

Bacterial Structure

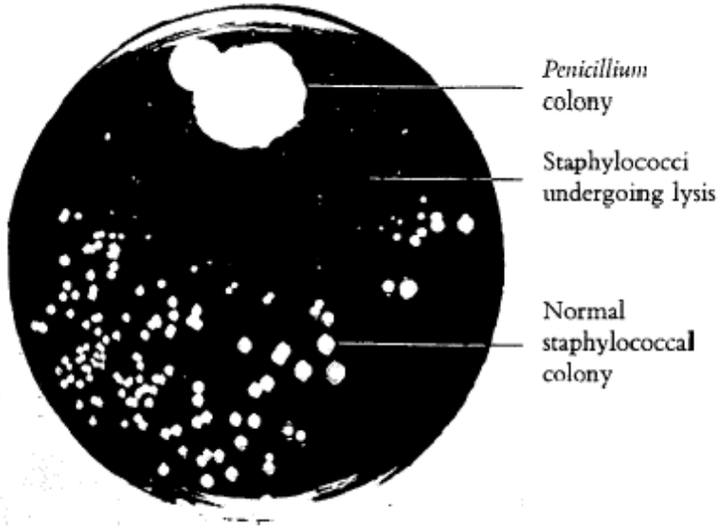


The bacterial structure showed as picture. The outer layer is the cell wall, some bacteria have the capsules on the outer layer from the wall, and some do not. There are also cilia on the wall, or with the movable flagellum. The cell membrane is in the wall, which coated the things like cytoplasm, ribosomes and the genetically related deoxyribonucleic acid (DNA) in the cells.

The Origin of Antibiotics.



(Alexander Fleming 1881 - 1955)



Antibiotic are the substances that specifically in inhibit or kill bacteria. They are often used by doctors to treat bacterial diseases, those are also one of the greatest invention of modern medicine. Penicillin is the first antibiotic produced by humans, which is the most recognized antibiotic name. On September 28, 1928, is the day that Penicillin was discovered unexpectedly.

Alexander Fleming(1881 - 1955) is known as the father Penicillin. Penicillin was successfully purified and mass produced under the efforts of Fleming, Oxford University' s Ernst Chain and Howard Flore, they all saved a large number of frontline soldiers from World War II.

Drug-Resistant Bacteria

About 2 million people in the United Stated are infected with drug-resistant bacteria every year. And there are 20,000 people among them die unfortunately, with economic losses of up to 5 billion US dollars; the number of deaths in the EU is 25,000 and the economic loss is 1.5 billion EUR. According to the statistic from the National Health Research Institutes, the amount of antibiotic for inpatients in Taiwan can reach to 10 billion TWD yearly in the recent years, and the broad-spectrum antibiotic for drug-resistant treatment account for about 70%.

The infected inpatients with intensive care in Taiwan, those with the days and the expense in the hospital are much higher than other patients.

The drug resistance has had such big impact in recent years because there have no enough antibiotic listed to subdue these bacteria. There were also the drug resistance problems before the 1980s, why was there no such panic at that time? Because with various new antibiotics flourished at the time, there were many other antibiotics in the medical community to resist them. However, after 1980s, there are fewer pharmaceutical companies willing to invest the new antibiotics. The medical community gradually encountered the dilemma of no medicine after 2010.

Livestock and Fishery: One-Fifth of Drug-Resistant Bacteria come from Animal and Food

The use of antibiotics in animals is a problem that people can not ignore.
It will produce more bacteria in animal with many antibiotic in improperly. Those can also transmitted to people through foods, environment or animals.

The US Bureau of Diseases said in 2013 that about one-fifth of drug-resistant bacteria come from animal and food , including directly touching the drug-resistant bacteria from the animal, uncooked and untreated food.
This is a huge risk with highly valued in the world. The EU made the resolution in July that they would strictly limit the use of colistin antibiotic in animal husbandry, and aim to reduce the use of six or five percent.

The Top Drug-Resistance in the World- China Seafood

According to the UN data, the $900 billion aquaculture trade accounts for almost half of all seafood harvests. And the seafood supply from China accounts for almost 60% of the global, which is the world’ s largest exporter.

The US food regulatory authorities have been aware the antibiotic problems in China for more than 10 years.

The abuse of antibiotics in China, with the breeding method for both pig and fish rising. Those caused the extremely serious antibiotic residues in the water.

There are a large amounts of antibiotics while the feeding from the farmers, and even abused with the colistin, which is called “ the last line of drugs”. Those drugs abuses would be finally prohibit by China’ s government until November in 2016. However, the pig farmers still abusing other antibiotics, that one foreign media have seen the abuse of nine antibiotic from a small pig farm, and seven of which are drugs that the WHO thought those are extremely important for human health.

According to the research, 90% of the antibiotics fed to the pig flowing to the fish pond from the farms where rising both pigs and fish in China. In order to avoid the pathogens in the water, they added other antibiotics to the fish pond until flowing out to the river in the finally. Then it would be caused the serious antibiotic contamination throughout the entire river.

In the case of the Pearl River Estuary as the instance, approximately up to 193 metric tons of antibiotics were infused to the river each year from a study in 2013.

Invisible killer-Vibrio

Vibrio has always been the most important health management problem in shrimp farms. The reason why it is so tricky is that it has the following characteristics :

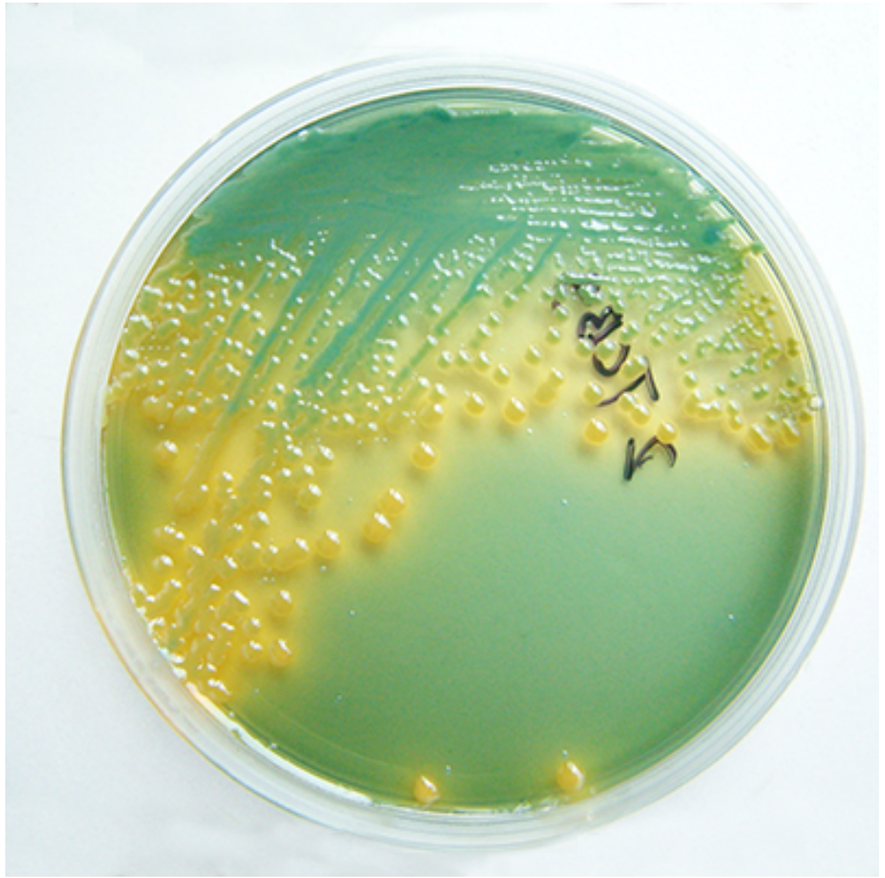
1. Vibrio is the dominant species in seawater, and its growth rate or adaptability is much better than other bacteria like probiotics.

2. Vibrio can spread to a considerable distance with the flow of air. It is very difficult to avoid the invasion of harmful Vibrio under the condition that it can not block and filter the outside air.

3. Almost all raw baits carry or are attached to Vibrio, such as brine shrimp, sea worms, oysters, squid and microalgae. The shrimps themselves also have Vibrio, so they cannot be eliminated.

4. Vibrio can form biofilms and use this mechanism to survive harsh environments. Even if it is disinfected with high concentrations of chemicals or antibiotics, it cannot be completely removed, not mentioning the possible drug resistance consequences of using antibiotics. Furthermore, based on the fast growth of Vibrio, even if 99.99% of Vibrio has been removed, the remaining 0.01% can grow to original amount within five or six hours.

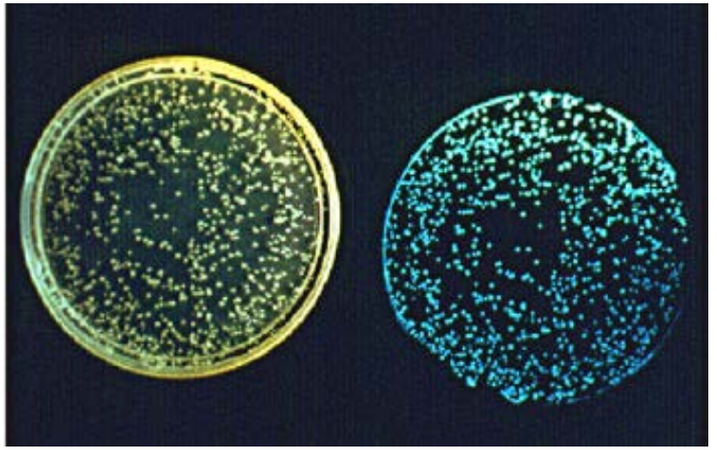
5. Vibrio is not the same as virus because it can survive and multiply by itself. Therefore, Vibrio is a lingering nightmare for the health management of shrimp farms.



The consequence of Vibrio in shrimp farm

1.Vibrio harveyi

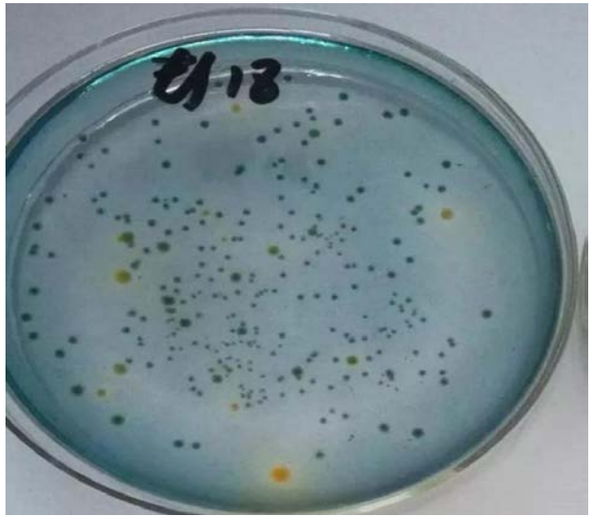
Vibrio harveyi is a luminescent marine bacterium that is an important pathogen of aquaculture animals that have been recognized in the recent 10 years. The shrimp seedlings are yellow when they were under tested on the medium, and one of the bacteriophage of the fluorescing disease from the shrimp seedlings was Vibrio harveyi. Symptoms and pathological changes of Vibrio harveyi: In the early stage of the disease, the larval activity is weakened, swimming in the lower middle layer of the water, eating less or not feeding, the body is whitish, and the larvae that died or almost died will fluoresce. In adulthood, the ventral surface of the head and chest and the abdomen are fluorescing, and the whole body emits light when it is severe that is common in shrimp farm, especially in the shrimp phase.



Vibrio harveyi

2. Vibrio parahaemolyticus

Vibrio parahaemolyticus, also known as halophilic bacteria, appears green on the medium and can survive for 47 days in seawater. Vibrio parahaemolyticus is widely distributed, mainly living in seawater, fish and shrimp, shell crustaceans, usually causing gastrointestinal or intestinal infections. In recent years, the main pathogen from early death syndrome of P. vannamei is Vibrio parahaemolyticus. The disease happened in 7-30 days under shrimp culture. During the onset of shrimp, it has the symptoms oflosing appetite and emptying the stomach on the pond water slowly flowing or crouching on the slop of the pond. The disease spread rapidly and mortality rate is extremely high.

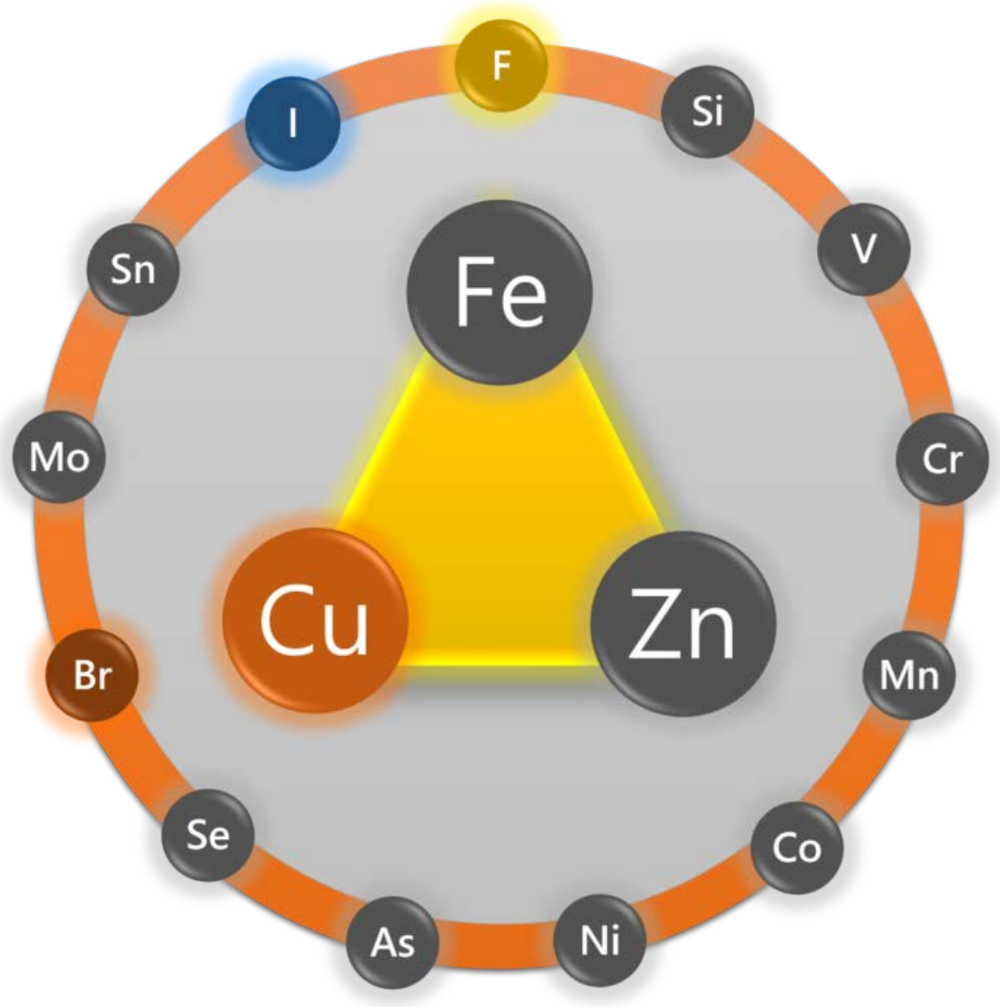


Vibrio Parahemolyticus

Introduction

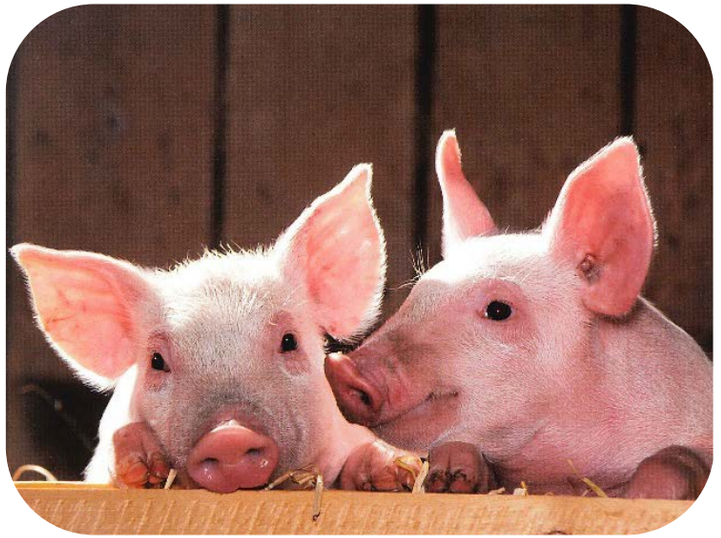
•Copper is one of the trace elements found in the human body. Although the daily demand for trace elements is small, it has a huge impact and is an indispensable element for good health.

•Copper is a trace element contained in the human body. Its content is second only to iron and zinc, which can maintain the normal metabolism of the human body.



Copper used in feed

Lack of copper can lead to growth and metabolism disorders. A small amount of copper can be allowed in livestock and poultry feed. Because poultry and human body need a small amount of copper, copper can help metabolism, and hematopoietic bone development can also help the gastrointestinal bactericidal effect, which can accelerate the growth of poultry. Promote physical health, thereby reducing production costs, improving operational efficiency and competitiveness.

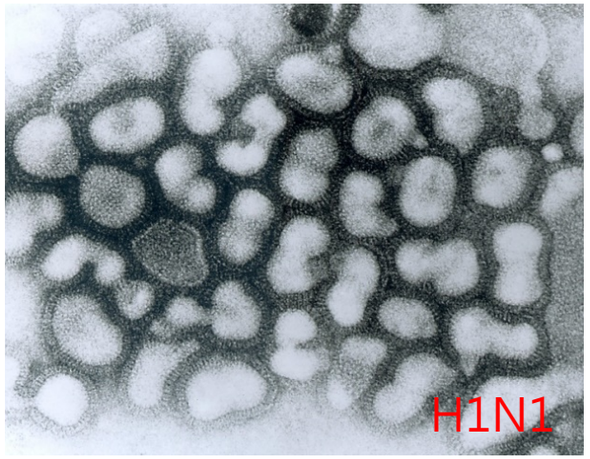


Antibacterial and antiviral copper

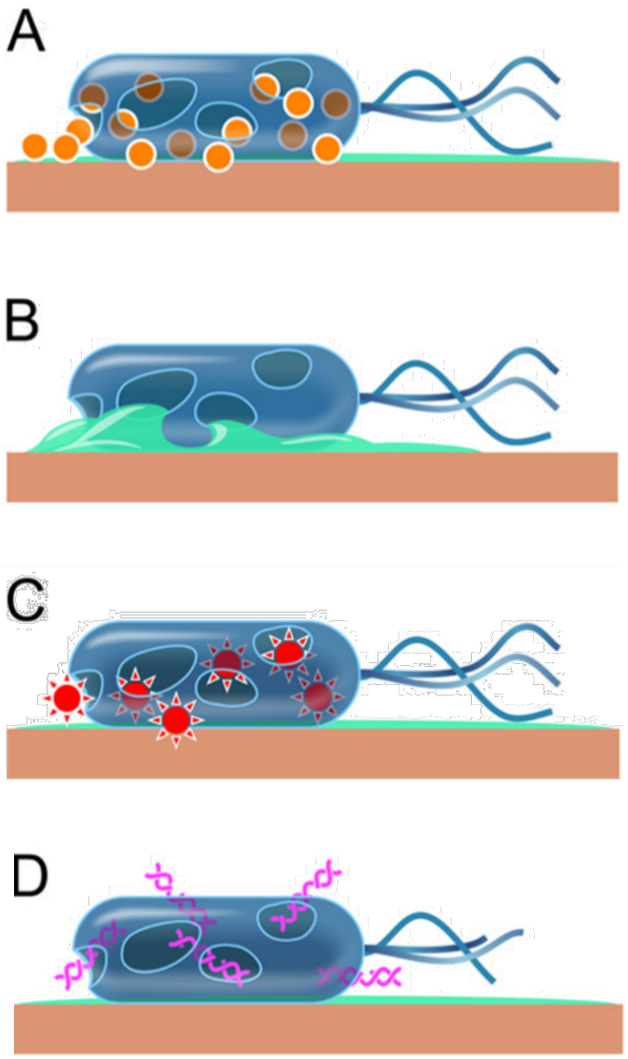
- In 2009, C. William Keevil, Department of Environmental Health Sciences, University of Southampton, UK, published a research report that copper can inhibit influenza A virus (A H1N1), killing 75% of influenza A virus in 1 hour on copper surface, 6 hours later Almost 99% of the flu virus was killed; while on the stainless steel surface, $5x10^6$ virions survived after 24 hours.

- The US Environmental Protection Agency (EPA) specification test showed that copper alloys killed 99.9% of super pathogens (MRSA) in two hours.

- According to a study published by the World Health Organization, the use of copper in hospitals can help reduce the incidence of nosocomial infections (HAI) by up to 40% and effectively kill 97% of bacterial, viral and fungal pathogens.



Copper antibacterial mechanism



A. Copper dissolves from the surface, causing cell damage

B. Cell membrane ruptures due to copper or other stress, leading to cell membrane potential and cytoplasmic loss

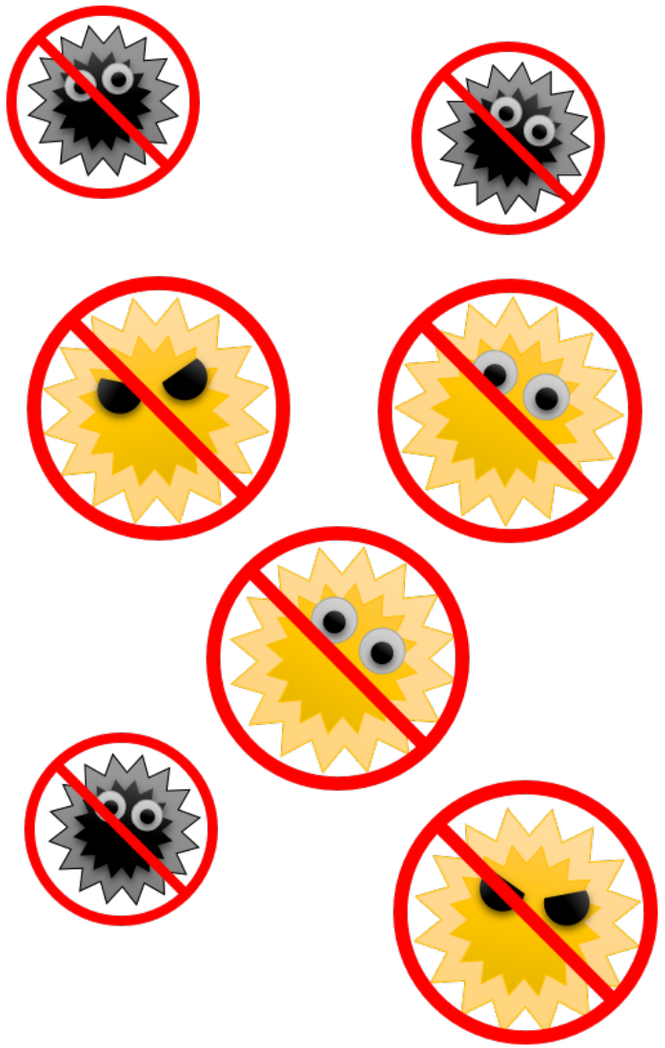
C. Copper ions induce the production of reactive oxygen species and cause further damage to

D. genes and DNA damage

Copper ions can destroy the cell membrane of bacteria and enter the bacteria, blocking metabolism and inactivating bacteria, destroying its genes, killing bacteria, and even preventing bacterial resistance mutations.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2011, p. 1541–1547

Copper antibacterial species



Species	Application method	Killing time, RT[a]
Salmonella enterica	Wet, 4.5×10^6 CFU[b]	4 h
Campylobacter jejuni	Wet, 4.5×10^6 CFU[b]	8 h
Escherichia coli O157	Wet, $(3–4) \times 10^7$ CFU[c]	65 min
Escherichia coli O157	Wet, 2.7×10^7 CFU[c]	75 min
MRSA[d] (NCTC10442)	Wet, $(1–1.9) \times 10^7$ CFU[c]	45 min
EMRSA-1[e] (NCTC11939)	Wet, $(1–1.9) \times 10^7$ CFU[c]	60 min
EMRSA-16[e] (NCTC13143)	Wet, $(1–1.9) \times 10^5$ CFU[c]	90 min
Listeria monocytogenes Scott A	Wet, 10^7 CFU[c]	60 min
Mycobacterium tuberculosis	Wet, 2.5×10^7 CFU[f]	5 to 15 days[g]
Candida albicans	Wet, $>10^5$ CFU[f]	60 min
Klebsiella pneumoniae	Wet, $>10^7$ CFU[f]	60 min
Pseudomonas aeruginosa	Wet, $>10^7$ CFU[f]	180 min
Acinetobacter baumannii	Wet, $>10^7$ CFU[f]	180 min
MRSA	Wet, $>10^7$ CFU[f]	180 min
Influenza A virus (H1N1)	Wet, 5×10^5 viruses[h]	6 h, 4-log decrease
C. difficile (ATCC 9689) vegetative cells and spores	Wet, 2.2×10^5 CFU[c]	24–48 h
C. difficile NCTC11204/R20291 vegetative cells	Wet, $(1\text{-}5) \times 10^6$ CFU[i]	30 min
C. difficile dormant spores	Wet, 8×10^6 CFU[i]	Unaffected in 3 h
C. difficile germinating spores	Wet, 8×10^6 CFU[i]	3 h, 3-log decrease
Pseudomonas aeruginosa PAO1	Wet, 2.2×10^7 CFU[j]	120 min
MRSA NCTC 10442	Wet, 2×10^7 CFU	75 min, 7 log decrease
Escherichia coli W3110	Dry, 10^9 CFU[i]	1 min
Acinetobacter johnsonii DSM6963	Dry, 10^9 CFU[k]	A few minutes
Pantoea stewartii DSM30176	Dry, 10^9 CFU[i]	1 min
Pseudomonas oleovorans DSM 1045	Dry, 10^9 CFU[k]	1 min
Staphylococcus warnerii DSM20316	Dry, 10^9 CFU[k]	A few minutes
Brachybacterium conglomeratum DSM 10241	Dry, 10^9 CFU[k]	A few minutes
Aspergillus flavus	Wet, $(2–300) \times 10^5$ spores[c]	120 h
Aspergillus fumigatus	Wet, $(2–300) \times 10^5$ spores[c]	>120 h
Aspergillus niger	Wet, $(2–300) \times 10^5$ spores[c]	> 576 h
Fusarium culmonium	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Fusarium oxysporum	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Fusarium solani	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Penicillium crysogenum	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Candida albicans	Wet, $(2–300) \times 10^5$ spores[c]	24 h
Enterococcus hirae ATCC 9790	Wet, 10^7 CFU[c]	90 min
Different *Enterococcus* spp.	Wet, 10^6 CFU[f]	60 min
Candida albicans	Dry, 10^6 CFU[k]	5 min
Saccharomyces cerevisiae	Dry, 10^6 CFU[k]	30 s

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Cu-PURE compared with other types of disinfectants

Disinfectant type	Bactericidal reducing	viral reducing	Fungicidal	Irritating	safety	duration	operational ease level	weakness
KlenCu+ (nano Copper)	Excellent	Excellent	Excellent	Low	High (non-carcinogenic, non-irritating)	long	Easy (Dilute with water to use)	Higher price
Chlorine dioxide	Excellent	Excellent	mediocre	Middle	Middle (non-carcinogenic, acid-activated, irritating)	short	Middle (Need to mix in two doses of AB)	Fast degradation rate, very short duration
Sodium hypochlorite (bleach)	Excellent	Excellent	mediocre	Middle	Middle (higher skin and respiratory irritation than chlorine dioxide)	short	Easy (Dilute with water to use)	Short duration, irritating
Sodium hydroxide solution (caustic soda)	Excellent	Excellent	Excellent	High	Low (strongly irritating and corrosive to skin and eyes)	mediocre	Hard (The dilution solution should be arranged in order and diluted in a safe area.)	Highly corrosive and irritating
70% ethanol	Excellent	Bad	Bad	Middle	Middle (allergenic to the skin, possibility of burning)	short	Easy (Dilute with water or buy directly)	High price, no effect on viruses and fungi
hydrogen peroxide	mediocre	mediocre	mediocre	High	Low (irritating to eyes and skin)	short	Hard (Due to irritation, it needs to be diluted in a safe area)	Highly irritating

KlenCu+ production

KlenCu+ is the world's highest concentration and most stable aqueous solution of nano copper.

The technology of KlenCu+ has been developed for 10 years. With the control of special stabilizers, nano copper can be stably dispersed in pure water for a long time. KlenCu+ nano Copper can accurately and stably release Copper ions, which has a very good killing effect on bacteria and viruses, and its performance is about 1000 times higher than that of ordinary copper sulfate.

Origin	Taiwan
Material	Nano Copper
Appearance	Blue Aqueous Solution
Solvent	Pure Water
Particle Size	4 nm
Concentration	150,000 ppm
Density	1.52
pH Value	3
Packing	25 KG

SGS test report for the nano copper:
Copper Content 15.42%=154200ppm

KlenCu+ ： 藍色液體

測試項目	單位	測試方法	方法偵測極限值	結果 No. 1
銅 (Cu)	%	酸消化後以感應耦合電漿原子發射光譜儀(ICP-AES)分析.	0.0002	15.42

SGS ICP Concentration Test Report : Copper Content 15.42% = 154,200 ppm

備註：

1. mg/kg = ppm；0.1wt% = 1000ppm
2. MDL = Method Detection Limit / 方法偵測極限值
3. n.d. = Not Detected / 未檢出

SGS report for SO_4^{2-} volume

SGS

KlenCu+

SGS test report does not contain sulfate, so it belongs to non-copper sulfate

測試項目	CAS NO.	測試方法	測試結果	定量極限/偵測極限	單位
硫酸根離子 (SO_4^{2-})	014808-79-8	本測試以離子層析儀(IC)檢測。	N.D.	50.0	ppm(mg/L)

備註：1. 測試報告僅就委託者之委託事項提供測試結果，不對產品合法性做判斷。

2. 本報告不得分離，分離使用無效。

3. 若該測試項目屬於定量分析則以「定量極限」表示；若該測試項目屬於定性分析則以「偵測極限」表示。

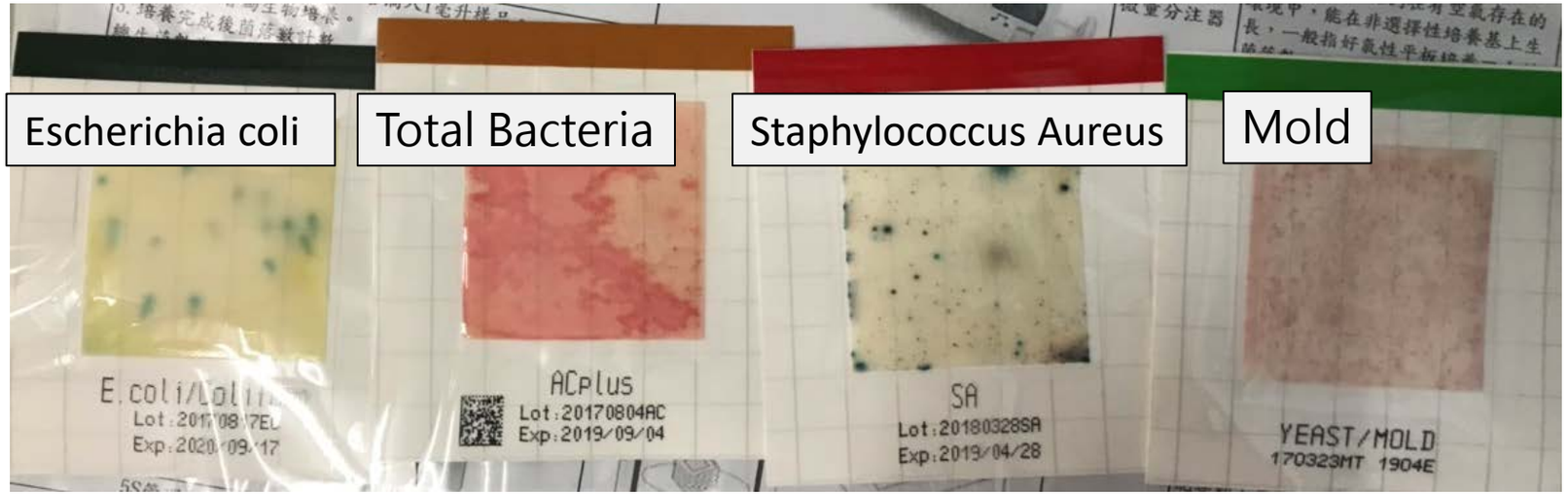
4. 低於定量極限之測定值以 "N.D." 表示；低於偵測極限之測定值以" 陰性 " 表示。

-END-

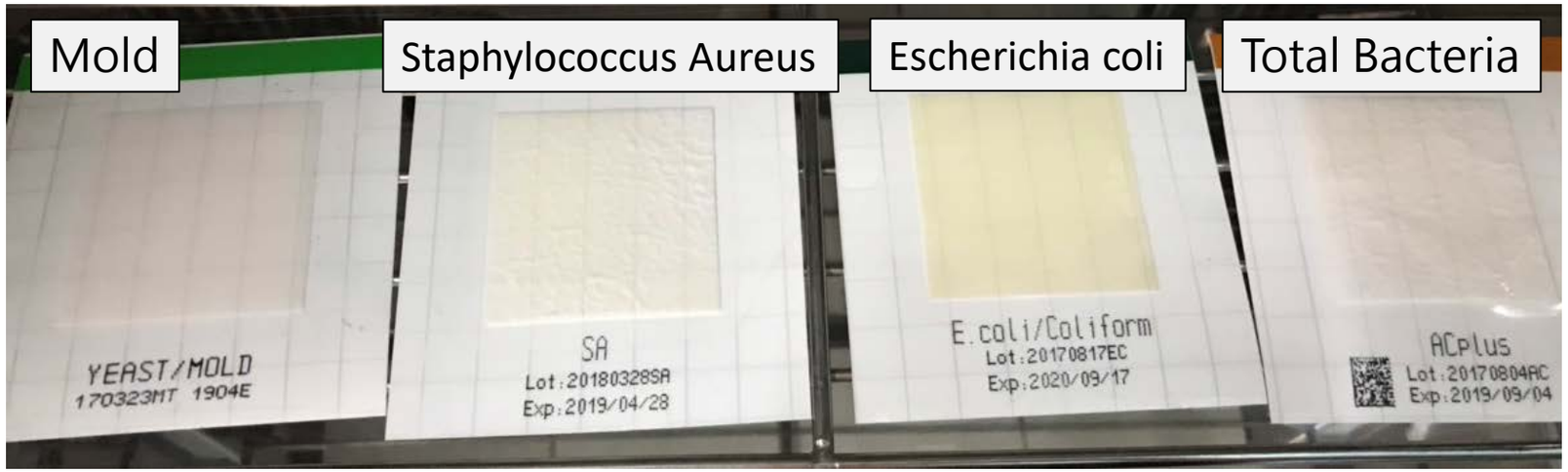
KlenCu+ Intertek RoHS 2.0 Test Report

Material	Unit	Conclusion
Cadmium (Cd) Content	ppm	ND
Lead (Pb) Content	ppm	ND
Mercury (Hg) Content	ppm	ND
Chromium VI (Cr6+) Content	ppm	ND
Polybrominated Biphenyls (PBBs) Monobrominated Biphenyls MonoBB) Dibrominated BiphenylsDiBB) Tribrominated Biphenyls TriBB) Tetrabrominated Biphenyls TetraBB) Pentabrominated Biphenyls PentaBB) Hexabrominated Biphenyls HexaBB) Heptabrominated Biphenyls HeptaBB) Octabrominated Biphenyls OctaBB) Nonabrominated Biphenyls NonaBB) Decabrominated Biphenyl DecaBB)	ppm	ND
Polybrominated Diphenyl Ethers (PBDEs) Monobrominated Diphenyl Ethers(MonoBDE) Dibrominated Diphenyl Ethers(DiBDE) Tribrominated Diphenyl Ethers (TriBDE) Tetrabrominated Diphenyl Ethers (TetraBDE) Pentabrominated Diphenyl Ethers (PentaBDE) Hexabrominated Diphenyl Ethers (HexaBDE) Heptabrominated Diphenyl Ethers (HeptaBDE) Octabrominated Diphenyl Ethers (OctaBDE) Nonabrominated Diphenyl Ethers (NonaBDE) Decabrominated Diphenyl Ether (DecaBDE)	ppm	ND
Phthalates Di(2-ethylhexyl) Phthalate (DEHP) Dibutyl Phthalate (DBP) Benzyl Butyl Phthalate (BBP) Diisobutyl Phthalate (DIBP)	ppm	ND

General Circulating Water



Circulating water added **KlenCu+** 1 hour later.



KlenCu+ application in fields of Livestock and Fishery

Prevention of bacterial and viral related diseases

Freshwater and marine Vibrio control

KlenCu+

Live fish and shrimp nurture process and transportation protection

Prevention of wound infection and inflammation

Performance- Shrimp nurture in Zhanjiang, China

location	Zhanjiang, China
Nurture measure	3000 acre
Nurture type	Vannamei
Nurture problem	Vibrio exceeds the standard, antibiotics are not effective
Performance	Before using KlenCu+, the number of Vibrio resistant bacteria was as high as 5000 CFU/ml. After using KlenCu+, the number of Vibrio resistant bacteria decreased to less than 500 CFU/ml, and the mortality of shrimp was greatly reduced.

Performance-Shrimp nurture in Andhra Pradesh, India

location	Andhra Pradesh, India
nurture measure	400、1000 acre
nurture type	Vannamei
nurture problem	Bacterial exceeds standard
performance	Because of drug abuse problems, antibiotics do not work well for bacterial control. Before using KlenCu+, the number of Vibrio resistant bacteria was as high as 7000 CFU/ml. After using KlenCu+, the number of Vibrio resistant bacteria was controlled below 500 CFU/ml.

Application- Crab farming in Malaysia

location	Malaysia
Nurture measure	1000 Mu
Nurture type	Crab
Nurture problem	Bacteria level exceeded standards
Performance	Because of drug-abuse problems, antibiotics do not work well for bacterial control. Before using KlenCu+, the number of Vibrio resistant bacteria was as high as 4000 CFU/ml. After using KlenCu+, the number of Vibrio resistant bacteria was controlled below 500 CFU/ml.

Application-a large-scale dealer for fish and shrimp drug in China

location	China
Nurture measure	Over 70,000 Mu
Nurture type	fish, shrimp, crab and shellfish
Nurture problem	Bacteria, vibrio exceeded standards
Performance	One of customer is a large-scale fish drug dealer via diluting distribution sales in China. Most customers have encountered problems with drug-resistant bacteria and Vibrio exceeded the standards. After using KlenCu+ continuously, bacteria and vibrio were under controlled that customers use 9 tons of KlenCu+ per year.

Application – a large-scale dealer for fish and shrimp drug in Taiwan

location	Taiwan
Nurture measure	Over 20,000 Mu
Nurture type	fish, shrimp, crab and shellfish
Nurture problem	Bacteria, vibrio exceeded standards
Performance	One of customer is a large-scale fish drug dealer via diluting distribution sales in Vietnam. Most customers have encountered problems with drug-resistant bacteria and Vibrio exceeded the standards. After using KlenCu+ continuously, bacteria and vibrio were under controlled that customers use 3 tons of KlenCu+ per year.

Aquaculture-suggested ways for using KlenCu+ in various conditions(I)

Ways for using KlenCu+

types	additive	normal additive amount	Normal frequency of adding additive	Additive amount under illness	frequency of adding additive under illness	remark
shrimp						
Shrimp nurture	Pool water	0.05 g / ton of water	supple KlenCu+ when changing water	-	-	Supple KlenCu+ every 2 weeks if not changing water
Shrimp delivery	Pool water	0.1 g / ton of water	1 time	-	-	
grown-up shrimp nurture	Pool water	0.05 g / ton of water	supple KlenCu+ when changing water	-	-	Supple KlenCu+ every 2 weeks if not changing water

When the aquaculture pond is more than one mu of land (666 tons of water), KlenCu+ and 20 liters of water should be mixed and diluted first, then pour KlenCu+ into three sections of the pond to make sure KlenCu+ can be evenly dispersed in water.

KlenCu+ will consume some probiotics, so customer should supple probiotics within 2~3 days to maintain the balance of bacteria in water.

Aquaculture-suggested ways for using KlenCu+ in various conditions(II)

Ways for using KlenCu+

types	additive	normal additive amount	Normal frequency of adding additive	Additive amount under illness	frequency of adding additive under illness	remark
FISH						
fry	Pool water	0.05 g / ton of water	supple KlenCu+ when changing water	-	-	Supple KlenCu+ every 2 weeks if not changing water
Fry delivery	Pool water	0.1 g / ton of water	One time	-	-	-
grown-up fish nurture	Pool water	0.05 g / ton of water	supple KlenCu+ when changing water	-	-	Supple KlenCu+ every 2 weeks if not changing water
skin disease of fish	Pool water	0.1 g / ton of water	supple KlenCu+ when changing water	-	-	Supple KlenCu+ every 2 weeks if not changing water
grown-up fish delivery	Pool water	0.1 g / ton of water	One time	-	-	-
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other types of aquaculture						
Seedling and grown-up	Pool water	0.05 g / ton of water	supple KlenCu+ when changing water	-	-	Supple KlenCu+ every 2 weeks if not changing water

KlenCu+

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