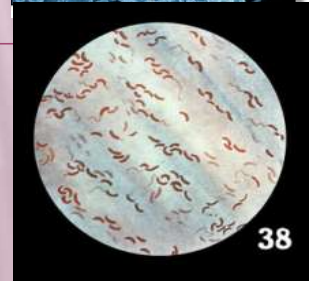
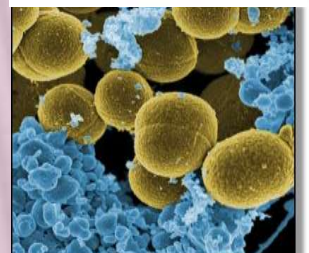


FISH AND FISHERY PRODUCTS MICROBIOLOGY- 3 (2 - 1)

PATHOGEN BACTERIA IN FISH

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DEFINITION

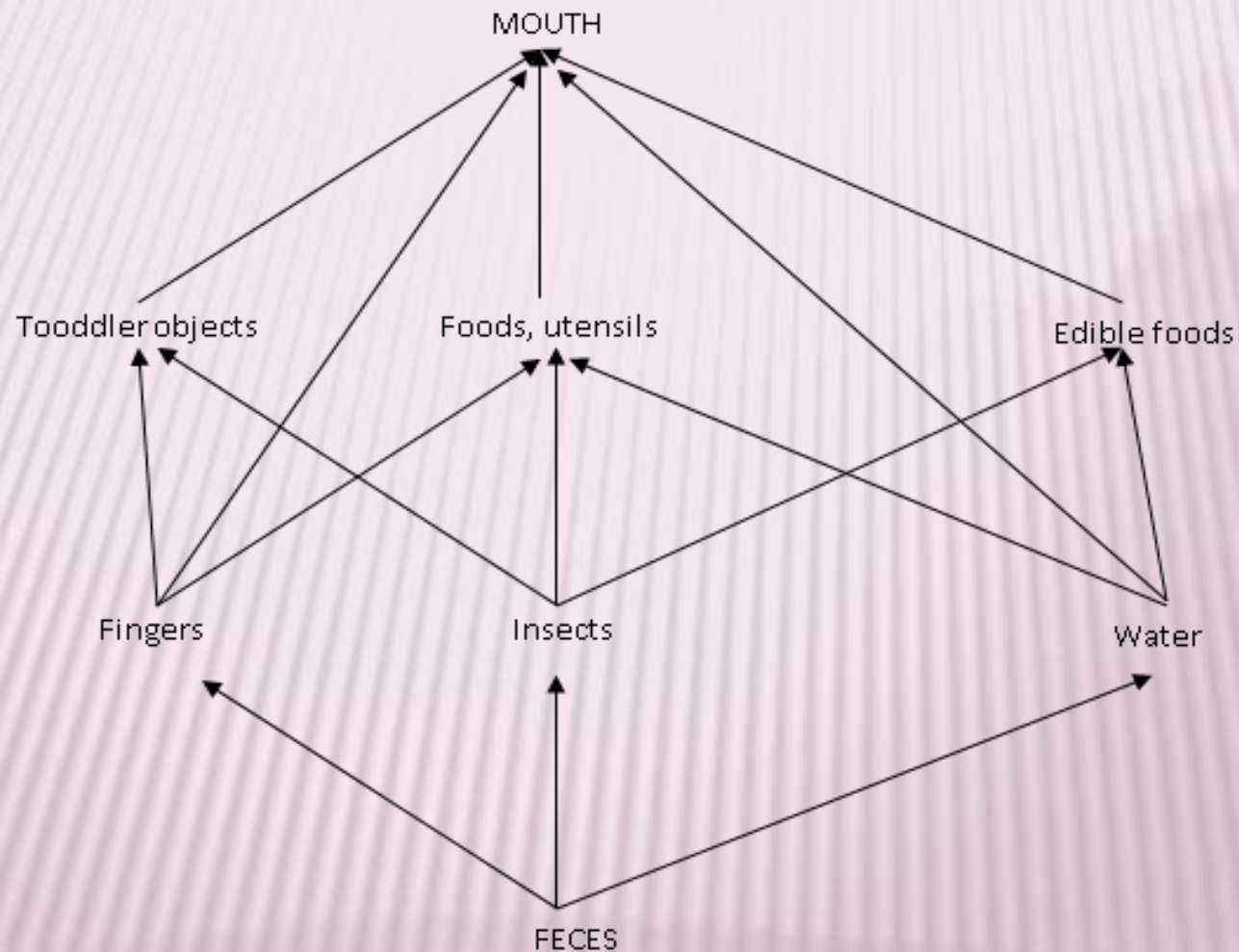
- ✖ Pathogens → MO that cause disease.
- ✖ Disease → any harmful change in the tissues and/or metabolism of a plant, animal & human that produces the symptoms of illness.
- ✖ Toxin → chemical substances produced by MO that are harmful to human tissues and physiology.
- ✖ Food poisoning → an acute (arising suddenly and of short duration) gastroenteritis caused by the ingestion of food

(source: Garbutt, 1997)

GROUPS OF FOODBORNE PATHOGENS

Flatworms	Bacteria
Flukes	Gram positive
Fasciola	Staphylococcus
Fasciolopsis	Bacillus cereus
Paragonimus	B. anhracis
Clonorchis	Clostridium botulinum
Tapeworm	C. perfringens
Diphyllobothrium	Lysteria monocytogenes
Taenia	Mycobacterium paatuberculosis
Roundworms	Gram negative
Trichinella	Salmonella
Ascaris	Shigella
Anisakis	Escherichia
Pseudoterranova	Yersinia
Toxocara	Vibrio
Protozoa	Campylobacter
Giardia	Aeromonas
Entamoeba	Brucella
Toxoplasma	Plesiomonas
Sarcocystis	Viruses
Cryptosporium	Hepatitis A
Cyclospora	Small round structured viruses (SRSVs)
Fungi – mycotoxin producers	Rotavirus
Afatoxins	Prions
Fumonisin	Creutzfeldt-Jakob disease (new variant form)
Alternaria toxins	Toxigenic Phytoplanktons
Ochratoxins	Paralytic shellfish poison
	Domoic acids
	Pfiesteria piscicida
	Ciguatoxins

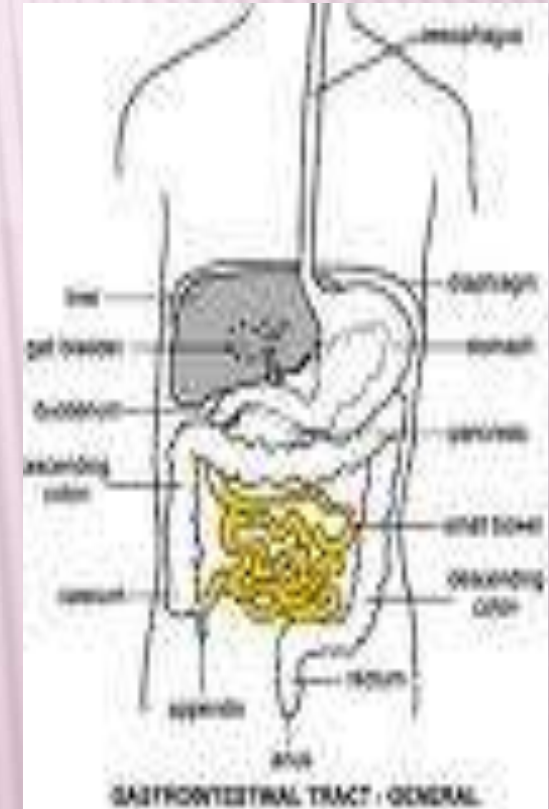
FECAL-ORAL ROUTES OF FOODBORNE INTestinal PATHOGENS



Source: Jay, 2000

PATHOGENESIS OF FOODBORNE & RELATED ORGANISM

- Skeletal muscle : *Trichinella spiralis*
- Stomach : *Helicobacter pylori*
- Liver : *Clonorchis*
- Small intestine :
Astroviruses, Bacillus cereus, Campylobacter jejuni, Clostridium perfringens, E. coli, Salmonellae, S.typhi, Vibrio cholerae, V.parahaemolyticus
- Large intestine/colon :
Campylobacter (small intestine), E.coli, Entamoeba histoytica, Salmonella eneritidis, Shigellae, especially S. dysenteridae.



PATHOGEN BACTERIA ON SEAFOOD

	Bacteria	Action model		Toxin stability	Minimum dose to infect
		infection	Toxin forming		
Indigenous bacteria	<i>Clostridium botulinum</i>		+	Low	-
	<i>Vibrio</i> sp	+			High
	<i>V. cholerae</i>	+			-
	<i>V. parahaemolyticus</i>	+			(> 10 ⁶ /g)
	<i>Aeromonas hydrophila</i>	+			NK
	<i>Plesiomonas shigelloides</i>	+			NK
	<i>Listeria monocytogenes</i>				NK
Non-indigenous bacteria	<i>Salmonella</i> sp	+			< 10 ²
	<i>Shigella</i>	+			➤10 ⁶
	<i>E. coli</i>	+			10 ¹ – 10 ²
	<i>Staphylococcus aureus</i>		+	High	10 ¹ – 10 ³

Source: Huss, 1994



Products	Preservation methods	General risk category	Main health hazard	
			Agent	Releasing factor
Raw or partially cooked shellfish	No or light heat treatment, refrigeration	High	Virus Biotoxins Vibrio spp. Salmonella typhi	Filter feeding Nature Temperature abuse, Inefficient Preservation strategy
Fresh/frozen fish	Refrigeration, MAP packing, frozen	Low	Histamine producers Marine toxins Parasites C. botulinum and indigenous pathogens	Temperature abuse, uncooked before consumption
Lightly preserved fish product	< 6% NaCl (wps), refrigeration (4– 8°C), (sorbate, benzoate, nitrite, smoke)	High	L. monocytogenes Indigenous pathogens	Poor GMP, Inefficient preservation Temperature abuse during storage
Semi-preserved fish	> 6% NaCl (wps) pH < 5; temp. <10°C (sorbate, benzoate, nitrate)	Low	C. Botulinum Histamine Producers	Poor raw material, NaCl < 10% wps, pH > 5 Poor raw material
Minimally processed seafood ('sous vide')	Mild cooking under vacuum Chill storage	High	C. botulinum L. monocytogenes S. Aureus E. coli, Salmonella spp., Vibrio spp.	Insufficient to eliminate C.botulinum post-process contamination
Pasteurized fish (e.g. hot-smoked fish)	Brined or drysalted, heat treatment (77.2– 98.8°C/1 min.), chill storage	High	C. botulinum L. monocytogenes S. aureus, E.coli, Salmonella spp.	>5C, <3% NaCl wps Post-process contamination Temperature abuse Post-process contamination
Sterilized (canned) fish	Heat treatment	Low	Histamine producing Bacteria C. botulinum	Poor raw material Post-process contamination, underprocessing

Source: Nilsson & Gram, 2002 (risk categories of seafoods)

MAJOR FOOD POISONING ORGANISMS ASSOCIATED WITH SEAFOOD ORGANISM CAUSING DISEASE (NILSSON & GRAM, 2002)

Organism causing disease	MID of toxin or live cells	Primary habitat
Bacteria of aquatic origin		
<i>Clostridium botulinum</i> type E	0.1–1 µg toxin	Ubiquitous in aquatic environment, soil, ocean sediment, intestinal tract of fish, surface of fish
Marine <i>Vibrio</i> spp		
<i>V. cholera</i>	10 ⁸ cfu/g	Estuarine and coastal warm waters (>15°C), intestines of shellfish-eating fish and tract of oysters
<i>V. parahaemolyticus</i>	10 ⁵ –10 ⁶ cfu/g	
<i>V. vulnificus</i>	Unknown	
Histamine producing bacteria	>100 mg histamine/100 g	Members of <i>Enterobacteriaceae</i> from the aquatic environment
Dinoflagellates; maybe bacteria associated with the algae	Paralytic shellfish poisoning (PSP) toxin	Aquatic environment, accumulated in bivalves (e.g. mussels, oysters)



CONTINUED:

Bacteria from the general environment		
<i>Listeria monocytogenes</i>	unknown–10 ⁸ cfu/g	Widespread in nature, soil, foilage, faeces, seafood processing environments
<i>C .botulinum</i> (mesophilic)		Widespread in soil
Bacteria from the human/animal reservoir		
<i>Shigella</i> spp.	10 ² –10 ⁵ cfu/g	Faecal polluted coastal regions or ponds; cause faecal contamination of seafood
<i>Salmonella</i> spp.	10–10 ⁶	
<i>Escherichia coli</i>	10–10 ⁸ cfu/g	
<i>Staphylococcus aureus</i>	0.14–0.19 µg toxin/kg bodyweight	Pond water, human carrier (cause postharvest contamination)
Viruses		
Hepatitis A	Living virus can infect humans	Faecal polluted water, accumulation in shellfish
Norwalk virus		
Algae		
Dinoflagellates	E.g. ciguatoxins, PSP, ASP, DSP, NSP toxins	Open waters, marine tropical waters; accumulation in shellfish (e.g. mussels, oysters)
Parasites		
	Some living parasites can infect humans	Fish and shellfish



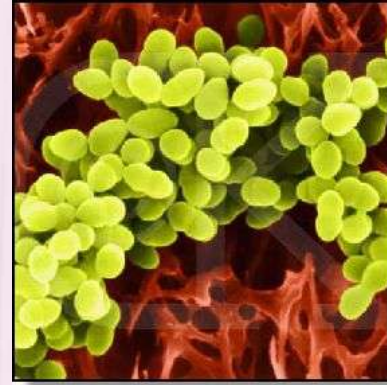
PATHOGEN BACTERIA



C. botulinum



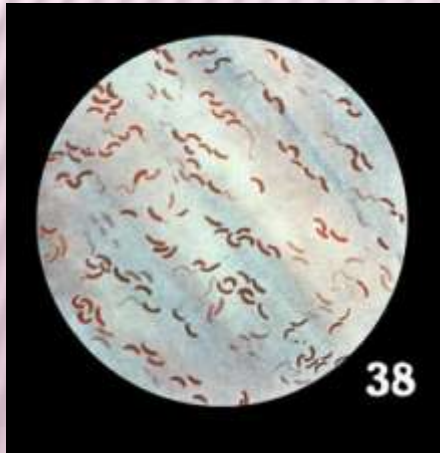
E. coli



Staphylococcus sp



Listeria sp



Vibrio sp



C. perfringens



Salmonellae sp



Shigella sp



AEROMONAS SPP

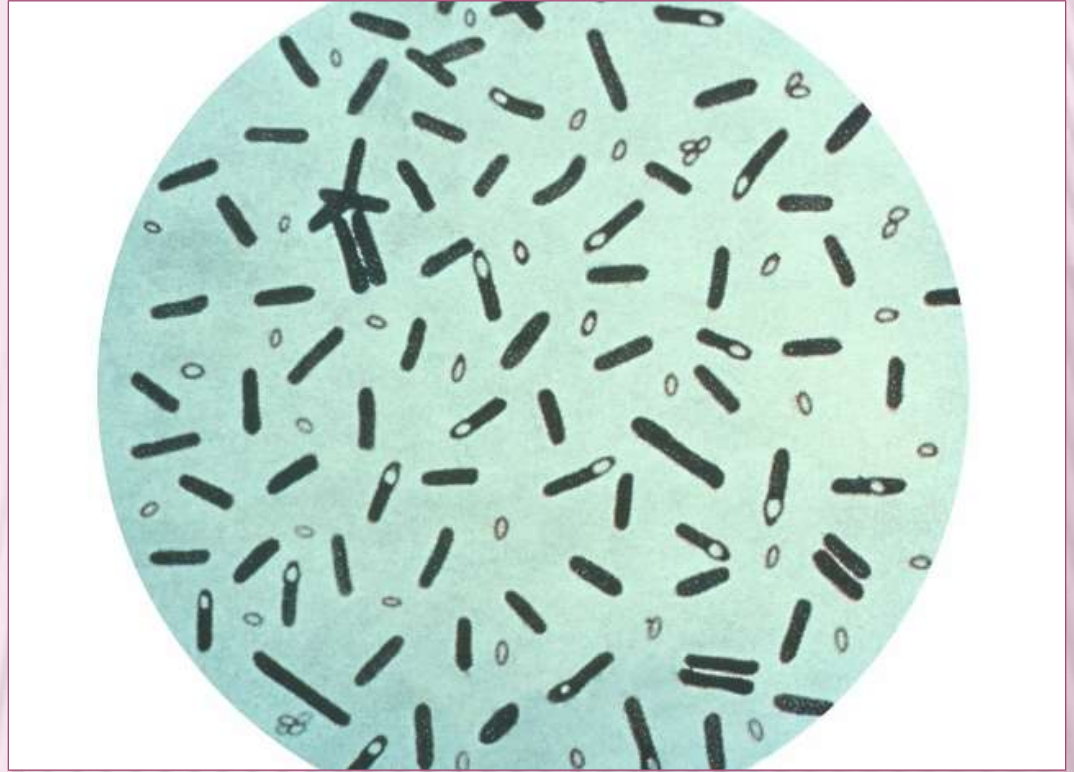
- ✖ Gram-negative rods, 0.3 - 1.0 x 1.0 - 3.5 μm
- ✖ Facultative anaerobe
- ✖ *Aeromonas* grows at temperatures of 2 - 45 °C
- ✖ The optimum growth temperature is 28 °C
- ✖ Aeromonads are sensitive to moderately high temperatures, and D-values at 45 and 51 °C have been reported as 29.5 minutes, and 2.3 minutes.
- ✖ *Aeromonas* has the potential to grow over a pH range of ca 4 – 10.
- ✖ Growth of *Aeromonas* is optimal in the presence of 1 - 2% sodium chloride (NaCl), and is sensitive to > 4.5% NaCl.

OCCURENCE IN FOODS

- ✖ *Aeromonas* species have been isolated from the following food commodities: fresh vegetables; salads; fish; seafood; raw meats including beef, lamb, pork and poultry; and raw milk.
- ✖ *A. hydrophilia* is more commonly isolated from meat, fish and poultry.

SOURCE OF AEROMONASS

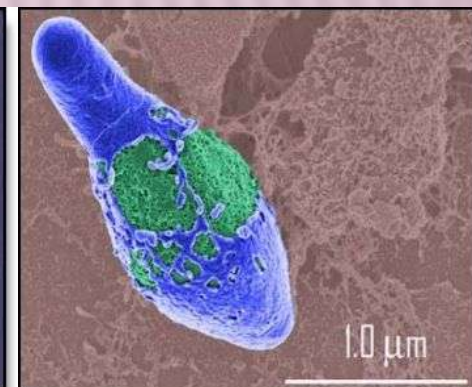
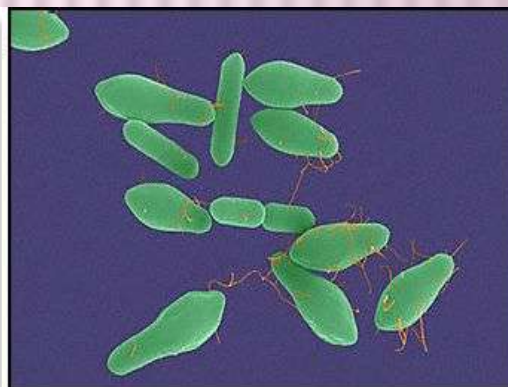
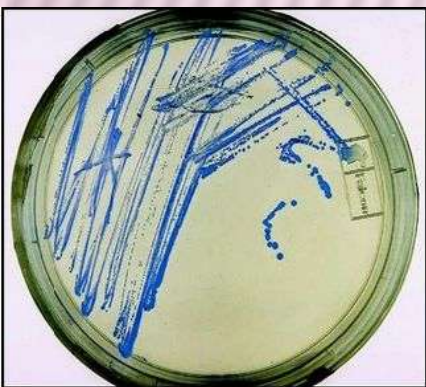
- × fresh water
- × in water supplies (including chlorinated water)
- × sewage
- × marine waters
- × estuaries
- × aquatic animals such as frogs, fish and leeches, in reptiles and in domestic animals such as pigs, sheep, poultry and cow



CLOSTRIDIUM BOTULINUM

CHARACTERISTIC OF *C. botulinum*

- × Gram positive, endospore-forming anaerobes.
- × Botulism is characterized as a rare paralytic disease caused by a nerve toxin produced by the pathogen.
- × The rod-shaped organisms grow best in low-oxygen environments.
- × Proteolytic *C. botulinum* is a highly dangerous pathogen.
- × Gram (+) spore-forming rod
- × Only serovar A, B, E & F cause botulism in human.



SUMMARY COMPARISON OF C. BOTULINUM STRAINS

Property	Serologic types						
	A	B	C	D	E	F	G
Year discovered	1904	1896	1960	1936	1960	1985	1969
Proteolytic (+), nonproteolytic (-)	+	+	-	-	+	-	+ (weak)
Group	I	I	II	II	I	II	IV
Primary habitat	Terrestrial	Terrestrial	Aquatic	Aquatic	Aquatic	Aquatic	Terrestrial
Minimum growth temp. (°C)	~10	~10	3.3	3.3	~10	3.3	~12
Maximum growth temp. (°C)	~50	~50	~45	~45	~50	~45	n.d.
Minimum pH for growth	4.7	4.7	4.7	4.8	4.8	4.8	4.8
Minimum Aw for growth	0.94	0.94	~0.97	~0.97	0.94?	~0.97	n.d.
Thermal D value for endospore	$D_{110} = 2.72 - 2.89$	$D_{110} = 1.34 - 1.37$	n.d.	$D_{110} = 0.80$	$D_{110} = 1.45 - 1.82$	$D_{110} = 0.25 - 0.84$	$D_{110} = 0.45 - 0.54$
Maximum NaCl for growth (%)	~10	~10	5 – 6	5 – 6	8 – 10	5 – 6	n.d.
Relative frequency of food outbreak	High	High	n.d.	Highest for seafoods	1 outbreak	1 outbreak	none
H ₂ S production	+	+	-	-	+	-	++
Casein hydrolysis	+	+	-	-	+	-	+
Lipase production	+	+	+	+	+	+	-
Manose fermentation	-	-	+	+	-	+	-

Source: Jay, 2000

CLOSTRIDIUM PERFRINGENS

- ✖ Gram-positive spore-forming rods; 0.3 - 1.9 x 2.0 - 10.0 µm.
- ✖ obligate anaerobe
- ✖ Its optimum temperature for growth is 43 - 45 °C, although *C. perfringens* has the potential ability to grow within the temperature range 15 - 50 °C.
- ✖ The D-value for *C. perfringens* in roast beef at 60 °C is 14.5 minutes.
- ✖ Limit growth pH values over the range $\text{pH} \leq 5$ and ≥ 8.3 , optimum 6-7.
- ✖ The lowest a_w recorded to support the growth of *C. perfringens* appears to be 0.93 and 0.97.
- ✖ Salt concentrations of 6 - 8% inhibit growth of most *C. perfringens* strains.
- ✖ 95 °C (D-values) range from 17.6 - 64.0 minutes for heat resistant spores to 1.3 - 2.8

SOURCE: *C. PERFRINGENS*

× Exist in:

- + soils,
- + water,
- + Foods products,
- + dust,
- + spices,
- + intestinal tract of humans & other animals.



LISTERIA SP

- ✖ Gram-positive short rods; 0.4 - 0.5 x 0.5 - 2.0 μm .
- ✖ Aerobe or microaerophilic
- ✖ Its optimum growth temperature, however, is between 30 and 37 °C.
- ✖ D-values for *L. monocytogenes* in crawfish tail meat at 55, 60 and 65 °C were reported as 10.23, 1.98 and 0.19 minutes.
- ✖ the organism is able to grow at pH values well below pH 5.
- ✖ *L. monocytogenes* is quite tolerant of high NaCl (10-12%)/low water activities (≥ 0.97).

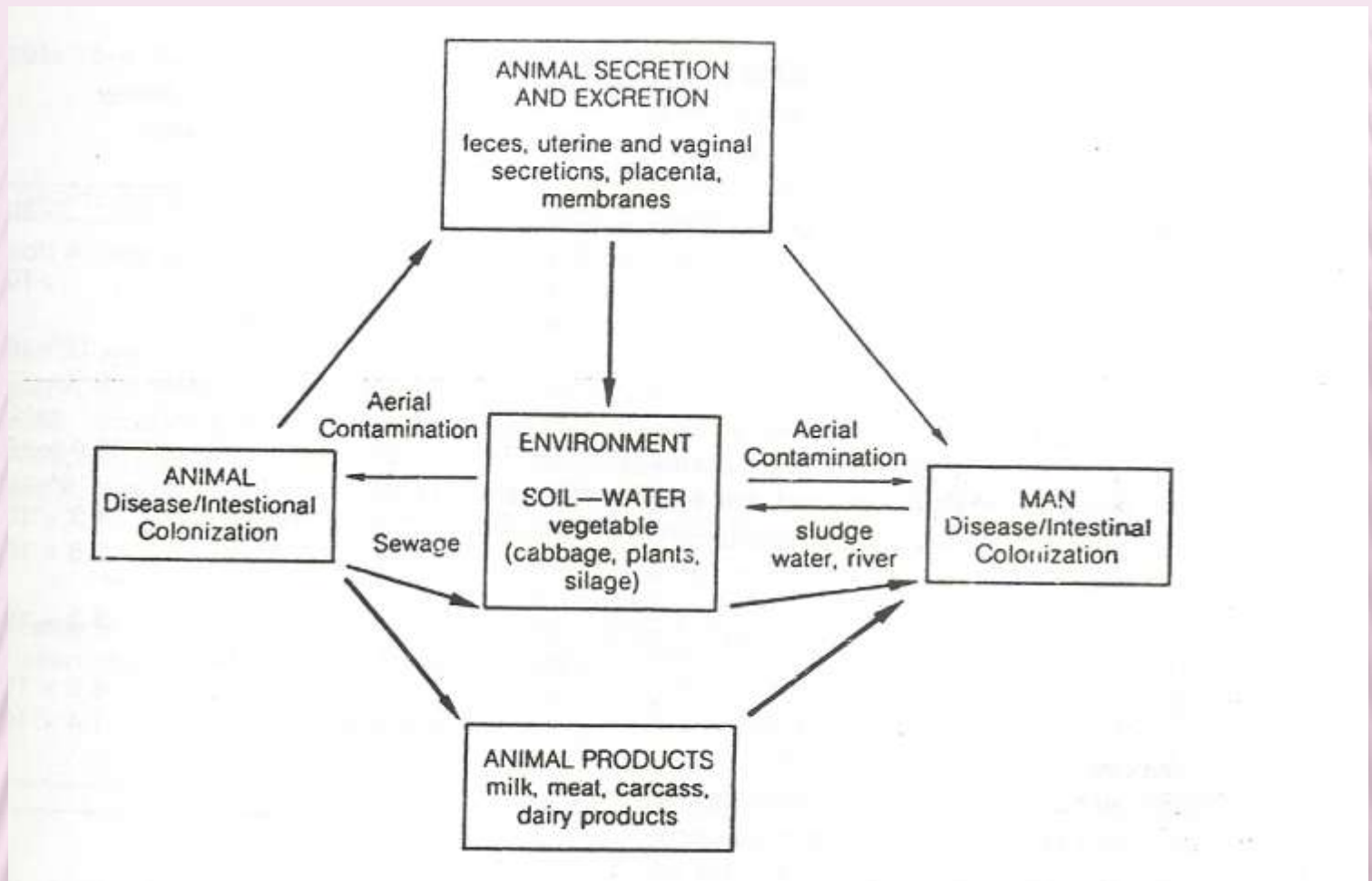
SOURCE OF LISTERIA

× Environment

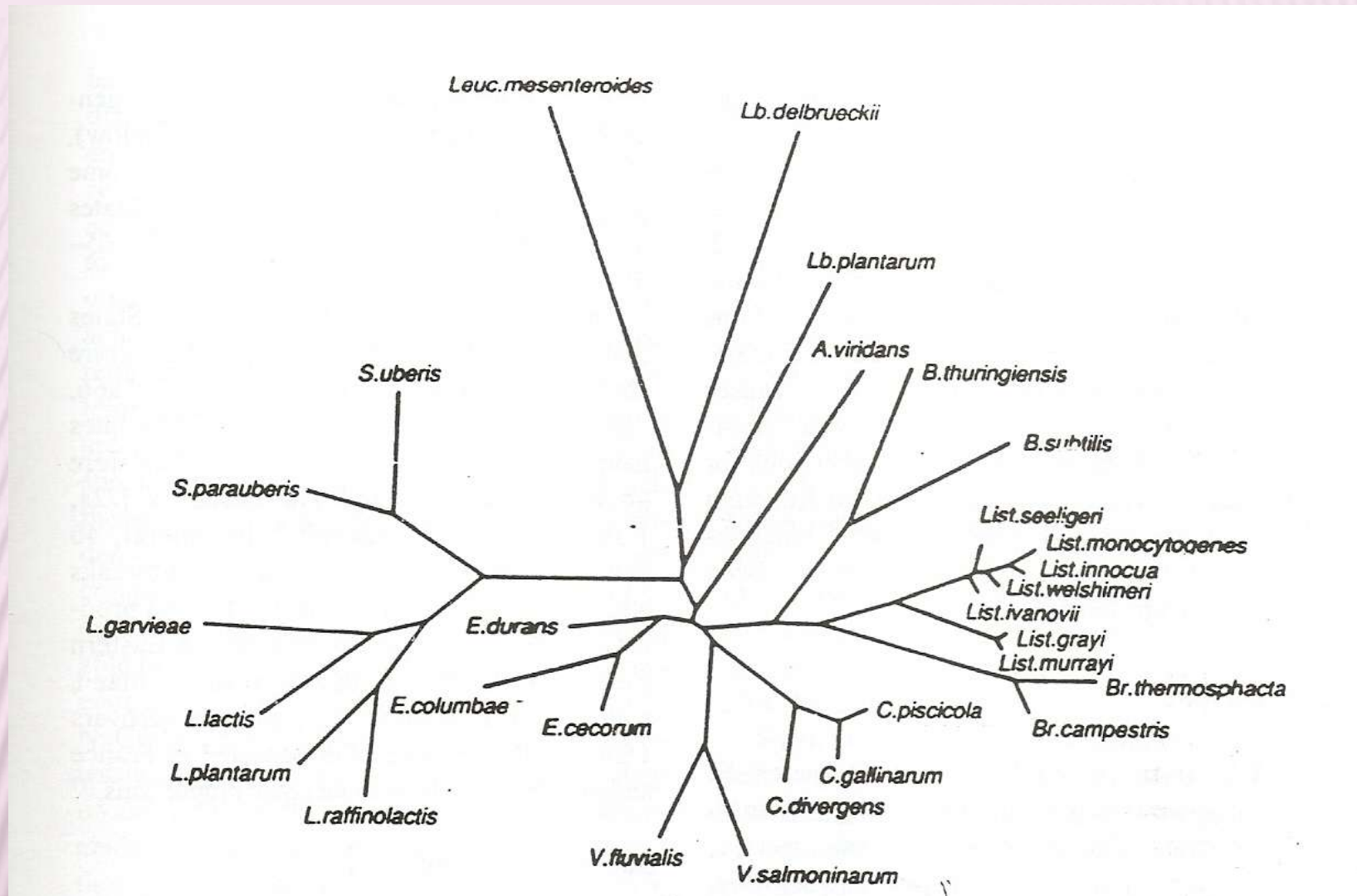
- + Decaying vegetation
- + Feces
- + Sewage
- + Silage
- + water

× Foods & human

- + Raw milk
- + Soft cheese
- + Fresh & frozen meat
- + Seafoods products (smoked fish)
- + Fruit & vegetable products
- + human



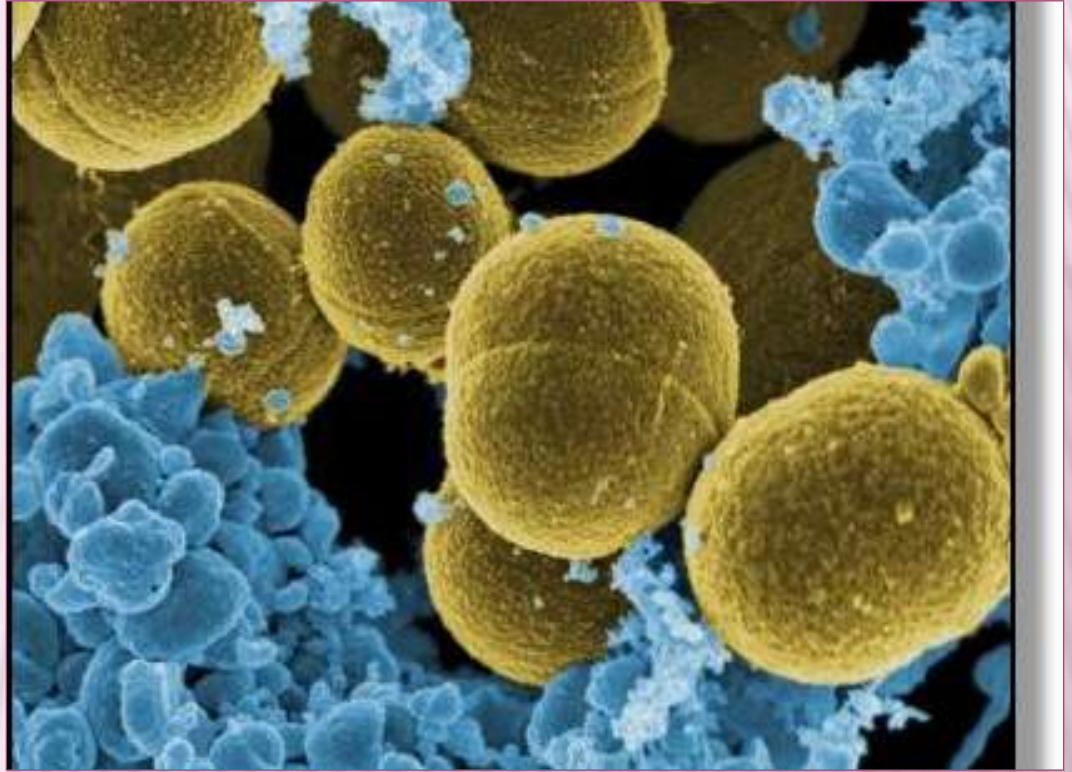
WAYS OF LISTERIA IS DISEMINATED IN ENVIRONMENT



PHYLOGENIC INTERRELATIONSHIPS OF LISTERIAE

PLESIOMONAS

- ✖ Gram-negative, 0.8 - 1.0 x 1 - 3 μm .
- ✖ Facultative anaerobe.
- ✖ *Plesiomonas* can grow in the temperature range of 8 - 45 °C, the optimum being 30 °C.
- ✖ Pasteurisation at 60 °C for 30 minutes has been reported as being effective in killing *Plesiomonas shigelloides*.
- ✖ *Plesiomonas* has a pH range for growth of pH 4.5 - 8.5.
- ✖ *Plesiomonas* can grow well at NaCl concentrations up to 4%.

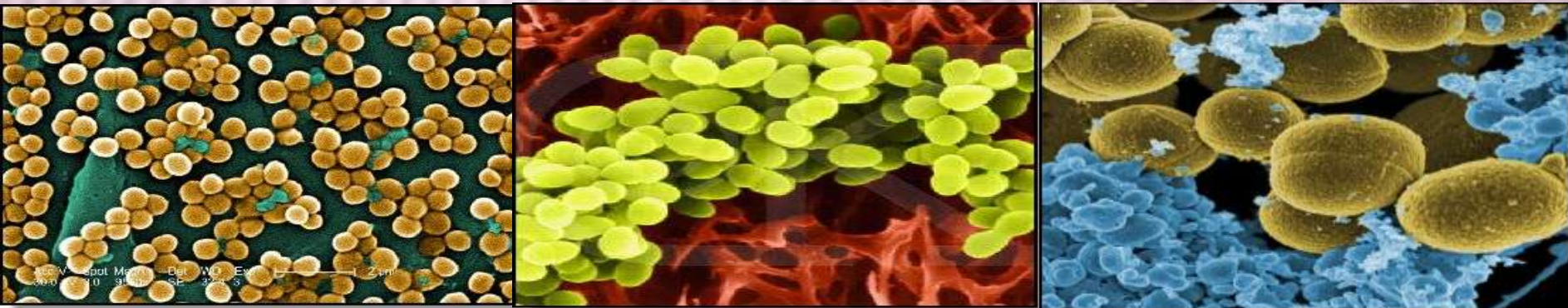


STAPHYLOCOCCUS AUREUS



CHARACTERISTIC OF THE ORGANISM

- ✗ Gram-positive cocci occurring in irregular clumps.
- ✗ Facultative anaerobe.
- ✗ Gram-positive cocci; 0.7 - 0.9 μm diameter
- ✗ Optimum growth temperature is 37°C (range of 6 to 48°C).
- ✗ Growth is inhibited in the presence of 0.1% presence acetic acid (pH 5.1) or at pH 4.8 with 5 % NaCl.
- ✗ Able to grow at Aw 0.86. it is capable to grow at Aw 0.83 in the presence of NaCl, sucrose, or glycerol humectants.



SOURCE OF MO

- ✗ Staphylococci are ubiquitous in air, dust, sewage, water, milk, & many foods & on food equipments, environmental surface, human & animals.
- ✗ 30 – 50 % of population are nasal & throat carrier.
- ✗ 15 % are skin carrier (hands esp. patients & staff in hospitals having a carrier rate 80%)
- ✗ Skin lesion esp. boils & infection of cuts & burns.
- ✗ Human faeces & clothing.
- ✗ Cows & goat.
- ✗ Food products with high protein is a good growth substrates for *S. aureus*.



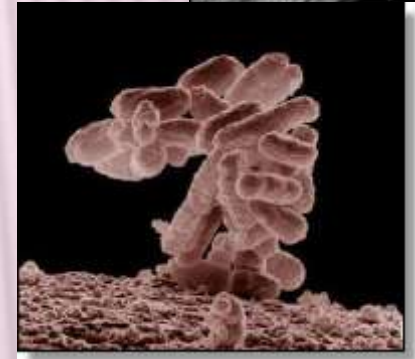


ESCHERICHIA COLI



THE CHARACTERISTIC OF ORGANISM

- × Gram negative rod.
- × Member family *Enterobacteriaceae* family.
- × Able to adapt & colonize a diverse array of environment & the gastrointestinal (GI)
- × *E. coli* bacteria are mesophilic organism
- × *E. coli* able to grow at temperature of 10-40°C with optimum tempt 37°C
- × Pathogen can replicate pH values of 4 – 10 & in the presence up t 8% NaCl.
- × Most strains of *E.coli* are not human pathogen



SOURCE OF DIARRHEAGENIC *E. coli*

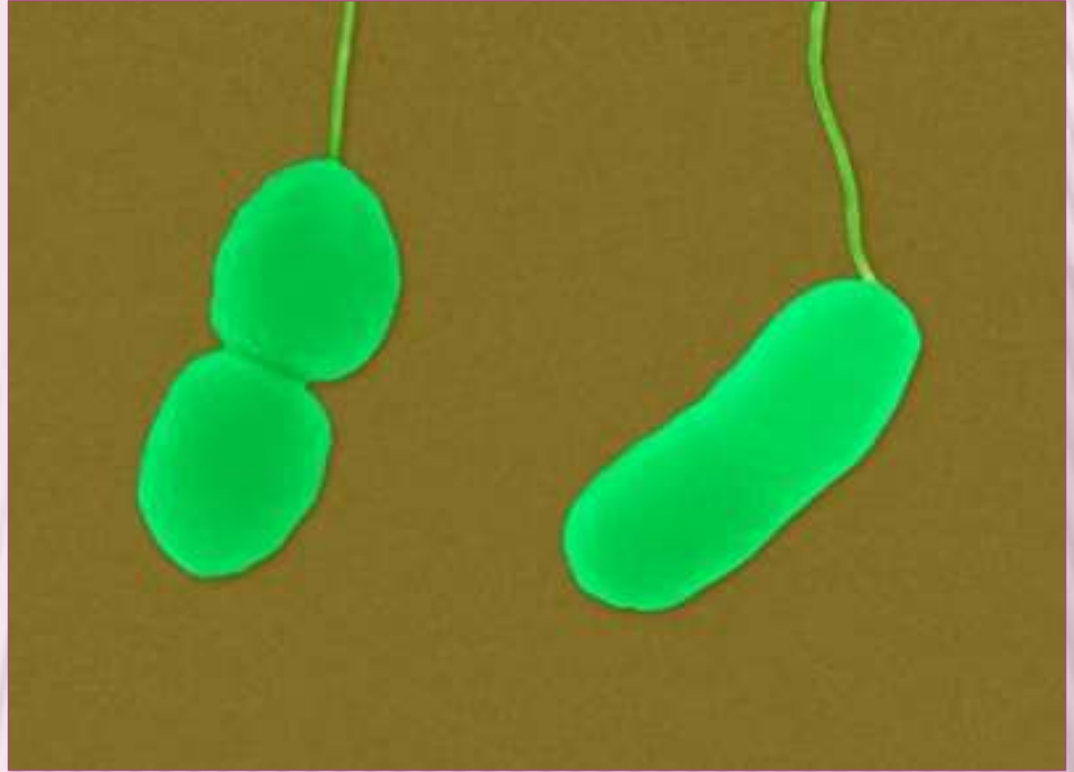
× Environment:

- + Water sources, compost, urban & rural soils & landscape, sewage, animals include beef & dairy cattle, sheep, swine, horses, rodents, dogs, horses, rodents.

× Foods:

- + Cross contamination to RM, processing water, equipments, & workers.





VIBRIO CHOLERAЕ

CHARACTERISTIC OF MICROORGANISM

- × Gram-negative curved rods; 0.5 - 0.8 x 1.4 - 2.6 μm .
- × Facultative anaerobe
- × Temperature range for growth of 10 - 43 °C.
- × The D-value for *V. cholerae* in crab meat homogenate at 60 °C is 2.65 minutes.
- × pH range for growth of pH 5 - 9.6, with optimum at 7.6.
- × a_w range 0.97 - 0.998, optimum 0.984.
- × It can grow in the presence of 0.1 - 4.0% NaCl.

VIBRIO PARAHAEMOLYTICUS

- ✖ Gram-negative curved rods.
- ✖ Facultative anaerobe.
- ✖ Range temperature 5 and 43 oC. Optimum 37oC.
- ✖ The D-values at 55 oC for clam and crab homogenate are 0.02 - 0.29 minutes.
- ✖ pH range for growth is 4.8 - 11, with an optimum pH of 7.6 - 8.6.
- ✖ Optimu Aw 0.98

VIBRIO VULNIFICUS

- × Gram-negative curved rods.
- × Facultative anaerobe.
- × temperature range for growth of 8 - 43 oC
- × it has been reported that cooking oysters for 10 minutes at 50 oC.
- × pH range for growth is 5 - 10, with an optimum pH of 7.8.
- × *V. vulnificus* grows over the range 0.5 - 5.0% NaCl, with an optimum of 2.5%.



***SALMONELLA* SP**

CHARACTERISTICS OF *SALMONELLA* SP

- ✗ Member of family *Enterobacteriaceae* .
- ✗ Gram-negative short rods; peritrichous flagella; 0.5 - 0.7 x 1.0 - 3.0 μm
- ✗ Occuring in the gut of man & animals in environment polluted with human or animals excreta.
- ✗ *Salmonella* can multiply & survive in the estuarine & freshwater envi
- ✗ Facultative anaerobe.
- ✗ Salmonellae can grow in the temperature range of 7 - 48 °C.
- ✗ $D_{60}^{\circ\text{C}}$ values normally range from about 1 to 10 minutes, with a z-value of 4 - 5 °C.
- ✗ *Salmonella* has a pH range for growth of pH 3.8 - 9.5.
- ✗ *Salmonella* has the potential to grow at a_w levels as low as 0.945

SOURCE

× Ubiquitous

- + Soil
- + Air
- + Water
- + Animals (rodents, wildlife, pets)
- + Humans
- + Food
- + Feed
- + Processing equipment
- + Some plant products



THANK YOU FOR ATTENTION