Pengantar Bioteknologi
(Introduction of Biotechnology)

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Topics

- Some applications of Biotechnology (general)
- Bioteknologi Pengembangbiakan Ikan
- Molecular Cytogenetics
- Cryopreservation-Gene Banking

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Beberapa aplikasi Bioteknologi

- Industrial Enzymes Production (Marine enzymes and their industrial and biotechnological applications)
- Application in Forensics
- Gene Therapy

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Bioteknologi pada pengembangbiakan ikan

- Reproduksi: GnRH tool
- Induksi kematangan gonad ikan melalui kontrol lingkungan;
- Induksi kematangan gonad ikan melaui hormonal
- Kontrol Fertilitas
- Poliploidisasi
- Kontrol proporsi sex pada keturunan
- Transplantasi Germ cell

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Molecular Cytogenetics

- Aplikasi dari Fluorescence *In Situ* Hybridization (FISH), *In situ* Hibridization (ISH), Immunohistochemistry untuk penelitian Akuakultur.

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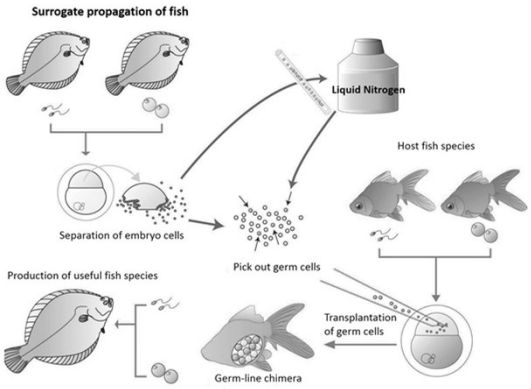
Cryopreservation -Gene Banking

- **Fish Gamete and Embryo Cryopreservation: State of the Art**



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Today's Topics

- Industrial Enzymes Production (Marine enzymes and their industrial and biotechnological applications)
- Application in Forensics
- Gene Therapy

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Introduction

Marine microorganisms are considered as a great source of biodiversity

The demand of marine enzymes is extensively and increasingly used in research and in various industries due to their diversity to survive under extreme conditions such as pH, temperature, light, atmospheric pressure, and the availability of nutrients

Despite the huge biodiversity and benefits present in marine enzymes, their biotechnology potentials remain largely unexplored

Marine enzymes can be produced and extracted from microorganisms, plants and animals using fermentation processes

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Introduction cont……

Some enzymes produced by marine microorganisms have potential application in various productions such as **pharmaceuticals**, **foods**, **textile**, **agricultural**, **chemical**, and **biomedical industries**.

- they offer new opportunities for new line of research and future biotechnological applications.
- Marine microorganisms are considered as a source of enzymes with potential interest to various commercial industries and many researchers

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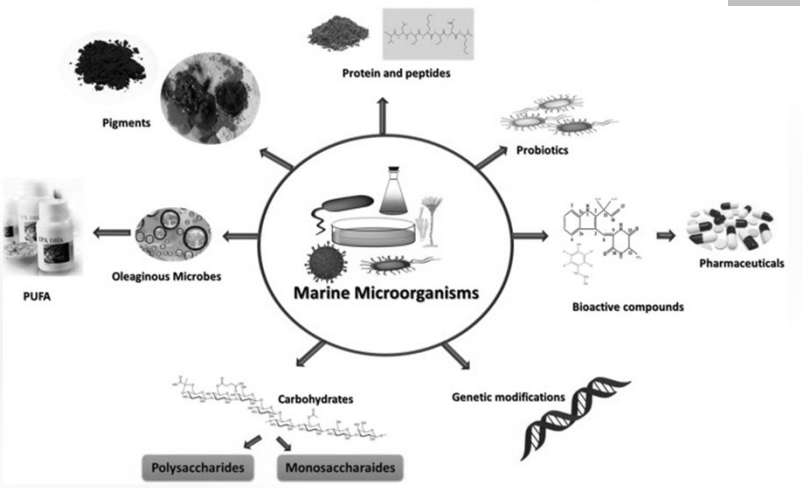
Marine Microorganisms



Marine microorganisms are considered as a great source of biodiversity (300,000 species)

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1. Industrial Enzymes Production

The use of microorganisms as a source material for enzyme production has developed for several important reasons:

- ✓ There is a high activity per unit dry weight of product.
- ✓ Seasonal fluctuations of raw materials and possible shortages due to climatic change or political upheavals do not occur.
- ✓ In microbes a wide spectrum of enzyme characteristics, as pH range and high-temperature resistance, is available for selection.
- ✓ Industrial genetics has greatly increased the possibilities for optimizing enzyme yield

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- ✓ The organism producing the enzymes should have a GRASstatus, which means that it is Generally Regarded as Safe.
- ✓ Most of the industrially used microorganisms have been genetically modified to overproduce the desired activity.
- ✓ Several thermostable enzymes, like the Taq polymerase have been identified and widely used in PCR from Thermus aquaticus .
- ✓ Cellulase is obtained from E . coli and degrades cellulose.
- ✓ Xylanase from fungus Trichoderma is used in paper industry

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- ✓ Bacterial proteases are still the most important detergent enzymes. Some products have been genetically engineered to be more stable in the hostile environment of washing machines.
- ✓ Protease and lipase containing enzyme solutions are used for lens cleaning.
- ✓ Some toothpaste contains glucoamylase and glucose oxidase.
- ✓ Also enzymes are also used for applications in skin and hair care products.
- ✓ The use of starch degrading enzymes, amylase, was the first large-scale application of microbial enzymes in food industry.

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Bioactive compounds of marine habitats products:

- Marine habitats products contain a wide range of bioactive compounds with various activities which include antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, antiviral, antitumor, and antioxidant properties

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Marine enzymes

- a large number of marine enzymes have been detected, isolated purified, and characterized for various industries such as:
- ✓ Proteases, Chitinases,
- ✓ Keratinases, Pullulanase,
- ✓ Amylases, Xylanases,
- ✓ Agarases, Lipases,
- ✓ Peroxidase, Tyrosinases,
- ✓ Polysaccharases, and Laccases.

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Marine enzymes cont…

- the characteristic of marine enzymes befits and attracts further research.
- The exploration of enzymes with novel extreme activities and improved stability, such as high salt tolerance,hyperthermostability, barophilicity, cold adaptivity, and large-scale cultivation, continues to be a priority objective in enzyme research interests to scientists. these properties may not be expected in terrestrial sources since marine organisms thrive in habitats such as hydrothermal vents, oceanic caves, and some areas where high pressure and absence of light are obvious. It is important to guide researchers towards a direct approach to explore more marine habitat especially organism that are able to produce enzymes.

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Method to produce enzymes

Fermentation is considered as the most common method used to produce enzymes from microorganisms, plants and animal sources

The important factors to be considered are:

the growth media used, type and source of microorganisms' diluents, preservatives, and stabilizers

Solid-state fermentation (SSF) processes are well established, but with low yield, high cost and generation of large amounts of wastewater; whereas, SSF is less demanding on process control, energy and aeration requirements, and it generates lower volumes of effluents

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Method to produce enzymes

several disadvantages of SSF, since it generates **excess effluents and consequently large volumes of wastewater** must be handled and disposed of during downstream processing. low water use in SSF results in low production of effluents and reduced growth of contaminating bacteria and yeasts

Alternative method is submerged fermentation (SmF) technique.

it is a technique in which the growth of microorganisms takes place in liquid medium. to produce enzymes, this process uses various parameters such as pH of medium, temperature, nutrient composition, inoculation concentration, dissolved oxygen concentration, and fermentation time

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Algae as source of enzymes

- the interest for the marine algae has increased considerably due to conditions to survive such as high salinity, heat, cold, varied light sources, osmotic pressure, and anaerobiosis
- They are considered as a source to produce cell and bioactive compounds useful in food, aquaculture, and pharmaceutical industries.
- Green, brown, red, and green algae such as **Chlorophyceae**, Chlorella vulgaris, Haematococcus pluvialis, Dunaliella salina, Saccharina japonica, Cylindrotheca, Closterium, Dunaliella salina, Chaetoceros muelleri, Polysiphonia species, Delesseria species, Adonostroma fuscum, and Porphyridium species are used as genera to produce enzymes with potential application in various industries.

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Bacteria and fungi as sources of enzymes

A summary of enzymes derived from marine fungi

TABLE I.—*A summary of enzymes derived from marine fungi.*[2, 3, 39-48]

Source	Enzyme	pH	Temperature (°C)	Time (h)	Agitation (rpm)	Enzyme activity	Application
Scopulariopsis spp.[39]	Alkaline protease	9.0	50	24	10,000	5.4 U/mg	Detergent formulations
Aspergillus awamori BTMFW032[40]	Tannase	8.0	30	24	10,000	436.72 U/mg	Useful in beer, and fruit juice clarification, to enhance antioxidant activity of green tea
Aureobasidium pullulans[3]	Protease	5.0	24.5	24	150	7200 U/L	Detergent formulations
Aureobasidium pullulans[2]	Glucoamylase	4.0	28	72	300	10.04 U/mL	Food industry
Aspergillus sp. AS58[41]	Glucosidase	5.0	45	96	120	80,000 U/L	Food, surfactant, biofuel, and agricultural industries
Cerrena unicolor[42]	Laccase	N*	30	24	N*	24,000 U/L	Detoxification of industrial effluents, paper and pulp, textile and petrochemical industries
Aspergillus niger[43]	Xylanase	4.5-8.5	28-30	168	N*	580 U/L	Biobleaching of paper pulp
Aspergillus oryzae[44]	Alginate lyase	6.5	35	72	12,000	67.24 U/mg	Biochemicals and biofuels
Plectosphaerella sp. MF-1[45]	Chitinase	3.0-4.0	37		10,000	0.01 U/mL	Polycyclic hydrocarbons
Penicillium melinii[46]	Nuclease	3.7	75	168	4226	41,250 U/mg	Manufacture of chemical
Aspergillus awamori BTMFW032[47]	Lipase	7.0	40	96	N*	495.0 U/mL	Several industrial applications
Kodamaea ohmeri BG3[48]	Phytase	5.0	65	24	7455	114.6 U/mg	Food industry

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Bacteria and fungi as sources of enzymes

A summary of enzymes derived from marine bacteria

TABLE II.—*Enzymes derived from marine bacteria.*[34, 36, 49-56]

Source	Enzyme	pH	Temperature (°C)	Time (h)	Agitation (rpm)	Enzymes activity	Application
Vibrio alginolyticus[34]	Amylase	6.0	60	2	10,000	N*	Implications in food, pharmaceutical, and chemical industry
Halophilic strain of SD11[36]	Alkaline protease	10.0	60	24	10,000	182.47 U/mg	Industrial applications
Oceanobacillus sp. PUMB02[49]	Lipase	8.0	30	24	2300	58.84 U/mL	Disruption of bacterial biofilms
Paenibacillus sp. CHE-N1[50]	Chitinase	7.0	34.3	56	300	11.8 U/mL	Hemolytic and anticancer activity
Vibrio sp. strain JT0107[51]	Agarase	8.0	25	10	600	625 U/L	Food industry pharmaceutical, and chemical industry
Saccharophagus degradans[52]	Alginases	7.6	25	36	N*	1690 alginase units	Biochemicals and biofuels
Paenibacillus sp. BME-14[53]	Endoglucanase (Cel9P)	6.5	35	2	N*	70%	Cellulase activity, cold-active mechanism, and industry applications
Rhodococcus sp. strain YM12[54]	N-acetyltransferase	8.0	35	2	12,000	60%	Herbicide detoxification by transgenic crops
Vibrio costicola[55]	L-glutaminase	7.0	25	24	N*	N*	Anticancer properties
Vibrio harveyi[56]	Protease	7.2	30	9	16,000	4.28 U/mg	Detergent formulations

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Industrial applications of enzymes from marine habitat

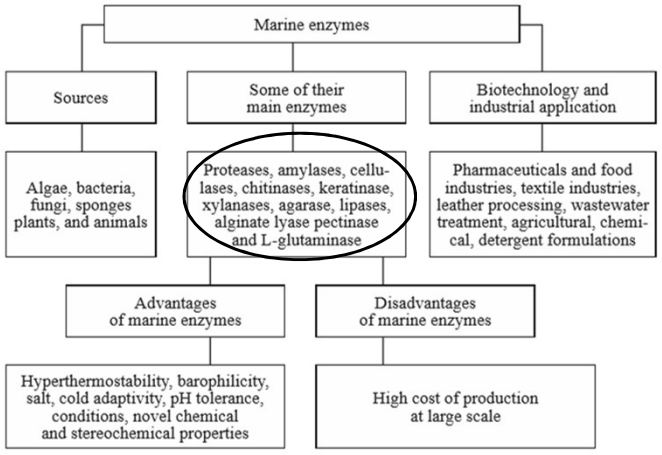

Figure 1.—Summary of potential applications marine enzymes.

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2. Application in Forensics

- ✓ Forensic science can be defined as the intersection of law and science.
- ✓ DNA profiling (also called DNA testing, DNA typing, or genetic fingerprinting) is a technique employed by forensic scientists to assist in the identification of individuals via their DNA profiles
- ✓ DNA profiling has helped to acquit or convict suspects in many of the most violent crimes, including rape and murder.
- ✓ Because DNA evidence is so specific, it is actually much easier to exclude a suspect than to convict someone based on a DNA match.

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How can DNA evidence be planted?

- ✓ Blood and seminal fluid
- ✓ Sneezing or coughing
- ✓ Person touches the mouth, nose or other part of the face.
- ✓ Hairs, fibers, or trace material from their clothing.
- ✓ Wind can carry in contaminants.

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Criminal Investigation Steps

- DNA Collection & Comparison
- PCR: Polymerase Chain Reaction
- Digestion of DNA using restriction enzymes.
- Separation of the DNA fragments by size using gel electrophoresis.
- Transfer of fragments to a nitrocellulose or nylon membrane.
- Hybridization of a probe to the fragment or fragments of interest.
- Probe detection (autorad development).

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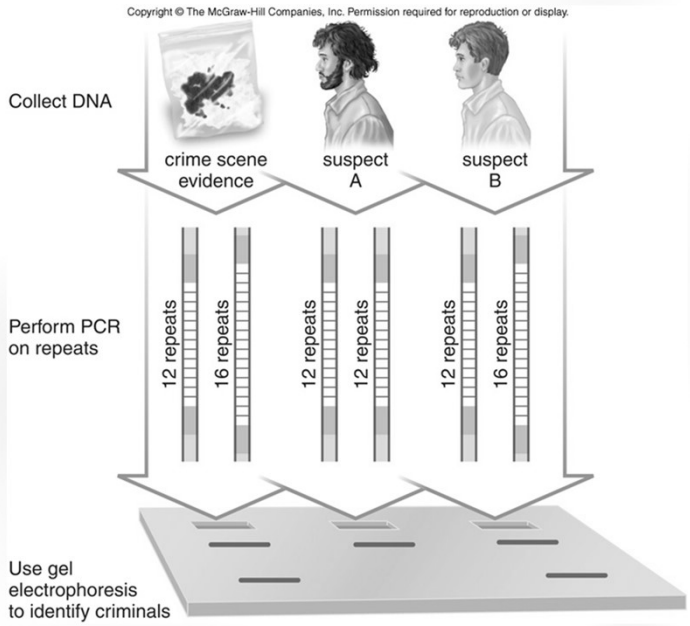

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3. Gene Therapy

Gene therapy is a process where cells are taken from the patient, alter their chromosomes by adding genes, and then replace the cells back into the patient using a carrier such as a virus.

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