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Role and Source of eDNA in fishereis and aquaculture

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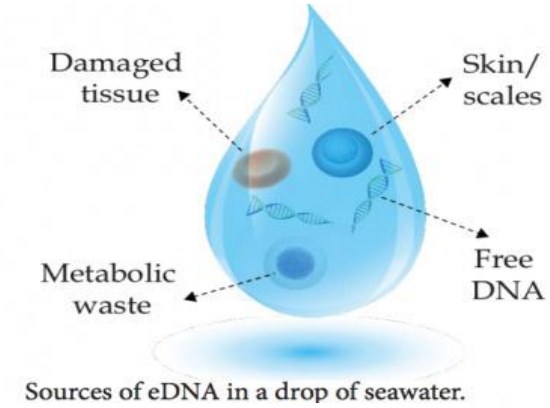
Introduction

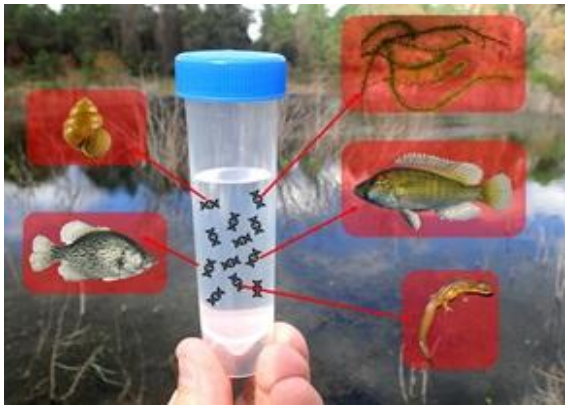
Rising Antibiotic Resistance Bacteria (ARB) are continuing to emerge as a global threat due to potential public health risk. Rapidly evolving AMR & its persistence in the environment (Salam *et al.,* 2023). Extracellular DNA (eDNA) is gaining increased attention as it can be one of the significant drivers for the transmission of extracellular ARGs (eARGs) via horizontal gene transfer (HGT) to competent environmental bacteria and diverse sources of ARGs in the environment.

Source of eDNA

eDNA is "located outside the cell and originating from intracellular DNA by active or passive extrusion mechanisms or by cell lysis. eDNA is released from dead plant or microorganisms and accumulates in human body, soil, aquatic, marine life, and sediment (Joseph *et al.,* 2022).



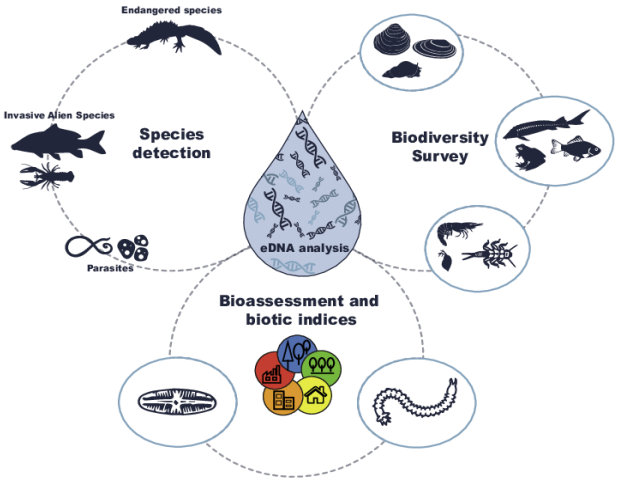
Sources of eDNA in a drop of seawater.



Functions of eDNA

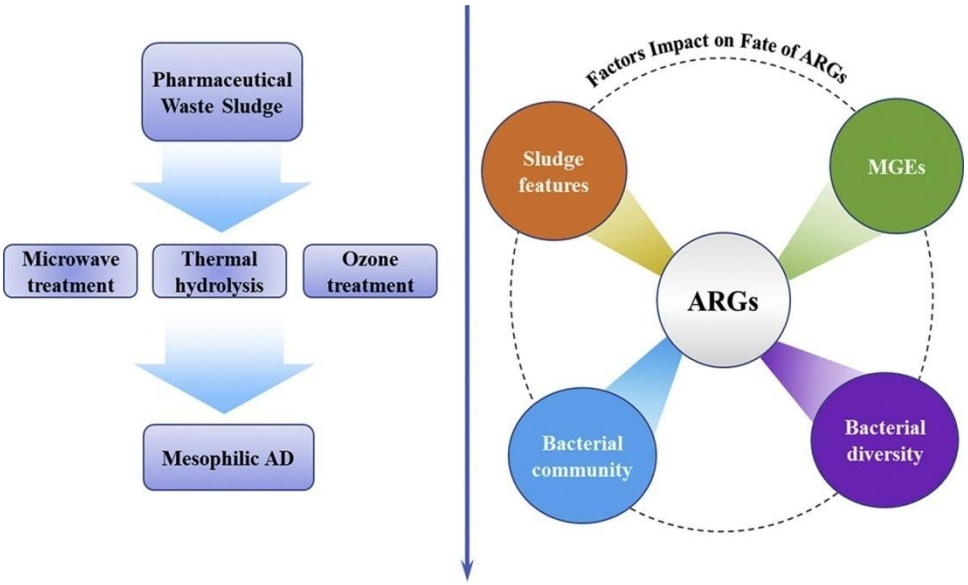
Bacteria actively release or secrete DNA, or it is released during bacterial lysis and outer membrane vesicle formation and eDNA is known to accumulate in many Gram-negative and Gram-positive bacterial biofilms (Rath *et al.,* 2025). Fate of exDNA may include biotic

degradation (mainly due to ubiquitous extracellular and cell-associated DNases) and abiotic (physical and chemical) decay, as well as environmental (vary with different environmental conditions) long-term preservation and possible incorporation by microbial cells or other living beings via horizontal gene transfer (HGT).



eDNA Persistence and natural transformation

When epithelial cells are shed or sloughed off through movement, excretion, and secretion, eDNA/eRNA is released into the environment. Their physiological stress, along with the size and number of individuals, affects the DNA production rate. When eDNA is released from cells, which may adsorbed into soil, sediments, clay minerals and humic substances and likely to be prevented from degradation by extracellular nucleases. On the other hand, eDNA that is not bound to the particle matrix can be present in the form of free–eDNA (f-eDNA) and can be degraded within several days. Moreover, the efficiency of bacterial uptake to f-eDNA is easier than adsorbed eDNA. For the physical persistence of eDNA in the environments, several mechanisms have been proposed.



Factors influence spread of ARGs in the environment

On the other hand, the efficient binding of eDNA onto clay content of organic matter, humic substances and proteins cause higher accumulation and the persistence in sediments by

reducing its decay rates and could contribute to horizontal gene transfer through natural transformation.

eDNA production and degradation in water

The link between the production and its degradation of eDNA is crucial for its transmission. When epithelial cells are shed or sloughed off through movement, excretion, and secretion eDNA/eRNA is released in the environment. The process of eDNA generation and degradation in different species and systems. A range of factors, including light, temperature, enzymatic activity, and pH, impact the breakdown eDNA and these factors' interactions and the effects they have on eDNA stability.

eDNA in Fish Disease

The ability of bacteria, archaea, and fungi cultures to release their genetic material into the extracellular medium, as well as in the context of multicellular microbial communities such as biofilms. Bacteria release their DNA in water by different methods including cell lysis and extrusion. The integrity of DNA released by cell lysis is usually more because the exonucleases cannot act fast to degrade the DNA (Bohara *et al.,* 2022).

Many environmental bacteria including Micrococcus, Acinetobacter, Bacillus, Flavobacterium, Azotobacter, Pseudomonas, and Alcaligenes release their genetic material while growing in the media. The amount of eDNA found depends on several factors (such as temperature, salinity, turbidity, and vegetation In water systems).

Transfer of antibiotic resistance from aquaculture settings

Aquaculture sites represent 'hotspots for antibiotic-resistant genes'. Bacteria harboring different genes of antibiotic resistance can grow according to environmental features, spreading genes in different sites. The aquatic environment may also contain human and animal bacterial pathogens, which act as agents in sharing genetic determinants between aquatic and terrestrial bacteria. The set of mobile genetic elements in a genome can spread among aquatic bacteria. The mobilome comprehends naked DNA, insertion sequences, insertion sequence elements with common regions, integrons mobilized by plasmids, transposons, and integrative and conjugative elements, genomic islands, transposons and conjugative transposons, conjugative and mobilizable plasmids and bacteriophages, including phage-like elements designated gene transfer agents.

Conclusion

The distribution of eDNA and its impact on environmental resistome, the source and fate in the environment largely remain overlooked. The extent to which eDNA contributes to HGT in allochthonous bacteria in the environment is still largely unknown and challenging. Despite

this, ARGs encoded in the eDNA in aquatic sediments and water can potentially provide insights concerning the diversity of eARGs for the environmental antibiotic resistome. The acquisition of clinically significant eARGs (such as ESBL and carbapenem-resistant genes) can transform environmentally harmless bacteria into pathogens and may pose a potential threat to human health. It is also noted that no investigations based from developing countries where inadequate environmental regulations contributing to intensive anthropogenic contamination represents the key to research and filling the knowledge gap considering the coordinated global action for the mitigation of environmental antibiotic resistance. There are no studies to date for the transformation of environmental extracted eDNA in natural competent cells in the influence of various biotic and abiotic stresses in more realistic conditions mimicking occurrences in natural environments. The study of the efficiency of environmental bacteria to take up eDNA via transformation will be of essential importance in future research which depends on advancements in methodological approaches. Overall, currently, novel and the most effective technology is needed to disinfect and remove ARBs and eARGs from wastewaters to control and limit the dissemination of antibiotic resistance and to maintain the ecological balance and decrease the risk to human health.

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