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Biomineralization – A Fundamental Approach for Reducing Heavy Metal Toxicity Induced by the Metagenomic Approach

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ABSTRACT: Bacteria, mainly belonging to the genus α-proteobacteria, synthesized and compartmentalized a wide range of diverse chemicals called biominerals. The molecular mechanisms behind the synthesis process of these biominerals are called biomineralization. Magnetotactic bacteria, one of the typical examples of the biomineralization process, synthesized crystals of the magnetic particles in the cell organelles called magnetosomes. This cell organelle further helps the bacteria orient themselves according to the magnetic field of the earth. These microbial communities have adapted themselves to survive in adverse environmental conditions, in this case it is heavy metal tolerance. Apart from the bacteria belonging to the genus α-proteobacteria, other organisms are also involved in the biomineralization process, such as deuterostomes, echinoderms, sea urchins (*Strongylocentrotus purpuratus*), and a few vertebrates. About 99% of microbes present in the soil are non-culturable in laboratory conditions, so these microorganisms' DNA samples are directly isolated from the soil sample through a metagenomic approach. The isolated bacterial genome contains many functional genes, and a suitable metagenomic library is prepared to screen and study various noble metabolites present in the bacteria present in the soil microenvironment.Thus, exploration and understanding of these microbes will enable us to unravel the mystery about them in biomineralization process, remediation of heavy metal and open the new avenues in the field of bioremediation. Hence, this review aims to comprehend the information about biomineralization and metagenomic approach. It discusses the potential application of metagenomics in biomineralization and bioremediation.

Keywords: Biomineralization, Metagenomics, Heavy Metal Toxicity, Magnetotactic bacteria, Humic acid contamination, Metagenomic library.

INTRODUCTION

Apart from this, two evolutionarily distinct groups of bacteria have been capable of biomineralization: 1) Several species of the cyanobacterium phyla, 2) Some Achromatium species in the Gammaproteobacterial class.Achromatism oxaliferum - a sulfur-producing bacteria, was the first bacteria found to have biomineralization properties and was describedby Schewiakoff in 1893. In nature, two pathways of the biomineralization process have been described: Specific gene-mediated biomineralization pathways in which genes, various cell structures, and various transcriptomes are involved (Dhami *et al*., 2013; Take *et al.,* 2019). The second is biologically induced mineralization, in which mineral precipitation outside the body of organisms occurs due to the chemical shift in the environment of the microorganisms (Marin *et al.*, 2004; Anbu *et al.*, 2016; Görgen *et al*., 2021). The process of biomineralization is focused not only on prokaryotes but also on eukaryotic organisms that also secrete large amounts of minerals (Vaghela and Pitroda 2019). However, the molecular mechanisms involved in that process remain a mystery among the investigators.

Apart from that, Microbial induced calcium carbonate precipitation (MICP) which is an extension of the biomineralization process, has a wide range of potential applications in various fields, and some of the applications are listed below:

1. Removal of heavy metals and radionucleotide from the groundwater
2. Sequestration of atmospheric carbon dioxide through chemical fixation of CO_2 in the form of calcite, aragonite, dolomite, and magnesite
3. Biomineralized carbonate is used in construction materials
4. Removal of calcium and polychlorinated biphenyls
5. MICCP technique has been recently reported toproduce a material used as filler in rubber and plastics, fluorescent particles in stationery ink, and a fluorescentmarker (Yoshida *et al.,* 2010).

Biomineralization is a widespread phenomenon occurring in many organisms, including marine invertebrates, echinoderms, various species of molluscs, and magnetotactic bacteria (MTB) (Lefèvre and Bazylinski, 2013). The biominerals synthesized by these organisms have the process of accumulation of

multiple minerals inside and outside of the organism. Secreted biominerals have various functions like body structure maintenance, which plays crucial roles in magnetic and gravity sensing and the storage of biominerals(Aggarwal *et al.,* 2021). The process of biomineralization is an enzyme-mediated metabolic pathway that involves a large set of genes in different organisms.

PhoK, PhoN, *Salmonella Enterica*, Urease gene of the *Sporosarcina pasteurii*, Isotig 02195, and Isotig00817 gene of *Pinctada fucata* are the names of a few genes that involve in biomineralization. Various bioinformatics tools have been employed to analyze the sequence of all these genes. Tools used are Blast, KEGG pathway database, Tremble (UniprotKB), Clustalw, and the Go annotations.Go Annotations of the uniport are used to analyze the protein transcriptomes of different cellular functions, molecular interaction, and subcellular localization. $CaCO_3$ crystal accumulation in MTB has been further analyzed using microscopic techniques like TEM and SEM(Liu et al., 2021). The sequence analysis of the genome is done by using various sequencing techniques, like the shotgun technique and next-generation sequencing technique. Both eukaryotes and prokaryotes synthesize a wide range of biominerals, and each molecular crystal has its specific function in the organisms. The functions of a few organic crystals can be summarized in Table 1.

Table 1: Enlist of different Organic Crystals and their unctions.

Name of the biominerals	Chemical Formula	Name of the organisms and relative function of the crystal structure
Calcite	$CaCO_3$	Algae/ part of the exoskeletons and eye lens
Aragonite	$CaCO_3$	Fish/ Gravity device. Mollusca/ exoskeleton
Vaterite Amorphous	$CaCO_3.nH_2O$	Ascidians/Spicules
Ca phosphate: Hydroxyapatite	$Ca_{10}(PO_4)\ (OH)_2$	Vertebrates/endoskeletons resent in the teeth, Ca store.
Octa- calcium phosphate Amorphous	$Ca_8H_2(PO_4)_6$	Vertebrate/precursor phase in the bone. Muscles/ Ca store
Calcium Oxalate Whewellite Weddellite	 $CaC_2O_4.H_2O$ $CaC_2O_4.2H_2O$	 Plants/ca store Plants/ Ca store
Group 11A metal sulfates: Gypsum Barite Celestite	 $CaSO_4$ $BaSO_4$ $SrSO_4$	Jellyfish larvae/Gravity device Algae/Gravity device Acantharia/ Cellular Support
Silicon dioxide: Silica	SiO_2	Algae/ Exoskeletons
Iron oxides: Magnetite Goethite Lepidocrocite Ferrihydrite	Fe_3O^4 alpha-FeOOH Gamma-FeOOH $5Fe_2O_3.9H_20$	Bacteria/magneto taxis Limpets/Teeth Chitions/(Molluscs) Teeth Animals and Plants /Fe Storage Proteins

Apart from the organisms mentioned above in the table, mollusks and arthropods also secrete various chemicals. Molluscs exoskeleton has almost 95% calcite or carbonate and argonauts, providing toughness and strength. All members of arthropods which make the largest phylum of the animal kingdom, also have a tough and rigid exoskeleton. This hard covering protects against harsh environmental conditions and resistance to desiccation (Colwell *et al*., 2005). Chitin, polysaccharides, structural proteins, and inorganic minerals such as carbonate and calcium (Bachmeier *et al.*, 2002). Therefore, the current eview intends to comprehend the information about biomineralization and metagenomic approach. And also discusses about the potential usage of metagenomics in biomineralization and bioremediation.

FUNDAMENTAL UNDERSTANDING OF METAGENOMICS

Metagenomics is a new emerging domain of environmental biotechnology that allows us to extract DNA from non-culturable microbes and further use it to study and express novel genes, enzymes, and its application in medicine, environmental biodegradation preparation of metagenomic libraries, and biosurfactants. In 1985, Pace and his colleagues were the first individuals who proposed the idea of cloning DNA samples directly from the environment. The DNA obtained from Environmental samples is identified as a metagenome and is widely used for discovering new species, Studying various cellular and metabolic pathways (Alves *et al.,* 2018). Multiple studies suggest that the microbial metagenomic community can act as a potential biomarker for identifyingvarious air, water, and soil pollution (Kisand *et al*., 2012). It has been observed that the accumulation of certain pollutants in the environment triggers the expression of specific microbial genes. For example, if any niche has cyclo-hexane accumulation, then it induces the expression of the cyclohexane degrading enzyme (Techtmann *et al*., 2016). Apart from this, various other bioactive compounds, bioremediation bacteria, and other enzymes with potential environmental pollution degradation activity have been identified and analyzed through a metagenomic approach. Carboxylesterase,

monooxygenase, laccases, Esterases, phenol degrading enzymes, polyaromatichydrolyzing enzymes, etc. are a few enzymes that have been discovered and analyzed through a metagenomic approach. (Ufarte *et al.,* 2015)

The gene content and organization of gene clusters in MTB bacteria have also been identified and analyzed through the environmental DNA sample and metagenomic approach applying the shotgun DNA sequencing technique.

1. The sequential steps one should follow during handling non-culturable microorganisms.
2. Selection of metagenomic samples directly from the environment containing a mix of microbes.
3. Genomic DNA Isolation
4. Construction of the metagenomic library containing a large no of short DNA sequence
5. Sequence information from these libraries
6. Identification of a metabolic pathway
7. Species identification through comparative analysis.
8. Identification of the new coding sequence
9. Genome Annotation
10. Development of a bioinformatics tool and server for storing these sequences and analyzing them.

The schematic representation of different metagenomic approaches used to understand microbial niches is illustrated in Fig. 1.

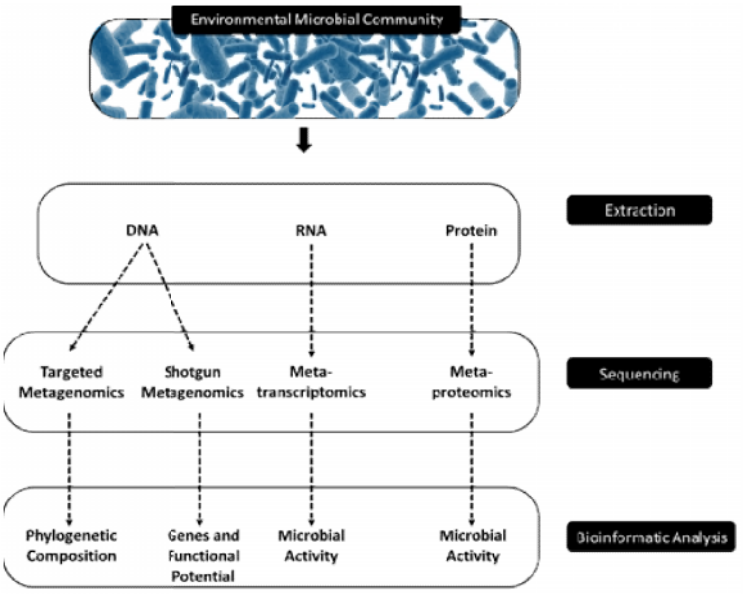

Fig. 1. Diagrammatic Illustration of Different Metagenomic Approaches used to understand the microbial niche.

LIMITATIONS OF THE METAGENOMIC APPROACH IN THE LIGHT OF BIOREMEDIATION & BIOMINERALIZATION

There are various disadvantages to using the metagenomic approach for studying genes and their synthesized enzymes. (Alves *et al.,* 2018).

In metagenomic DNA, there are fewer genes available that synthesize enzymes. And these Enzymes are not worked very well in environmental conditions, which further makes it difficult to understand their potential environmental pollution marker and their application in biomineralization and bioremediation.

Various enzymes do not show any expression in the express expression vector like *E. coli.*

CONCEPT OF THE METAGENOMIC LIBRARY

There have been many studies done in the areas of aquatic systems, soil microbiota, and plant microbiota to understand the significance of the construction of the metagenomic library and its screening of a particular gene or an enzyme. Apart from this metagenomic DNA library from the soil or aquatic microbiota have many the functional gene from the unculturable bacteria, further opening the way for discovering various novel metabolites (Daniel *et al*., 2007). 99% of the soil bacteria cannot be cultivated under laboratory conditions which further indicates microbial diversity and the Nobel functional insert in the metagenomic library (Courtois *et al.,* 2003). The construction of metagenomic libraries plays an essential role in functional genomics, allowingus to study the function of a particular gene involving DNA extraction from the soil sample, expressing the genes in the host, and further screening and checking its enzymatic activity.

GENOMIC DNA ISOLATION PROTOCOL

The reagent's requirements and their respective concentrations will differ for the different organisms. The source from which extract genomic DNA mainly includes soil bacteria, plants, fungi, and mammalian cell lines.

— Bacterial Genomic DNA isolation: 0.2% of SDS used along with lysozyme enzyme responsible for the breakdown of the peptidoglycan layer of the cell wall.

— Animal Genomic DNA isolation: Macrozymes are used as cell lysis and tissue buffer along with 0.1% of SDS detergent.

— Fungi genomic DNA isolation- 1% of SDS along with Zymolyase T, the mixture of different types of chitinase enzymes.

— Plant genomic DNA Extraction - Cellulase enzyme is used to break the cellulose component of the cell wall.

— Humic Acid Removal methods used in Metagenomic DNA extraction protocol.

Various contaminants like urea, humic acid, and polysaccharides are present in the soil with similar solubility propertiesto DNA. All these contaminants interfere with DNA isolation and analysis. Humic acids are not easily removed through classical DNA extraction protocols such as Phenol extraction. Various methods are applied to remove the humic acid from the soil sample,including microwave-based methods (Orsisni *et al*., 2001) and bead beading lysis (Miller *et al.*, 1999). Besides these methods, cesium chloride density centrifugation (Leff *et al.*, 1995) and hexadecyltrimethylammonium bromide (Cho *et al.*, 1996) also shows a very good amount of DNA on the agarose gel, which is free from the humic acid. Moreover, Aluminum sulfate is also used to remove the humic acid from the soil.

CONNECTING THE LINK BETWEEN BIOMINERALIZATION AND METAGENOMIC

The metagenomic approach has been studied that in modern microbialites the non-cyanobacterial lineage plays a crucial role in Calcium carbonate precipitation. The 16s and 18s rRNA genes collected from metagenomic samples and sequencing through Illumina suggest the presence of broad diversity of bacteria and eukaryotes and a minor presence of archaea (Couradeau *et al*., 2013). Also, the Cyanobacteria belonging to order pleurocapsales are present in abundant numbers in deeper sections of microbialites (Couradeau *et al*., 2013). The metagenomic libraries of the Magnetotactic bacteria such as *Magnetospirillum gryphiswaldense* and freshwater magneto spirillum suggest that genes controlling the Magento tactic property are located within four significant operons (mamAB, mamGFDC, mms6, and mamXY). (Fukuda *et al.*, 2006). In the recent study, deletion of the operon region of the mamGFDC and mms6 responsible for the reduction and various types of size and morphological defects of magnetite crystals, and further if anyhow the Magnetotactic bacteria get devoid of mamAB operon then it will have resulted in the loss entire magnetite crystals. (Muruat *et al.*, 2010).

HEAVY METAL TOXICITY AND BIOREMEDIATION THROUGH BIOMINERALIZATION

Heavy metals are defined as metallic elements having a high molecular weight and having a specific density that is five times greater than water (Fergusson *et al*., 1999). The excessive use of heavy metals in numerous areas such as the industry, domestic, agriculture, and medical application have created an adverse impact on human health and metabolisms and environmental implications. (Bradl *et al.*, 2000). There are various heavy metals such as copper (Cu), nickel (Ni), iron (Fe), chromium (Cr), magnesium (Mg), and Selenium (Se) are very useful and essential for numerous metabolic pathways, but exposure to some other heavy metal such as cadmium (Cd), lead (Pb), Chromium (Cr) and arsenic (As) has a very negative impact on the health of living organisms (Jen *et al*., 2017; Silva *et al.*, 2005).

After understanding the harmful effect of heavy metals on public health and the environment, removing these toxic elements from the environment becomes critical and essential. Remediation of the heavy metals from the groundwater through radionuclides in recent times has become a very effective measure to remove the various heavy metals from the groundwater. (Fujita *et al.*, 2004). Through biomineralization, the radionuclide and the contaminant metals are incorporated into the calcite lattice via a competitive co-precipitation reaction.

$$90Sr_2^{+} + OH^{-} + HCO^{-3} = SrCO_3\,(s) + H_2O$$

The calcium ions are substituted into the mineral precipitate, and cations and radionuclides integrate into the calcite structure, which further forms the strontium carbonate with low solubility in the water.

CONCLUSION AND FUTURE SCOPE

The environment demands immediate response to regulate the industrial discharge and heavy metal contamination, which requires an effective remediation approach and routine monitoring. Considering the economy and efficiency of these approaches, the implementation of metagenomics and bioremediation still imposes a challenge. The remediation of metals from soil and water with the help of microbes is an effective method as microbes have the potential to assimilate carbon, nitrogen, and phosphorus. Moreover, biodegradation is a remarkable choice for ameliorating, cleaning, and restoring the contaminated environment. Owing to their adsorption potential for heavy metal traces, microbes-mediated technique, *i.e*., metagenomics, is an ideal method for heavy metal removal. The properties of microbes like colonization, growth, and biofilm formation are used to develop new bioremediation processes. Thus, now extensive research is being done in this direction to scale up the lab-scale process to industrial-scale by maintaining the optimum condition and substrate mixture. Furthermore, with the progressive development in genetic techniques, approaches like metagenomics, metaproteomics, metatranscriptomics, and metabolomics will be used in combination to explore the potential of microbial strain, metabolites, and putative genes for bio-transforming the contaminants from the environment.

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Conflict of Interest. The authors declare no conflict of interest

REFERENCES

Aggarwal, A., Kaur, I., Kaur, V., Kaur, J., Tyagi, M., Dhanjal, D. S., Singh, R. and Chopra, C. (2021). Microbial Biomineralization: Creating Wealth from Waste. *Biological Forum – An International Journal, 13*(1): 554-566.

Alves, L. D. F., Westmann, C. A., Lovate, G. L., de Siqueira, G. M. V., Borelli, T. C., & Guazzaroni, M. E. (2018). Metagenomic approaches for understanding new concepts

in microbial science. *International Journal of Genomics*, *2018*.
Alves, L. D. F., Westmann, C. A., Lovate, G. L., de Siqueira, G. M. V., Borelli, T. C., & Guazzaroni, M. E. (2018). Metagenomic approaches for understanding new concepts in microbial science. *International Journal of Genomics*.
Anbu, P., Kang, C. H., Shin, Y. J., & So, J. S. (2016). Formations of calcium carbonate minerals by bacteria and its multiple applications. *Springerplus*, *5*(1): 1-26.
Bachmeier, K. L., Williams, A. E., Warmington, J. R., & Bang, S. S. (2002). Urease activity in microbiologically induced calcite precipitation. *Journal of Biotechnology*, *93*(2): 171-181
Bradl, H. (Ed.). (2005). *Heavy metals in the environment: origin, interaction, and remediation*. Elsevier.
Cho, J. C., Lee, D. H., Cheol, C. Y., Cho, J. C., & Kim, S. J. (1996). Direct extraction of DNA from the soil for amplification of 16S rRNA gene sequences by polymerase chain reaction. *Journal of Microbiology*, *34*(3): 229-235.
Colwell, F. S., Smith, R. W., Ferris, F. G., Reysenbach, A. L., Fujita, Y., Tyler, T. L., & Watwood, M. E. (2005). Microbially mediated subsurface calcite precipitation for removal of hazardous divalent cations: microbial activity, molecular biology, and modelling
Couradeau, E., Benzerara, K., Gérard, E., Estève, I., Moreira, D., Tavera, R., & López-García, P. (2013). Cyanobacterial calcification in modern microbialites at the submicrometer scale. *Biogeosciences*, *10*(8): 5255-5266.
Courtois, S., Cappellano, C. M., Ball, M., Francou, F. X., Normand, P., Helynck, G., & Pernodet, J. L. (2003). Recombinant environmental libraries provide access to microbial diversity for drug discovery from natural products. *Applied and environmental microbiology*, *69*(1): 49-55.
Daniel, R. (2004). The soil metagenome–is a rich resource for the discovery of novel natural products. *Current opinion in biotechnology*, *15*(3): 199-204.
Dhami, N. K., Reddy, M. S., & Mukherjee, A. (2013). Biomineralization of calcium carbonates and their engineered applications: a review. *Frontiers in microbiology*, *4*, 314.
Fergusson, J. E. (1990). *The heavy elements: chemistry, environmental impact, and health effects\Jack E. Fergusson* (No. 628.53 F4.).
Fujita, Y., Ferris, F. G., Lawson, R. D., Colwell, F. S., & Smith, R. W. (2000). Subscribed content calcium carbonate precipitation by ureolytic subsurface bacteria. *Geomicrobiology Journal*, *17*(4): 305-318.
Fujita, Y., Redden, G. D., Ingram, J. C., Cortez, M. M., Ferris, F. G., & Smith, R. W. (2004). Strontium incorporation into calcite generated by bacterial ureolysis. *Geochimica et cosmochimica Acta*, *68*(15), 3261-3270.
Fukuda, Y., Okamura, Y., Takeyama, H., & Matsunaga, T. (2006). Dynamic analysis of a genomic island in Magnetospirillum sp. strain AMB-1 reveals how magnetosome synthesis developed. *FEBS Letters*, *580*(3): 801-812.
Görgen, S., Benzerara, K., Skouri-Panet, F., Gugger, M., Chauvat, F., & Cassier-Chauvat, C. (2021). The diversity of molecular mechanisms of carbonate biomineralization by bacteria. *Discover Materials*, *1*(1): 1-20.
Jen, J. J. S., & Chen, J. (Eds.). (2017). *Food safety in China: Science, Technology, management, and regulation*. John Wiley & Sons.
Kisand, V., Valente, A., Lahm, A., Tanet, G., & Lettieri, T. (2012). Phylogenetic and Functional Metagenomic Profiling for Assessing Microbial Biodiversity in Environmental Monitoring. *PLoS ONE*, *7*(8): e43630.
Lefèvre, C. T., & Bazylinski, D. A. (2013). Ecology, diversity, and evolution of magnetotactic bacteria. *Microbiology and Molecular Biology Reviews*, *77*(3): 497-526.
Leff, L. G., Dana, J. R., McArthur, J. V., & Shimkets, L. J. (1995). Comparison of methods of DNA extraction from stream sediments. *Applied and Environmental Microbiology*, *61*(3): 1141-1143.
Liu, P., Liu, Y., Ren, X., Zhang, Z., Zhao, X., Roberts, A. P., Pan, Y., & Li, J. (2021). A novel magnetotactic alphaproteobacterium producing intracellular magnetite and calcium-bearing minerals. *Applied and Environmental Microbiology*, *87*(23): e01556-21.
Marin, F., & Luquet, G. (2004). Molluscan shell proteins. *Comptes Rendus Palevol*, *3*(6-7): 469-492.
Miller, D. N., Bryant, J. E., Madsen, E. L. & Ghiorse, W. (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Applied and environmental microbiology*, *65*(11): 4715-4724.
Murat, D., Quinlan, A., Vali, H., & Komeili, A. (2010). Comprehensive genetic dissection of the magnetosome gene island reveals the stepwise assembly of a prokaryotic organelle. *Proceedings of the National Academy of Sciences*, *107*(12): 5593-5598.
Orsini, M., & Romano-Spica, V. (2001). A microwave-based method for nucleic acid isolation from environmental samples. *Letters in applied microbiology*, *33*(1): 17-20.
Silva, A. L. O. D., Barrocas, P. R., Jacob, S. D. C., & Moreira, J. C. (2005). Dietary intake and health effects of selected toxic elements. *Brazilian journal of plant physiology*, *17*(1): 79-93.
Take, S., Igarashi, Y., Yoshitake, K., Asakawa, S., Maeyama, K., Nagai, K., Watabe, S., & Kinoshita, S. (2019). Gene expression profiles at different stages for formation of pearl sac and pearl in the pearl oyster *Pinctada fucata*. *BMC genomics*, *20*(1): 1-21.
Techtmann, S. M., & Hazen, T. C. (2016). Metagenomic applications in environmental monitoring and bioremediation. *Journal of Industrial Microbiology and Biotechnology*, *43*(10): 1345-1354.
Ufarté, L., Potocki-Veronese, G., & Laville, E. (2015). Discovery of new protein families and functions: new challenges in functional metagenomics for biotechnologies and microbial ecology. *Frontiers in microbiology*, *6*, 563.
Vaghela, K., & Pitroda, J. (2019). Microbial Mineral Precipitation to Develop the Properties of the Concrete–A Review. *International Journal on Emerging Technologies*, *10*: 335-344.
Yoshida, N., Higashimura, E., & Saeki, Y. (2010). Catalytic biomineralization of fluorescent calcite by the thermophilic bacterium Geobacillus thermoglucosidasius. *Applied and environmental microbiology*, *76*(21): 7322-7327.

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