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REVIEW ARTICLE

Unravelling the Role of Rhizospheric Plant-Microbe Synergy in Phytoremediation: A Genomic Perspective

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> Abstract: Background: Accretion of organic and inorganic contaminants in soil interferes in the food chain, thereby posing a serious threat to the ecosystem and adversely affecting crop productivity and human life. Both endophytic and rhizospheric microbial communities are responsible for the biodegradation of toxic organic compounds and have the capability to enhance the uptake of heavy metals by plants via phytoremediation approaches. The diverse set of metabolic genes encoding for the production of biosurfactants and biofilms, specific enzymes for degrading plant polymers, modification of cell surface hydrophobicity and various detoxification pathways for the organic pollutants, plays a significant role in bacterial driven bioremediation. Various genetic engineering approaches have been demonstrated to modulate the activity of specific microbial species in order to enhance their detoxification potential. Certain rhizospheric bacterial communities are genetically modified to produce specific enzymes that play a role in degrading toxic pollutants. Few studies suggest that the overexpression of extracellular enzymes secreted by plant, fungi or rhizospheric microbes can improve the degradation of specific organic pollutants in the soil. Plants and microbes dwell synergistically, where microbes draw benefit by nutrient acquisition from root exudates whereas they assist in plant growth and survival by producing certain plant growth promoting metabolites, nitrogen fixation, phosphate solubilization, auxin production, siderophore production, and inhibition or suppression of plant pathogens. Thus, the plant-microbe interaction establishes the foundation of the soil nutrient cycle as well as decreases soil toxicity by the removal of harmful pollutants.

> *Conclusion:* The perspective of integrating genetic approach with bioremediation is crucial to evaluate connexions among microbial communities, plant communities and ecosystem processes with a focus on improving phytoremediation of contaminated sites.

Keywords: Phytoremediation, plant-microbe synergy, transgenic plants, endophytes, CRISPR, pollutants.

1. INTRODUCTION

Accumulation of both organic and inorganic pollutants like polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCB), explosives, metals, metalloids and radionuclides [1-3] in soil and water resources is a global concern. These contaminants possess serious threats to the living organisms and the environment owing to their toxicity and recalcitrant nature, thus remaining in the soil for a long period [4]. They can get access to groundwater and surface water by leaching, thus contaminating water bodies. Consumption of water contaminated with toxic pollutants causes various severe anomalies in living beings. Chemical pesticides have been reported to be toxic for humans, livestock, plants, soil microbes and other insects of the crop fields [2]. For instance, the presence of hydrocarbons and heavy metals in soil adversely affects seed germination, plant growth [5-8], soil microbial population and activities [9, 10], and the ability of plants and microbes to absorb water and nutrients from soil. Furthermore, these organic contaminants even at very low concentrations can inhibit the growth activities of soil-associated microbes and upon access to the food chain, they could cause carcinogenicity in humans and animals [4, 10, 11]. Therefore, the removal of these toxic substances from the soil is critically important for sustainability in the environment [10, 12]. The traditional methods of remediating contaminated soil and water systems by chemical and physical methods are often very costintensive and destruct the natural environment. Thus, phytoremediation comes into play that is considered costeffective, solar energy-driven, eco-friendly, and in-situ technology to clean up the contaminated soil and restore the degraded soil system [13].

Microbial population dwelling in the zone of rhizosphere and endosphere is capable of aiding in the development of

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the root system of plants growing under the stressed conditions, thereby resulting in greater efficiency of plants to mitigate the pollutants from soil [14, 15]. Rhizobacteria are recognized to colonize in the proximity of the root system, while the endophytes colonize the internal tissues of the plant root or shoot systems, without causing any pathogenicity to the host plants [4]. Endophytic bacteria have received substantial consideration to amend the proficiency of phytoremediation as they have an intimate connection with the host plant species and are conspicuous plant growth promoters and hence can lead to enhanced forbearance and removal of the pollutant from the environment [4, 16, 17]. Microbial-assisted phytoremediation is sustainable green technology by which plants and their associated rhizosphere/endophytic communities work together in the reclamation of degraded soils [14, 15, 18, 19].

In order to ameliorate the competence of hyperaccumulator plants to remove pollutants from the environment, there has been an emphasized research on the genetic perspectives of manipulating the genes from plants, microbes, endophytes and rhizospheric microorganisms [18, 19]. With the expression of specific bacterial genes in the transgenic plants, pollutants like mercury (Hg) and selenium (Se) can be converted into less toxic forms. Overexpression of mammalian genes encoding cytochrome P450s in plants is responsible for enhanced metabolism and elimination of a range of organic pollutants like herbicides from the polluted soil [20]. Phytoremediation efficiency is the consequence of synergistic interactions between the plant's system and surrounding microbial diversity. This technology has gained importance as a feasible remediation alternative due to its eco- friendly nature and cost-effectiveness but there is always uncertainty associated with phytoremediation that whether full or rapid soil remediation would happen or not under dynamic field conditions [21].

2. ROLE OF RHIZOSPHERIC AND ENDOPHYTIC MICROBES IN PHYTOREMEDIATION

The most predominant rhizospheric microbial communities include Firmicutes, Proteobacteria, Actinobacteria, Bacillus, Pseudomonas and Arthrobacter [22]. It is also well reported that certain heavy metal tolerant rhizobia are capable of carrying out symbiotic nitrogen fixation. Apart from this, the symbiotic association of leguminous plants and rhizobium is popularly known for detoxification of heavy metal contaminated sites [23, 24]. Metal-microbe interaction in the rhizospheric zone is dependent upon type and concentration of metal, physicochemical parameters of soil, diversity of rhizospheric microbes and their metabolic activity [24]. Rhizospheric microorganisms residing in the close vicinity of plant roots can alter the bioavailability of metals and organic contaminants by complexation, precipitation, acidification, chelation and redox reactions. Commonly, metal bioavailability is increased by the secretion of organic acids and metal-chelating compounds, favoring the adsorption of HMs by microbial/plant biomass in the rhizosphere [24].

Rhizospheric and endophytic microbial communities can also induce biofilm formation in the vicinity of roots of host plants. Biofilm formation by the microbes enhances their ability to survive in the presence of toxic pollutants [25, 26]. Microorganisms employ various molecular mechanisms like the expression of stress-responsive genes, production of reactive oxygen species (ROS) and synthesis of certain metabolites in response to a particular type of stress to combat various abiotic stresses such as salinity, drought, extreme temperatures, heavy metal toxicity and oxidative stress [27-31]. They also secrete a slimy substance called extracellular polymeric substance (EPS) composed of carbohydrates, lipopolysaccharides, glycoproteins, and soluble proteins that possess various functional groups that help to remove/ sequester metal ions from the rhizosphere through biosorption process [25, 26]. The microbiome of the plant endosphere is significantly influenced by the particular species and genotype of the host plant [19]. It also influences the alteration of the endophytes dwelling in the stress environment of the soil. Moreover, endophytes can have more close interaction with the host plant as compared to rhizobacteria because they dwell inside the plant tissues [4, 19].

Madhaiyan et al. (2007) stated that endophytic bacterial species, Methylobacterium oryzae and Burkholderia sp. isolated from rice plants, can considerably reduce the toxicity and accumulation of Ni and Cd along with enhancement in plant growth in Lycopersicon esculentum (tomato) plants [32]. Wan et al. (2012) revealed that endophytes can also prevent metal-induced oxidative damage by modulating the activity of antioxidative enzymes and lipid peroxidation [33]. Sessitsch et al. (2012) used metagenomic analysis to find out the phytoremediation potential of certain endophytic bacteria, isolated from the roots of Oryza sativa plants (rice plants), that can degrade aromatic and aliphatic hydrocarbons [34]. Also, the genes encoding for the production of quorum sensing molecules, specific enzymes for degrading plant polymer and various detoxification pathways for the organic pollutants were identified. This metagenomic analysis decoded the information about various degradation genes that were found naturally in abundance even though the plant and endophytes were not subjected to a contaminated environment for the study [34]. This perhaps is indicative of the ability of endophytes possessing particular genes and enzymes for the breakdown of complex organic molecules which may be secreted by plant or present in the soil as organic contaminants. The endophytic microbes play key roles in reducing the phytotoxicity of toxic metal contaminants via intracellular accumulation, extracellular precipitation, metal chelation or biotransformation into less toxic forms [24, 35].

Additionally, certain reports also revealed about particular microbes that can degrade toxic organic contaminants which are recalcitrant in nature. In a study by Mitter et al. (2013), they reported the comparative genome analysis of Burkholderia phytofirmans PsJN, a natural plant growthpromoting endophyte, and eight other bacterial strains, and revealed that this strain has detoxification mechanisms for degrading hydrocarbon compounds such as phytosynthates. It possesses particular genes for alkane monooxygenase (alkB), and CYP450 alkane hydroxylase (AHs) enzymes employed in degrading particular organic contaminants [20]. Additionally, this strain PsJN possess several cell surface signaling and secretion systems that permit it to interact with a diversity of plant species which helps those plants to adapt to different stress conditions [20]. Thus, this strain plays a dual role in combating the stress as well as promoting the

growth of plant species. Apart from this, it is also wellstudied fact that endophytic bacteria that have the capability of degrading toxic pollutants can display preferential colonization in specific plant tissues [36]. It is also well reported that certain alkane-degrading root endophytic bacteria showed better colonization in the root, while shoot endophytic bacteria showed better colonization in the shoot [4, 16, 36]. It is evident from the previous reports that smaller population of these alkane degrading bacteria were observed at the initial period of plant development signifying that predominantly it is crucial to establish the bacterial population in the proximity of roots and later it plays role in the degradation of organic pollutants [4, 37]. However, a large number of pollutant-degrading endophytic bacteria were observed inside roots as compared to the shoot and leaves of the host plants [4, 38].

Other than rhizospheric and endophytic bacterial communities, the most commonly reported microbes from contaminated sites are fungi, particularly genera belonging to phyla Basidiomycota and Ascomycota. It has also been observed that soils that are nutrient deficient and heavily polluted are primarily colonized by arbuscular mycorrhizal fungi (AMF) [39]. AMF are known to inhabit under various stress conditions like extreme pH, high salinity, drought, nutrient deficiency, and presence of toxic pollutants. These fungi can develop a mutual symbiotic relationship with the root system of terrestrial plant species, and facilitate the uptake of water, nutrients, metal ions, and assist plant survival under stress conditions [40]. They are known to transform a large number of metals and alter the metal speciation, toxicity, mobility, dissolution and transformation in the soil environment [41]. Thus, by inoculating AMF in the root zone of certain hyperaccumulator plants of the importance could be a holistic approach to enhance the efficiency of phytoremediation. Another report by Yang et al. (2016) investigated the effect of AMF on phytoremediation of Pb from contaminated soil, with or without legume plants. They revealed that AMF and plant synergistic interaction influences nutrient acquisition, rate of photosynthesis and increase in plant biomass [40].

3. GENETICALLY MODIFIED MICROBES FOR BIO/ PHYTOREMEDIATION

Microbes contain a plethora of genes that can aid in detoxification of contaminants by various metabolic pathways in aerobic or anaerobic conditions. Through recombinant DNA technology (RDT), the genes for degradation or accumulation of pollutants can be expressed in microbes in order to transform the contaminants to less/nontoxic form or for their removal. RDT can be used to engineer the complete microbe, the biodegradation pathway and the enzymes that play a key role in the mineralization of any pollutant [42]. These constructed superbugs are more robust and can adapt to an extremely toxic environment and also expected to provide an environmentally friendly and economical technology for heavy metal removal [43].

Genetic engineering can be used to produce bacterial strains with improved ability to remediate the toxic environment (organic and inorganic contaminants) by a number of approaches:

- 1) Modification of catabolic pathways for bioremediation [44].
- 2) Engineering microbes for improved bioremediation [44].
- 3) Modification of genes encoding the enzymes involved in the degradation of pollutants [44].

In a study by Yang *et al.* (2010), a native soil bacterium, *Stenotrophomonas* YC-1 strain was genetically modified to produce organophosphorus hydrolase (OPH) enzyme in order to degrade a varied range of organophosphates. It was reported that a mixture of 6 organophosphate pesticides was degraded within 5 hr of activity of these engineered microbes [45].

Many PGPR are able to stimulate the growth of plants directly or indirectly by the synthesis of certain metabolites, and such PGPR possess certain consortia of genes that enable them to survive in heavy metal contaminated environments and use such metal ions as terminal electron acceptors [40]. PGPR enhances plant growth under stress conditions by producing substances such as siderophores, phytohormones, and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Also, bacterial cells can compartmentalize recalcitrant organic pollutants (or their metabolite) and heavy metal ions in their cell walls, and vacuoles, thus immobilizing the pollutants [41].

4. GENETICALLY MODIFIED PLANTS FOR PHY-TOREMEDIATION

To develop effective and economic phytoremediation plants for soil metals and pollutants, molecular biology tools are used to modify certain metal hyperaccumulator plants, their genes and tissue-specific promoters [46]. The plants that can be considered for genetic engineering approach for phytoremediation should have high biomass and should possess the intrinsic ability for phytoremediation and should be friendly for genetic transformation.

Significant progress has been made in the identification of particular genes in plant and microbial systems that encode for the specific metal ion transporters and manipulation of such genes into the desired target host genome offers an influential means to improve the capability of such plants to remediate the contaminated soils [46, 47]. The various genetic and molecular approaches have been exploited to explore a range of gene families that are involved in detoxification pathways of hyperaccumulator plants. Although there have a variety of hyperaccumulator plants that have natural metal tolerance mechanisms, there are numerous reports of transgenics being created by overexpressing the specific genes involved in the detoxification pathways or associated with the synthesis of metal-binding proteins and peptides. To improve the detoxification process by the transgenic plants, the approaches that should be envisioned include: increasing the number of uptake sites and binding sites, and alteration of the specific intracellular binding sites to reduce competitive inhibition by unwanted cations [47-49].

Some plants including *Nicotiana tobbacum*, *Populus angustifolia* and *Silene cucubalis* have been engineered to overexpress the gene responsible for glutamylcysteine synthetase, thereby enhancing heavy metal accumulation as compared with a corresponding wild type plant [46]. Over-

expression of the γ -ECS gene isolated from bacterial source that encodes for glutamylcysteine synthetase enzyme *in planta* has been known to reduce oxidative stress in plants growing in contaminated soils. This gene also enhances the tolerance and accumulation of metals and herbicides in the plant species [50-52].

Some of the popular hyperaccumulator plants for which fortification protocols are pre-established include *Brassica juncea* (Indian mustard), *Lycopersicon esculentum* (tomato), *Helianthus annuus* (sunflower), and *Liriodendron tulipifera* (yellow poplar) [48, 49]. One of the hyperaccumulator plants *Brassica juncea* was genetically modified to overexpress the desired gene *E. coli* gshII that encodes glutathione synthetase (GS) in the cytosol of the plant cell in order to increase tolerance and accumulation of cadmium (Cd) and the production of phytochelatins and its precursor glutathione. The transgenic seedlings and mature plants with the expressed gene were able to exhibit improved accumulation of Cd as compared to wild type plants and could produce increased concentrations of phytochelatins, glutathione and non-protein thiols, Ca and S [53].

Some particular genes that have been introduced in *Arabidopsis thaliana* (Brassiciaceae) include SMT (selenocysteine methytransferase), LAC1 (secretory laccase), CdPCS1 (phytochelatin synthase), AsPCS1 and GSH1 (synthases) for enhanced phytovolatilization of Se, increased resistance to 2,4,6-trichlorophenol (TCP), increased accumulation of Cd and As and enhanced phytochelatins production, respectively [54-57].

There are certain examples of transgenic hyperaccumulator plants for effective phytoremediation, as tobacco plant was transformed with *CAX-2* gene from *A. thaliana* (involved in vascular transporters) to improve the accumulation of Ca, Mn and Cd from the contaminated soil [58]; *A. thaliana* was modified with gene *Glutathione-S-transferase* from tobacco to enhance tolerance towards Al and Cu [59]; tobacco and rice plants species have been genetically engineered to overexpress *Ferretin* gene from soybean to increase iron (Fe) accumulation in plant tissues [60]; Also, *B. juncea* (Indian mustard) have been transformed with a bacterial glutathione reductase by expressing the gene in cytosol and chloroplast to make the plant tolerant to higher levels of selenium [61].

Eapen & D'Souza (2005) have also reported that *A. thaliana* engineered with *ars C* and *y-ECS* genes (from *E. coli*) can transport and reduce the oxyanion arsenate (high toxicity) to arsenite (less toxic) form, within shoot tissues, where thiol peptide complexes could sequester arsenite easily [48]. In order to achieve enhanced phytovolatilization of elemental Hg, *MerA* and *MerB* genes have been expressed in certain hyperaccumulator plants which eventually increased mercury tolerance and phytovolatilization potential by several folds [62, 63]. Also, overexpression of the ACC (1-aminocyclopropane-1-carboxylic acid) deaminase gene can lead to an enhanced accumulation of several heavy metals [48, 64].

5. GENOMIC PERSPECTIVE FOR ENHANCEMENT OF PHYTOREMEDIATION

Several plants have been characterized as phytoremediator plants for several pollutants and the genomes of these plants are considered as models since they have been partially or fully sequenced. These model phytoremediators include *Thalspi caerulscens*, now called as *Noccaea caerulescens* (Zn, Ni, and Cd hyperaccumulator), *Pteris vittata* (As hyperaccumulator), *Arabidopsis halleri* (Cd and Zn hyperaccumulator, rhizostimulation of PAHs), *Brassica juncea* (capable of detoxifying Pb, Cd, Ni, As, rhizostimulation of organosphosphates) and some other plants species [65-67]. The knowledge of genomic sequences can be used to identify and characterize the key genetic determinants important for the phytoremediation processes like phytostabilisation, phytotransformation, phytoextraction, phytovolatilization, *etc.*

One such important and revolutionary technology of genome engineering is CRISPR (clustered regularly interspaced short palindromic repeats). This tool has the potential to enhance the targeted traits in plants. CRISPR technology could be used to transfer a target set of information which is desired to be integrated into the plant genome in a candid mode [68-70]. Moreover, two variant technologies of this ground-breaking genome editing tool, i.e. CRISPR-Cas9 and CRISPR- Cpf1 can be employed for generating knock-outs plants (removing a particular target gene from the plant genome), targeting transcriptional regulation or making genesubstitutions in the particular plant genome of interest [70, 71]. CRISPR systems can be established for enhancing phytoremediation by targeted engineering of mechanisms involved in the complexation, accumulation, volatilization and degradation of pollutants [69] (Fig. 1).

The main focus for enhanced phytoremediation of the contaminated soil is the expression of desired genes (through CRISPR) in order to increase the synthesis of metal-binding proteins such as metallothioneins (MT) and phytochelatins (PC), metal transporter proteins (from the MATE, CDF, HMA, ZIP and YSL families), plant growth hormones (like auxins, cytokinins, gibbrellic acids), and root exudates [71]. Several studies since the early 2000s have revealed that particular plant and bacterial genes when integrated with the target plant genomes could enhance the phytoremediation ability. For example, expression of NAS1 gene (responsible for encoding the enzyme Nicotianamine synthase-1) in tobacco and Arabidopsis thaliana plants resulted in enhanced metal tolerance for Ni, Cd, Cu, Zn, Mn and Fe [72, 73]. Similarly, the overexpression of MT encoding genes like MT1, MT2 and MTA1 in tobacco, Arabidopsis and poplar plants have enhanced the ability to accumulate heavy metals like Cd, Cu and Zn [69, 74]. The gene MT2b is also involved in the up-regulation and down-regulation of genes associated with abscisic acid synthesis and is well reported to increase the accumulation of Pb by H. incana plant [69, 75, 76]. Apart from the role of a genomic perspective in phytoremediation by plants, the CRISPR technology can be applicable for competent PGPR microbes as well [77]. Various literature studies have been reported on the genes responsible for the synthesis of particular plant hormones [78], phosphate solubilization, Siderophore production [79], free nitrogen fixation, etc. [80], therefore manipulating these PGPR strains with these genes of interest could beneficially affect the ability of plant-microbe synergy to combat the abiotic stress of the pollutants (Table 1). Altogether the up-gradation in the



Fig. (1). The genomic perspective employing CRISPR technology in plant-microbe synergy for enhanced phytoremediation. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

S. No.	Plants	Synergistic Microorganisms	Target Pollutant	Enhanced Effect on Phytoremediation	Refs.
1.	Poplar	Pseudomonas putida W619- TCE	TCE	Rapid TCE degradation	[4, 15]
2.	Yellow Lupine	Bacillus cepacia VM1468	TCE, Ni	Enhances degradation rate for TCE and increases Ni resistance in plants	[4, 15]
3.	Triticum aestivum (Wheat)	Enterobacter sp. 12J1 (endo- phyte)	Pyrene	Degrades pyrene and promotes plant growth by IAA and Siderophore produc- tion	[4]
4.	Lycopersicon esculen- tum (tomato)	Methylobacterium oryzae, Burkholderia sp.	Ni, Cd	Increases accumulation of Ni and Cd and simultaneously promotes plant growth	[32]
5.	Populus deltoids	Agrobacterium radiobacter	As	Increases phytoremediation and pro- motes plant growth by IAA and Sidero- phore production	[42]
6.	Zea mays (Maize)	Microbacterium sp., Exophiala pisciphila	Cr (VI)	Enhances phytoremediation and pro- motes plant growth, Reduces Cd phytotoxicity	[42, 30]
7.	Spiruline platensis	<i>Burkholderia</i> sp. D54	Pb, Cd, Zn, Cu	Increased uptake of metals and in- creased production of IAA, Siderophore, ACC deaminase, phosphate solubiliza- tion.	[43]

Table 1.	Plant-microbe synergy	for phytore	mediation of o	rganic and	inorganic pollutant
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S. No.	Plants	Synergistic Microorganisms	Target Pollutant	Enhanced Effect on Phytoremediation	Refs.
8.	Pteris vittata	Pseudomonas, Stenotrophomo- nas	As	Enhanced phytoremediation, Sidero- phore production	[44]
9.	Oryzae sativa (Rice)	Bacillus aryabhattai, Pseudo- monas stutzeri A15	As	Alleviates As toxicity in rice, PGP properties	[45, 30]
10.	Helianthus annusvar (Sunflower)	Pseudomonas fluoroscens	Pb	Increases phytohormone production, Siderophore and phosphate solubiliza- tion	[81]
11.	<i>P. trichocarpa</i> (Hybrid cottonwood)	Pseudomonas sp.	2, 4-dichloropheno- xyacetic acid, toluene, naphthalene	Enhances phytoremediation	[81]
12.	Pisum sativum (Pea) plants	Pseudomonas putida VM1441	Naphthalene (NAH)	40% higher degradation rate for NAH (a probable carcinogen)	[81]

plant-microbe synergy *via* genomic approaches would take phytoremediation to the higher levels, allowing the effective reclamation of the degraded natural soil systems in stipulated time frames.

CONCLUSION

The opportunities for exploiting plant-microbe synergy for remediation and detoxification of contaminated environment are numerous and diversified. The use of hyperaccumulators and other plants, along with their correlated rhizospheric microbiota and endophytes has an amplitude of potential to degrade toxic contaminants and enhance the uptake potential as well. From the genomic perspective, various approaches could be administered to produce transgenic plants and associated microbes that can degrade toxic pollutants like pesticides, polyaromatic hydrocarbons, and persistent organic pollutants or uptake heavy metals and radionuclides effectively, within a stipulated time frame. However, there are extensive possibilities for manipulation of the rhizospheric environment by engineering the plant metabolites as well as bacterial response mechanisms, as the majority of the plant-microbe interactions in this zone are complex, dynamic and influenced by a number of factors. The precedence of the CRISPR technology (targeting plants and PGPR) are vast and unexplored in the field of phytoremediation. In a similar way, the impediment associated with these genomic approaches to produce transgenic for phytoremediation should be assessed by conducting field trials to know their ecological impact. Hence, the advancement in the role of plant-microbe synergy would ameliorate the phytoremediation prospects as well as result in efficacious reclamation of the deteriorated soil and water systems.

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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