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Cell free supernatant for sustainable crop production

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The increase in demand for food production due to the ever-increasing human population across the world requires that food production should grow exponentially. For agricultural food production to meet the needs of human requirements and demands there is a need for sustainable practices that will ensure production and availability of food without affecting soil health, soil biota and soil fertility. Over the years, many plant growth promoting bacteria (PGPB) strains have been identified and reported to provide a number of benefits to plants, including enhanced nutrient uptake, growth, and development as well as increased resistance to biotic and abiotic stress. However only a small number of them, are sold today, mostly due to the formulations' inability to support bacterial survival both during and after application in agroecosystems. PGPB strains that present these difficult constraints can be employed in the production of cell-free supernatants (CFSs), which are broth cultures that have undergone various mechanical and physical procedures to eliminate cells. The available literature suggests that CFS may be a reliable source of secondary metabolites for sustainable agriculture. This review therefore discusses cell free supernatant of various soil microorganisms that have been used in crop production and offered pertinent information about CFS for upcoming studies on CFSs as bio stimulant and biocontrol agents in sustainable agriculture. The significance, sources, applications, mechanisms of action of CFS and benefits of studies on CFS agricultural applications—both as a bio fertilizer and a biocontrol agent were studied.

KEYWORDS

agrobiotechnology, biofertilizers, bioinoculants, bioremediation, sustainable food production

Introduction

Conventional agriculture employed to increase agricultural production and meet the food demand of the ever-increasing human population heavily relies on excessive use of agrochemicals such as fertilizers, herbicides and pesticides (Li et al., 2022). However, this agricultural system, although increasing production, has proven unsustainable in the long run. Further nutrient input for instance, can result in a decline in soil fertility (Kopittke et al., 2019). The excessive and inappropriate use of agrochemicals pollute soil, air and water through nutrient leaching, causing a chemical imbalance across multiple ecosystems (Chandini et al., 2019). These chemical fertilizers and pesticides can also lead to soil acidification, greenhouse gas emissions, negative impacts on human health and harm to non-target organisms resulting in a loss of biodiversity (Li et al., 2020). Chemical fertilizers have considerable deleterious effects on soil, plants, humans and environmental sustainability increasing the costs and reducing profitability (Alori et al., 2019).

Therefore, there is a need for a more eco-friendly and sustainable methods that may be practiced without health hazards. The use of microbial inoculants holds great promise to improve crop yield without negative environmental and health risks (Alori et al., 2019). However, under harsh environmental condition, the impact of microbial inoculant diminishes (Naamala et al., 2023).

Cell-free supernatant is a liquid fraction obtained from microorganism cultures after centrifugation. It contains various plant growth-promoting compounds, enzymes and secondary metabolites such as antibiotics (Naamala et al., 2023). Microbial cell-free supernatant (CFS) has been reported to be associated with the strong proliferation of resident beneficial soil microbes that activate beneficial soil microorganisms; therefore, it is a safe, efficient and environmentally friendly approach to minimize shortfalls related to the technology of microbial inoculation (Morcillo et al., 2020).

The use of cell-free supernatant in sustainable crop production has garnered attention for its potential to promote plant growth, increase yield, and reduce the negative impact of chemical fertilizers and pesticides on the environment. The compounds are less likely to experience diminished effects under harsh environmental conditions. Additionally, they are generally required in low concentrations and are easier to store compared to live microbial cells (Naamala et al., 2022).

Cell-free supernatants can be extracted via several methods depending on the purpose of use of the supernatant. These methods include centrifugation and filtration that are appropriate for general applications (Subramanian et al., 2021; Zhou et al., 2022), solvent extraction and precipitation are useful for isolating specific metabolites (Gudiña et al., 2015; Santoyo et al., 2021), and Lyophilization method which is preferred for long-term storage (Sornsenee et al., 2021).

Extracted CFS has been stored by several researchers using various techniques. The following methods have reported. Refrigeration (4°C) Storage (Koohestani et al., 2018). Freezing (−20°C to −80°C) (Gunal Köroglu et al., 2024). Lyophilization (Freeze-Drying) Storage Technique: Šuchová et al. (2022) Spray Drying (Gullifa et al., 2023). Storage in Preservative Solutions (Hamad et al., 2022). Encapsulation Technique (Bassani et al., 2019).

Several researchers have reported the use of CFS in medicine and food. For example, Arrioja-Bretón et al. (2020), investigated the antimicrobial activity and storage stability of CFS from lactic acid bacteria and its applications with fresh beef. Mani-López et al. (2022) studied CFS production, composition, and the antimicrobial activity of CFS from LAB *in vitro*, on foods, and in active packaging. Mao et al. (2023) explored the impact of CFS of lactic acid bacteria on *Staphylococcus aureus* biofilm and its metabolites.

In agriculture, Wang et al. (2018) examined the application and mechanism of Bacillus subtilis in biocontrol of plant disease. Khamsuk et al. (2024) investigated the anti-microbial activities of CFS of plant growth promoting bacteria from rhizospheric soil of rice plant. Li et al. (2023), studied if the application of Bacillus subtilis promotes growth and quality of cucumber. Pellegrini et al. (2020) reviewed the application of CFS from plant growth-promoting bacteria in sustainable agriculture.

However, there is limited information on the comprehensive application of microbial CFS (from both bacteria and fungi) in agriculture and environmental remediation. This research aims to discuss the application of microbial CFS to various soil microorganisms for crop growth and yield improvement, crop protection, and crop response to abiotic stressors including the remediation of polluted soils. It emphasizes the significance of CFS in sustainable crop production. Sources of CFS, mechanisms of action, challenges and limitations were thoroughly explained.

Significance of cell-free supernatant in sustainable crop production

Cell-Free Supernatants are significantly important in sustainable crop production as follows:

Improving soil health

Cell-free supernatant contains various secondary metabolites, including organic acids, enzymes, and siderophores, which can enhance soil health. These compounds can stimulate the growth of beneficial microorganisms such as mycorrhizal fungi, rhizobia, and other nitrogen-fixing bacteria, ultimately improving soil fertility (Pellegrini et al., 2020). Microbial CFS supports the proliferation of resident beneficial soil microbes (Morcillo et al., 2022).

Enhancing plant growth and yield

Cell-free supernatant contains various plant growth-promoting compounds, including indole-3-acetic acid (IAA), gibberellins, cytokinins, and auxins. These compounds can enhance plant growth by promoting cell division, elongation, and differentiation (Naamala et al., 2023). Additionally, cell-free supernatant can improve nutrient uptake by plants by increasing the solubility and availability of essential minerals such as nitrogen, phosphorus, and potassium (Islam et al., 2016). *Bacillus subtilis* and *Bacillus licheniformis* can enhance plant growth and biomass production through the synthesis of various phytohormones, nitrogen fixation, phosphate solubilization, and ammonium ion production (Khan et al., 2018). Moreover, microbial CFS can suppress the growth of plant pathogens, thereby reducing the need for chemical pesticides (Islam et al., 2016). Plants treated with CFS exhibited a higher fruit yield than control plants (Imran et al., 2023).

Sources of cell-free supernatants

Cell free supernatants as shown in Figure 1 are obtained from bacteria, fungi, plant tissue, and animal tissue cultures. Bacterial cultures are the most common source of cell-free supernatant. Bacteria produce various secondary metabolites such as antibiotics, enzymes, and plant growth-promoting compounds, which are present in the cell-free supernatant (Kumar et al., 2020).

Fungal cultures are another source of cell-free supernatants. Fungi produce various secondary metabolites such as mycotoxins, antibiotics, and enzymes, which are present in the cell-free supernatant (Dozolme and Moukha, 2020; Nasrollahzadeh et al., 2022). These compounds are secreted by these microorganisms into the surrounding environment. Plant tissue cultures are also a potential source of cell-free supernatants. Plant cells produce various secondary metabolites such as phenolics, flavonoids, and alkaloids, which are present in the cell-free supernatant (Yadav and Yadav, 2023). These compounds are secreted by plant cells into the surrounding environment to defend against herbivores or pathogens.

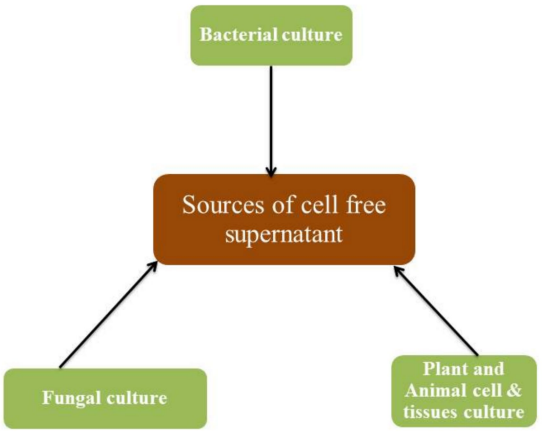

FIGURE 1
Sources of cell free supernatant.

Animal cell cultures are another potential source of cell-free supernatant. Animal cells produce various secondary metabolites such as cytokines, growth factors, and antibodies, which can be found in the cell-free supernatant (Feder-Mengus et al., 2008; Bourebaba et al., 2022). Figure 1 illustrates the various sources of cell free supernatant for agricultural purposes.

Methods for extracting microbial cell-free supernatants

To ensure only extracellular metabolites are obtained without microbial cell contamination, the extraction method of culturing the desired microorganism in the appropriate growth medium is critical. The type of bioactive compounds, desired purity, and intended application of CFS determine the choice of extraction method.

The first step is to culture the desired microorganism in the appropriate growth medium. The cells in culture can then be removed or separated using various methods.

For general applications, centrifugation and filtration are sufficient, while solvent extraction and precipitation are useful for isolating specific metabolites. For long-term storage, Lyophilization will be more appropriate.

i Centrifugation: The culture is centrifuged at 5,000–10,000 rpm for 10–20 min to pellet microbial cells (Majeedras et al., 2018). Differences in density allow centrifugation to separate cells from the liquid culture. The supernatant is then carefully collected without disturbing the pellet (Subramanian et al., 2021). This method is simple, cost-effective and fast. However, some microbial cells may remain in the supernatant, requiring additional filtration.

ii Filtration: This process involves using a membrane filter with an appropriate pore size to eliminate microbial cells and debris. Following centrifugation, the supernatant is filtered through 0.22 µm or 0.45 µm membrane filters to eliminate any remaining cells (Zhou et al., 2022). Sterile filtration guarantees the absence of live microorganisms, ensuring complete removal of microbial cells. This method is ideal for heat-sensitive compounds (Rashid et al., 2021). However some bioactive molecules may adhere to the filter membrane, and highly viscous cultures can lead to filter clogging.

iii Dialysis and ultrafiltration: This method allow for the removal of small molecules or concentrated-bioactive compounds using semipermeable membranes. The CFS is placed in a dialysis bag with a molecular weight cutoff (MWCO) to eliminate salts and low-molecular-weight impurities. Ultrafiltration membranes, with MWCOs (such as 3 kDa, 10 kDa, or 30 kDa) can selectively concentrate larger bioactive molecules (Khan et al., 2020). This process effectively eliminates unwanted small molecules while retaining bioactive compounds, and ultrafiltration enables the fractionation of compounds based on molecular weight. However this technique can be time-consuming and may result in the loss of some metabolites during dialysis.

iv Solvent extraction: This method involves using organic solvents to extract bioactive compounds based on polarity differences. The CFS is combined with an organic solvent like ethyl acetate, methanol, or chloroform at a 1:1 ratio. The mixture is then shaken and allowed to separate into two phases. The organic phase, which contains bioactive metabolites, is collected and evaporated under reduced pressure (Singh et al., 2016). The technique is effective for extracting non-polar bioactive compounds such as antibiotics and secondary metabolites. It can be utilized for selective extraction of various classes of metabolites. However, it's important to note that organic solvents may degrade heat-sensitive compounds, and some solvents can be toxic and should be handled with caution.

v Precipitation (ammonium sulfate or ethanol precipitation): In this method, salts or alcohol are used to precipitate proteinaceous bioactive molecules by altering their solubility. Ammonium sulfate is added to the CFS to achieve 40–80% saturation, leading to protein precipitation (Gudiña et al., 2015). The precipitate is collected by centrifugation and dissolved in buffer for further analysis. Ethanol or acetone can also be used to precipitate extracellular proteins and peptides (Santoyo et al., 2021). This method is suitable for purifying enzymes and antimicrobial peptides as well as concentrating bioactive molecules. However, it requires optimization of precipitation conditions and some bioactive molecules may be lost in the process.

vi Lyophilization (freeze-drying) is a process where water is removed from supernatants through sublimation under vacuum while preserving bioactive compounds. The CFS is frozen at −80°C or in liquid nitrogen before being subjected to a vacuum in a lyophilizer to remove ice through sublimation. The resulting dry powder can be stored for future use, maintaining bioactivity for long-term storage and being suitable for thermostable compounds. However, this method can be expensive and time-consuming, and there is a risk of losing some volatile compounds.

Applications of cell-free supernatant in agriculture and environmental remediation

Cell-free supernatant has various potential applications in agriculture, and environmental remediation. In agriculture, it can

be used as a natural substitute for chemical fertilizers and pesticides, because it contains plant growth-promoting compounds and can suppress the spread of plant diseases (Kaewchomphunuch et al., 2022).

CFS as bio-fertilizer

Several microbial CFS have been reported to enhance plant growth. This technology is desirable amidst the threat of climate change because it is sustainable and environmentally friendly. CFS obtained from *Lactobacillus helveticus* EL2006H enhanced the growth of corn, soybean and potato (Naamala et al., 2023). *Azospirillum brasilense* CFS increased the number of root branches and nodules in soybean (Rondina et al., 2020). According to Posada et al. (2016), CFS of *Bacillus subtilis* EA-CB0575 significantly increased the dry weight of banana plants. The inclusion of CFS from *Bradyrhizobium diazoefficiens* strain USDA 110 and *Rhizobium tropici* strain CIAT 889 increased root diameter by up to 1.6%, root length by 28.5%, root volume by 19.7%, root surface area by 17.8%, number of nodules by 29%, nodule dry weight by 27.2%, root dry weight by 13.5%, and shoot dry weight by 3.8% resulting in a yield increase of by 485 kg ha^{-1} (Moretti et al., 2020). The soil biostimulant products CFS obtained from *Lactobacillus rhamnosus* are made up of lactic acid, peptides, and free amino acids modified by microbial biodiversity, favoring bacterial genera recognized as growth plant promoters (Caballero et al., 2020). CFSs of *Bacillus* sp. strains (U35, U47, U48, U49, and U50) increased all the growth traits of corn (Yaghoubian et al., 2022).

CFS as bio-control agents

CFS containing lipopeptides from *Bacillus subtilis* have shown potent inhibitory activity against plant pathogenic fungus *Fusarium oxysporum* even at very low concentration. They inhibited spore germination by up to 26% and mycelium growth by up to 49% compared to the control (Mihalache et al., 2018). In environmental remediation, cell-free supernatant can be used to degrade pollutants or promote the growth of beneficial microorganisms (Xu et al., 2021). Soil biostimulant products such as (CFS obtained from *Lactobacillus rhamnosus*) consist of lactic acid, peptides, free amino acids, and protein hydrolysates, exhibiting biocontrol activity against some phytopathogenic microorganisms (Caballero et al., 2020). When applied to potato tubers, the filtrated supernatant of *Streptomyces* TN258 24 h before significantly decreased pathogen penetration (*Pythium ultimum*) by 62% and reduced the percentage of weight loss by 59.43% (Sellem et al., 2017). CFS from *Bacillus subtilis* GLB191 containing cyclic lipopeptides fengycin and surfactin is highly active against downy mildew in grapevines through the induction of defense gene expression and callose production (Li et al., 2019).

CFS as abiotic stress ameliorator

Globally, abiotic stresses such as drought, salinity, extreme pH, and heavy metals pose a significant threat to crop productivity. Improving crop tolerance to abiotic stress with microbial CFS is a sustainable strategy to meet the increasing demand for food. Cell-free supernatant may also mitigate abiotic stressors including heavy metal toxicity, salinity, and drought (Ibitoye and Kolawole, 2022). This can occur through various mechanisms such as osmotic adjustment, ion homeostasis, and antioxidant defense. For example, some bacteria can produce exopolysaccharides that protect plants from drought stress by increasing water retention in soil and improving soil structure. CFS produced from *Acinetobacter calcoaceticus* AC06 and *Bacillus amyloliquefaciens* BA01 contains biostimulants that induce osmotic tolerance and metabolic changes in groundnuts under drought stress (Eswaran et al., 2024).

CFS can also be used for bioremediation, which is the process of using microorganisms to degrade or remove environmental contaminants (Pacwa-Płociniczak et al., 2011). Many bacteria in the rhizosphere have the ability to degrade pollutants, such as heavy metals, organic compounds, and pesticides (Alori and Fawole, 2017). These bacteria can be cultured to produce CFS containing enzymes and other molecules that can degrade pollutants. Some examples of bacteria associated with the remediation of environmental pollutants include *Pseudomonas fluorescens* F113, *P. cepacia* ATCC 29351 (Alori and Babalola, 2018), and *Rhodopseudomonas palustris* (Sravya and Sangeetha, 2022). Additionally, CFS also stimulate the growth of indigenous microorganisms, enhancing the natural attenuation of contaminants. Table 1 shows some microorganisms whose CFS has been used to remediate polluted soil. The CFS of these soil microorganisms consists of various types of biosurfactants, glycolipids, exopolymeric substances, and siderophores, which have the potential to reduce and remove soil pollutants ranging from hydrocarbons to heavy metals. The percentage of pollutants removed ranged from 24 to 93%.

Mechanisms of action of microbial cell-free supernatants

Cell-free supernatant exerts various effects including pest and disease control, plant-growth-promoting, bioremediation of polluted soil and defense against abiotic stressors (such as acidity, and salinity) through various mechanisms. Detailed examples of these mechanisms are provided below.

Mechanisms of CFS as bio-control agents (disease control)

Antibiosis mechanism

Cell-free supernatant contains various antibiotics that can inhibit the growth of bacterial and fungal pathogens. These antibiotics can act by inhibiting protein synthesis, cell wall synthesis, DNA replication, or cell membrane integrity of the target microorganism (Borges et al., 2021). The mechanism of action depends on the specific antibiotic present in the cell-free supernatant. The growth inhibition of *Rhizopus oryzae* by CFS from *Trichoderma* spp. could be due to the secretion of harmful extra-cellular compounds like antibiotics such as gliotoxin, and glyoviridin (Alka and Prajapati, 2017).

Many antibiotics exert their antimicrobial activity by inhibiting the synthesis of cell wall components such as peptidoglycan or chitin. This can lead to the weakening or rupture of the cell wall, causing the death of the microorganism (Borges et al., 2021). Additionally, many antibiotics can inhibit the synthesis of bacterial proteins by binding to bacterial ribosomes and preventing the formation of peptide bonds between

amino acids. This can lead to the disruption of essential cellular processes such as DNA replication, transcription, and translation, ultimately causing the death of the microorganism (Sarathy et al., 2019). Moreover, cell-free supernatant may also modulate plant-microbe interactions, leading to improved plant health and yield (Santoyo et al., 2021).

Some bacteria can produce siderophores, which bind iron and make it unavailable to pathogenic fungi (Zhang et al., 2023). The antifungal activity of *Lactobacillus* spp. is associated with the synthesis of organic acids, fatty acids, esters of fatty acids, hydrogen peroxide, bacteriocins, and other secondary metabolites (Perczak et al., 2018). The antifungal activity of CFS containing lipopeptides produced by *Bacillus subtilis* is related to the inhibition of spore germination and the irreversible damage of the hyphae cell wall of plant pathogenic fungi *Fusarium oxysporum* (Mihalache et al., 2018). Microbial CFS activates plants and protects them against harmful bacteria and fungi (Pellegrini et al., 2020). The bioactive compounds in CFS inhibit fungal and bacterial pathogens by disrupting cell membranes (Kumar S. et al., 2021). The presence of amino acids, vitamins, and other nutrients in the supernatant can also contribute to its efficacy in disease resistance (Kumar S. et al., 2020).

CFS from *Trichoderma simmonsii, Aspergillus westerdijkiae*, and *Bacillus* sp. prevents dieback disease on apple rootstocks both when applied and in combination. This suggest additive or synergistic effects between the two biocontrol agents (M'henni et al., 2022).

Enzymatic activity

Lactobacillus plantarum CFS can prevent spore development of pathogenic fungi Fusarium oxysporum for up to 6 days by producing extracellular hydrolytic enzymes (Di Rico et al., 2025). Some CFS contain chitinases or glucanases, which degrade the cell walls of fungi (Veliz et al., 2017). The growth inhibition of *Rhizopus oryzae* could be due to the secretion of cell wall-degrading enzymes such as glucanases, endochitinases, and chitinases (Alka and Prajapati, 2017). The production of hydrolytic enzymes (such as chitinase, glucanase, protease, and cellulase; and antibiotics such as 2,4-diacetyl phloroglucinol, amphisin, oomycin A, hydrogen cyanide, phenazine, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, oligomycin A, zwittermicin A, kanosamine, and xanthobaccin) by beneficial microbes is an important mechanism against phytopathogens for sustainable plant disease management. These enzymes break down the cell wall of fungal pathogens, leading to cell death (Jadhav et al., 2017).

Quorum sensing inhibition

Cell-free supernatant contains various compounds that can interfere with quorum sensing (QS), a mechanism of communication among bacteria that regulates gene expression in response to cell population density and virulence. This mechanism is relied upon by many pathogenic bacteria to control virulence, biofilm formation, and antibiotic resistance. These compounds n CFS can either block the synthesis of quorum sensing molecules or inhibit their binding to receptors, thus disrupting the quorum sensing network (Escobar-Muciño et al., 2022). The bioactive compounds in microbial CFS inhibit fungal and bacterial pathogens by interfering with quorum sensing and inducing systemic resistance in plants (Kumar S. et al., 2021). Mechanisms of Quorum Sensing Inhibition by Microbial CFS include the following:

a Enzymatic degradation of QS signals

Enzymes such as lactonases, acylases, and oxidoreductases found in microbial CFS produced by certain microbes can breakdown or alter quorum sensing signals (autoinducers). For example, AHL-lactonases produced by *Bacillus* and *Pseudomonas* species can breakdown acyl-homoserine lactones (AHLs), which are important QS signals in Gram-negative bacteria (Uroz et al., 2009). Some *Pseudomonas* species can also produce enzymes that can breakdown N-acyl homoserine lactones (AHLs), a QS signaling molecule, effectively disrupting QS communication, and thereby reducing the virulence of plant pathogens (Dong et al., 2000).

b Disruption of biofilm formation

Some microbial CFS disrupts biofilms by interfering with QS pathways. Biofilms are structured bacterial communities that contribute to antimicrobial resistance. For example CFS from *Bacillus subtilis*, produces surfactin, this inhibits *Pseudomonas aeruginosa* biofilm formation. This alters cell adhesion and signaling (Bodini et al., 2007).

c Competitive binding to QS receptors

Certain metabolites in CFS can prevent native autoinducers from activating virulence pathways mimic QS signals by competitively binding to bacterial QS receptors. For example, phenolic compounds in CFS from *Lactobacillus* spp. interfere with QS-controlled biofilm formation in *Pseudomonas aeruginosa* (Rasamiravaka et al., 2015).

d Downregulation of QS gene expression

CFS from *Streptomyces* spp. have been reported to down regulate luxI/R genes in *Chromobacterium violaceum*. This disruption affects QS-regulated pigment production and biofilm formation ultimately reducing virulence and biofilm formation (Lade et al., 2014).

Induction of systemic resistance

Cell-free supernatant can induce systemic resistance in plants, which is a heightened defense response to subsequent pathogen infections (Sivojiene et al., 2021). This can enhance plant resistance to a wide range of pathogens, leading to improved crop health and yield. It is a mechanism by which plants can boost their resistance to a broad range of plant pathogens by activating plant defense pathways, resulting in the accumulation of phytohormones and other defense-related compounds.

For example, treatment with cell-free supernatant of *Trichoderma asperellum* SKT-1 induces systemic resistance against cucumber mosaic virus (CMV) by regulating the expression of various pathogen-related genes, enhancing the defense mechanism against CMV infection (Elsharkawy et al., 2013). Cell free supernatant may contain plant signal molecules such as lipo-chitooligosaccharides (LCO), chitooligosaccharides (CO), chitinous compounds, flavonoids, jasmonic acid, linoleic acid, linolenic acid, and karrikins (Bywater Ekegard and Fitzsimmons, 2020).

Certain compounds derived from CFS can trigger induced systemic resistance (ISR) and systemic acquired resistance (SAR) in plants, making them more resilient against biotic and abiotic stresses (Köhl et al., 2019). This preemptive defense mechanism enhances plant immunity, reducing disease incidence and improving overall crop health. ISR requires jasmonic acid (JA), ethylene (ET) signaling pathways, and nonexpressor of pathogenesis-related protein 1 (NPR1)

(Pieterse and Van Loon, 2007). The two major branches of the JA signaling pathways are controlled by the transcription factor MYC2 and the ethylene response factor (ERF). The ERF branch of the JA pathway is associated with enhanced resistance to necrotrophic pathogens that is regulated by members of the APETALA2/ethylene response factor (AP2/ERF) family, including the JA-responsive marker gene plant defensin1.2 (PDF1.2) (Lorenzo et al., 2003). JA also mediates resistance against herbivores (HIR). After leaf wounding, an increase in the JA derivative Jasmonoyl-isoleucine (JA-Ile) is perceived by a complex consisting of the protein Coronatine insensitive1 (COI1) and Jasmonate ZIM-domain (JAZ) protein. When the hormone is perceived, JAZ repressors are degraded by the proteasome releasing MYC2 and allowing the activation of JA responses such as the accumulation of vegetative storage protein2 (VSP2) (Pieterse et al., 2014). It has been demonstrated that volatiles derived from the linoleic pathway increase sensitivity to methyl jasmonate and induce several defense-related genes, such as chalcone synthase (CHS), allene oxide synthase (AOS), hydroperoxide lyase (HPL), and lipoxygenase 2 (LOX2) (Hirao et al., 2012).

Table 1 summarizes the application and implication of CFS used as biocontrol agents against pests and diseases. It shows that CFS has been studied on various crops including cereal crops like maize, legumes such as soybeans, Lotus japonicas, and chickpeas; vegetables like pepper, tomato, and potato, tuber crops like cassava, and ornamental crops. CFS has been produced from both bacteria, such as the genus Streptomyces, Bacillus, etc., and fungi, such as the genus *Penicillium, Trichoderma*, etc. Components of such CFS include harzianic acid, linoleic acid, hydroxymethylfurfural, gliotoxin, glyoviridin, siderophores, lipopeptides, enzymes such as glucanases, endochitinases, chitinases, etc.

Mechanisms of CFS as bio-fertilizer (plant growth-promotion)

Table 2 summarizes the use and application of CFS for plant growth promotion. It shows that CFS as biofertilizers has been studied on cereal crops such as maize, legumes including soybeans, Lotus japonicas, and chickpeas; vegetables such as pepper, tomato, and potato; tuber crops like cassava; and ornamental crops. CFS has been produced from both bacteria such as genus Lactobacillus, Bacillus etc.

TABLE 1 Microorganisms and their CFS as bio-control agents.

Crop	Organism	Effects	Active component of CFS/mechanism	References
Tomato	*Streptomyces rimosus*	Induces resistance in tomato against *Fusarium oxysporum* f. sp. Lycopersici	By activating the phenylpropanoid pathway	Abbasi et al. (2019)
Tomato	*Trichoderma* spp.	Reduced disease severity	Gliotoxin, glyoviridin, glucanases, endochitinases, chitinases	Alka and Prajapati (2017)
Peanut and maize	*Bacillus albus strains*	reducing disease incidence (DI) and disease severity index (DSI)	Diketopiperazines, macrolactins, siderophores lipopeptides,	Trinh et al. (2025)
Maize (*Zea mays*)	*Bacillus subtilis MF497446* and *Pseudomonas koreensis MG209738*	Controlling *Cephalosporium maydis* in Maize Plant	Siderophore	Ghazy and El-Nahrawy (2021)
Lotus japonicas	*Pantoea eucalypti M91*	Promote morphological and biochemical changes and induce improved photosynthesis and iron translocation	Siderophores	Campestre et al. (2016)
Ornamental bulb plants	*Bacillus subtilis*	Disease suppression	Lipopeptides	Mihalache et al. (2018)
Chili	*Trichoderma* spp.	Inhibits the growth of pathogen	Unknown	Nurbailis et al. (2019)
Tomato	*Trichoderma* sp.	Reduced the disease severity (%)	Harzianic acid, linoleic acid, hydroxymethylfurfural	Imran et al. (2023)
Tomato	*Bacillus amyloliquefaciens*	Reduction in disease incidence (70.00%) and disease severity	IAA production, siderophore production, lytic enzyme	Gautam et al. (2020)
Sugar beet	*Bacillus amyloliquefacien*	Inhibited the appearance of tissue necrosis (up to 92%)	Lipopeptide extracts	Nikolić et al. (2019)
Banana	*Bacillus* sp. EA-CB0959	Reduced incidence of Moko disease for up to 35%	Lipopeptides (surfactins, iturins and fengycins)	Villegas-escobar et al. (2018)
Grape	*Bacillus subtilis*	Reduced the leaf sporulating area by 97%	Surfactin and fengycin	Li et al. (2019)

TABLE 2 Microorganisms and their CFS as plant growth promoter (bio-fertilizers).

Crop	Organism	Effects	Active component of CFS	References
Maize (*Zea mays*), Soybean (*Glycine max*) L. Merill and Potato (*Solanum tuberosum*)	*Lactobacillus helveticus* EL2006H	Enhanced radicle length in corn, mean percentage germination in soybean and photosynthetic rate, greenness and mean fresh weight in potato	Unknown	Naamala et al. (2023)
Pepper (*Capsicum annuum*)	*Alternaria alternate*	Stimulated root growth, induced fruit development, and enhanced the number fruits per plant and yield	Volatile organic compounds	Baroja-Fernández et al. (2021)
Cassava	*Bacillus* sp. *CaSUT007*	Increased root and shoot lengths and total biomass of cassava stalks	Extracellular proteins	Buensanteai et al. (2013)
Maize (*Zea mays*)	*Bacillus Strains* (U35, U47, U48, U49, and U50)	CFSs of Bacillus strains increased all the growth traits of corn seeds and reduced the negative effects of salinity, especially severe salinity		Yaghoubian et al. (2022)
Banana	*Bacillus. subtilis EA-CB0575*	Enhanced dry weight of banana plants	Lipopeptides and siderophores	Posada et al. (2016)
Soybean	*Bacillus. amyloliquefaciens strain KPS46*	Promoted soybean growth	Antibiotic surfactin and proteins	Buensanteai et al. (2008)
Sesame	*Penicillium* spp.	Enhanced shoot and root length as well as the biomass	Enriched in amino acids	Radhakrishnan et al. (2014)
Pepper	*Trichoderma. Harzianum*	Promoted root growth, enhanced fruit yield and altered composition of fruits	Volatile organic compounds (VOCs)	Baroja-Fernández et al. (2021)
Chickpea	*Trichoderma* species	Enhanced germination percentage and increased fresh and dry weight	Unknown	Ali et al. (2014)
Arabidopsis thaliana	*Bradyrhizobium japonicum*	Stimulus effect on root growth and development	Lipo-chitooligosaccharides	Khan et al. (2011)
Lotus japonicas	*Pantoea eucalypti M91*	promote morphological and biochemical changes and induce improved photosynthesis and iron translocation	Siderophores	Campestre et al. (2016)
Soybean	*Bacillus amyloliquefaciens*	Increased root and shoot lengths and plant biomass	Indole-3-acetic acid and surfactin	Buensanteai et al. (2008)
Helianthus annuus	*Pseudomonas citronellolis* strain SLP6 H	Enhance the chlorophyll content, production of antioxidant enzymes, and plant growth	Hydroxamate siderophore	Silambarasan et al. (2020)
Maize	*Bacillus amyloliquefaciens and Bacillus subtilis*	Significantly enhanced length growth of maize seedlings	Indole-3-acetic acid (IAA)	Idris et al. (2004)
Cassava	*Bacillus* sp. CaSUT007	Enhanced growth	Phytohormone and extracellular proteins	Buensanteai et al. (2013)
Alfalfa	*Sinorhizobium meliloti* U143 and *Delftia* sp. JD2	Increased shoot and root matter and hence increased yield	Phenolic compounds (including flavonoids), organic acids, and volatile compounds	Morel et al. (2015)
Pigeon pea	*Bradyrhizobium* strain IC-4059	Significant enhancement in shoot length, root length, dry weight, protein content, and nodule number	Phosphatidylserine, phosphatidylcholine, FMC-5, pantoic acid, ascorbic acid, benzoic acid, 4-pentyl-4-formylphenyl ester, benzimidazole, and phosphatidylethanolamine	Tewari et al. (2020)

and fungi such as genus Penicillium, Trichoderma etc. Mechanisms of action of CFS as a biofertilizer are as follows:

Production of growth hormones

Cell-free supernatant contains various plant growth-promoting compounds such as auxins, cytokinins, and gibberellins. These compounds can stimulate plant growth and development by promoting cell division, elongation, and differentiation (Naamala et al., 2023). They can also enhance nutrient uptake, water retention, and stress tolerance of plants, thus improving their productivity and quality (Kumar S. et al., 2021). For example, some bacteria can produce indole acetic acid (IAA), which promotes root growth and lateral root formation. CFS contains phytohormones that can promote the expansion and maturation of plants, including gibberellins, cytokinins, and auxins. Phytohormones regulate numerous processes, such as cell division, elongation, and differentiation, leading to improved shoot and root growth, nutrient uptake, and stress tolerance (El Sabagh et al., 2022).

Cell-free supernatants that promote plant growth may contain biostimulants that enhance metabolic or physiological processes such as respiration, photosynthesis, nucleic acid uptake, ion uptake, and nutrient delivery. Examples of biostimulants found in CFS include humic acids, fulvic acids, myo-inositol, and glycine (Bywater Ekegard and Fitzsimmons, 2020). Additionally, organic acids and siderophores in CFS improve nutrient solubilization and uptake, ultimately enhancing plant nutrition and yield (Patel and Saraf, 2017).

According to Glick (2020), plant growth-promoting substances like auxins, gibberellins, and cytokinin-like compounds, which can stimulate root elongation, seed germination, and overall plant vigor, are often present in microbial CFS. Cell-free supernatant can also enhance nutrient uptake by plants by increasing the solubility and availability of essential minerals such as nitrogen, phosphorus, and potassium (Yuan et al., 2020). The presence of amino acids, vitamins, and other nutrients in the supernatant can also contribute to its efficacy in promoting plant growth (Kumar S. et al., 2020).

Improvement of soil structure

CFS can also contain exopolysaccharides (EPS) or biofilms, which can increase soil aggregation and water holding capacity. This can improve soil aeration, water infiltration, and nutrient retention, leading to improved plant growth and yield (Ibitoye and Kolawole, 2022).

Enzymatic activity

Cell-free supernatant contains various enzymes such as lipases, amylases, and proteases. These catalysts can hydrolyze different macromolecules like proteins, lipids, and carbohydrates, releasing nutrients that can be utilized by microorganisms or plants (Cruz-Casas et al., 2021).

Regulation of gene expression

Cell-free supernatant may also regulate gene expression in plants, leading to improved plant growth and yield (Kumar S. et al., 2021). For example, some bacteria can produce small RNA molecules that can target specific plant genes and regulate their expression. This can affect various plant processes such as photosynthesis, nutrient uptake, and stress response, leading to improved plant performance.

Mechanism of CFS in the mitigation of abiotic stresses

Table 3 shows some microorganisms that mitigate the effects of abiotic stresses such as drought, salinity, and heavy metals on crops and their components. The active components in these CFS include growth hormones, antioxidants, proteins, amino acids, vitamins, and osmolytes.

Advantages of microbial cell-free supernatants in crop production

Microbial cell-free supernatants (CFS) have emerged as a promising alternative to traditional agricultural inputs due to their bioactive properties and hence exhibit the following advantages:

Reduction in dependence on chemical inputs

By acting as biofertilizers and biostimulants, CFS can reduce the reliance on synthetic fertilizers and pesticides. This contributes to sustainable agriculture by minimizing chemical residues in the soil and preventing environmental pollution (Chowdhury et al., 2015). Furthermore, CFS-based treatments are often compatible with organic farming practices. The incorporation of natural resistance inducers in pest management programs of woody crops, alone or in combination with classical methods, could be a reliable method for reducing the amount of chemical residues in the environment. Cell-free supernatant can reduce the use of agrochemicals by promoting natural plant growth and suppressing the growth of plant pathogens (Yuan et al., 2020).

Compatibility with soil and rhizosphere microbiota

Unlike synthetic agrochemicals that may disrupt soil microbial communities, CFS typically supports beneficial rhizosphere interactions. The absence of live microbial cells reduces competition and interference with native soil microbiota while still promoting beneficial interactions, such as increased phosphate solubilization and nitrogen fixation (Garg et al., 2020). CFS contains antimicrobial compounds such as lipopeptides, enzymes, and volatile organic compounds that effectively suppress plant pathogens without harming beneficial microbes (Olanrewaju et al., 2019). This makes CFS an eco-friendly alternative to synthetic pesticides.

Improving soil health

The compounds present in them can promote the growth of beneficial microorganisms such as mycorrhizal fungi, rhizobia, and other nitrogen-fixing bacteria, which can enhance soil fertility (Kumar S. et al., 2020).

Potential for scalable and cost-effective production

Compared to live microbial inoculants, CFS offers a more stable and easily scalable solution for agricultural applications. It

TABLE 3 CFS of soil microorganisms that mitigate crop abiotic stresses.

S/N	Microorganism	Component of CFS	Stress type	Effect of the CFS	References
1	*Pseudomonas* and *Stenotrophomonas* spp.	Biosurfactant	Heavy metal (As)	24.6% of As removal (678 $mg.kg^{1}$)	Araújo et al. (2021)
2	*Bacillus Strains* (U35, U47, U48, U49, and U50)	Unknown	Salinity	Increased growth traits and reduced the negative effects of salinity	Yaghoubian et al. (2022)
3	*Aspergillus tubingensis* (STSP 25)	Not known	Heavy metals [Pb (II), Ni (II), Cu (II), and Zn (II)]	Removed more than 90% of heavy metals	Mahanty et al. (2020)
4	*Aspergillus carneus* and *Aspergillus niger*	Biosurfactant	Heavy metala (Polyaromatic hydrocarbons)	Removed the lubricating oil from contaminated soil	Mahmoud et al. (2024)
5	*Acinetobacter calcoaceticus AC06* and *Bacillus amyloliquefaciens*	Osmolytes (including proline, salicylic acid, trehalose and glycine betaine)	Drought-stress	Displayed distinct osmotic-adjustment abilities groundnut during drought-stress	Eswaran et al. (2024)
6	*Lysinibacillus* sp. NOSK	Not known	Heavy metals (Ni(II), Cr(VI) and Reactive black 5)	Removed Ni(II) by 70 ± 0.2%, Cr(VI) by 58 ± 1.4% and Reactive black 5 by 82 ± 0.8	San Keskin et al. (2018)
7	*Nocardiopsis* sp.	Biosurfactant (glycopeptide type)	Oil-Contaminated Soils (Hydroarbon)	29 and 35% decrease in the content of hydrocarbons	Biktasheva et al. (2024)
8	*Pseudomonas aeruginosa*	Not known	Tetracycline adsorption	Adsorption of 526.32 mg/g	Debnath et al. (2020)
9	*P. ostreatus*	Not known	Bisphenol-A and carbamazepine	Degradation of bisphenol-A = 90% and carbamazepine	Ji et al. (2017)
10	*Lactobacillus helveticus*	Unknown	Salinity	Enhance mean percentage germination and mean radicle length in the presence of NaCl stress	Naamala et al. (2023)
11	*Pseudomonas aeruginosa*	Glycolipids	Hydrocarbons	Emulsification of hydrocarbons and vegetable oils; removal of metals from soil	Sifour et al. (2007)
12	*Devosia* sp. *(strain SL43)*	Hormones, antioxidants, amino acids, and vitamins	Salinity	Mitigates the adverse effects of salt stress on soybean (*Glycine max* L.) seed vigor index	Monjezi et al. (2023)
13	*Mycobacterium tuberculosis, Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia* sp., *Corynebacterium* sp.	Glycolipids	Hydrocarbons	Enhancement of the bioavailability of hydrocarbons	Franzetti et al. (2010)
14	*Bacillus subtilis SNW3*	Lipopeptides	Crude oil	Displayed great physicochemical properties of surface tension reduction value in bioremediation of crude oil	Umar et al. (2021)
15	*Pseudomonas aeruginosa* and *Pseudomonas putida*	Exopolymeric substances containing alginic, glucuronic acid, galacturonic acid, and uronic acid	Heavy metals (Chromium)	Chromium bioavailability, solubility, and transport or sorption behavior in subsurface system	Kantar et al. (2010)
16	*P. azotoformans*	Siderophores	Arsenic	Detoxify heavy metals	Nair et al. (2007)
17	*Pseudomonas aeruginosa*	Siderophores (ferric iron chelating compounds)	Heavy metals	Detoxify heavy metals such as Cr^{3+}, Al^{3+}, Cu^{2+}, Eu^{3+}, and Pb^{2+}	O'Brien et al. (2014)
18	*Azotobacter chroococcum*	Siderophores	Heavy metals	Alleviate heavy metals	Rizvi and Khan (2018)

(Continued)

TABLE 3 (Continued)

S/N	Microorganism	Component of CFS	Stress type	Effect of the CFS	References
19	*Agrobacterium radiobacter*	Siderophores	Arsenic	Removing 54% of arsenic from polluted sites	Wang et al. (2011)
20	*Bacillus subtilis BL-27*	Lipopeptides	Hydrocarbons, heavy metals	Enhanced biodegradation of hydrocarbons and chlorinated pesticides; removed heavy metals from a contaminated soil	Wang et al. (2018)
21	*Brevibacillus* sp.	Biosurfactant	Phenanthrene	Degraded 93% of phenanthrene in 6 days	Reddy et al. (2010)
22	*Pseudomonas citronellolis*	Biosurfactant	hydrocarbon	Collapsed oil drop within the interval of 10–20 min	Ismail et al. (2018)

eliminates concerns regarding microbial survival, colonization, and environmental adaptability (Patel and Saraf, 2017). Additionally, CFS formulations can be stored and transported more efficiently than live microbial cultures, making them more practical for commercial use.

Ease of application

CFS can be easily applied to plants through foliar sprays or soil drenches, making them convenient for farmers to use.

Shelf life

CFS can be stored and transported more easily than live microbial cultures, making them more practical for large-scale applications.

Storage techniques for cell-free supernatants

To maintain the biological activity and stability of the bioactive compounds contained in CFS, proper storage techniques are essential. The choice of storage method depends on the nature of the bioactive compounds, the intended application, and the duration of storage. The following are some storage technique options that can be explored in the storage of CFS.

Refrigeration (4°C) storage

Refrigeration at 4°C is a commonly used method for short-term storage (up to a few weeks). This method slows down microbial contamination and enzymatic degradation and is hence effective at preserving heat-sensitive compounds. CFS containing organic acids, bacteriocins, and quorum sensing inhibitors can remain stable for 1–2 weeks under refrigeration (Koohestani et al., 2018).

Freezing (−20°C to −80°C)

Freezing is a widely used method to store CFS containing proteins, enzymes, and other temperature-sensitive metabolites. Standard freezing −20°C is suitable for antimicrobial peptides, biosurfactants, and quorum sensing inhibitors (Simpson, 2010). However, repeated freeze–thaw cycles can lead to protein degradation. Ultra-low freezing at −80°C preserves heat-sensitive biomolecules like bacteriocins and enzymes without significant loss of activity (Simpson, 2010).

Lyophilization (freeze-drying) storage technique

Lyophilization also known as freeze-drying removes water from CFS while preserving bioactive compounds. CFS is frozen at −80°C and then subjected to vacuum sublimation to remove ice without affecting bioactivity (Ge et al., 2024). This process prevents degradation of heat-sensitive compounds and extends their shelf life to months or years at room temperature (Kristensen et al., 2020). Lyophilized powder is stored in airtight vials at room temperature or 4°C. Šuchová et al. (2022) used the lyophilization storage technique for bacteriocins, biosurfactants, plant growth-promoting compounds, and quorum sensing inhibitors. Lyophilized bacteriocins from *Lactobacillus* spp. retain activity for over 12 months at 4°C (Cheikhyoussef et al., 2009).

Spray drying

This method is suitable and cost-effective for industrial-scale storage. CFS is atomized into fine droplets and rapidly dried using heated air (80–150°C) (Huang et al., 2017). It can also be encapsulated with maltodextrin or gum arabic to enhance stability (Barcelos et al., 2014). It can be used for probiotic metabolites, biosurfactants, and microbial fertilizers (Gullifa et al., 2023). Biosurfactants from *Bacillus* spp. stored via spray drying remain stable for over a year at room temperature (Marchant and Banat, 2012).

Storage in preservative solutions

Preservatives such as glycerol (10–30%) will protect proteins and bacteriocins during freezing (Corral et al., 2014). Ethyl alcohol (10–50%) was used for organic solvent-stable metabolites such as quorum sensing inhibitors (Hamad et al., 2022).

Encapsulation technique

To protect CFS bioactive compounds from environmental degradation, they can be microencapsulated with polymers (such as alginate and chitosan) (Bassani et al., 2019). Gunal Köroglu et al. (2024) discussed encapsulation of hydrophobic compounds in yeast cells for use in food industries.

Challenges and limitations

Several limitations hinder the widespread adoption of microbial cell-free supernatants (CFS) in sustainable crop production despite their promising applications.

i **Variability in composition**: CFS is a complex mixture of different compounds, and its composition can vary significantly depending on the microbial strain, growth conditions, and extraction methods used. This variability makes it difficult to standardize CFS formulations for large-scale agricultural applications and ensure consistent performance (Compant et al., 2005; Olanrewaju et al., 2019). Standardization and optimization remain critical issues for ensuring reproducibility in field applications.

ii **Inadequate understanding of the mode of action**: The incomplete full understanding of the mode of action makes it difficult to optimize CFS formulations for specific applications and to predict their efficacy in different environmental conditions (Santos et al., 2017).

iii **Compatibility with other inputs**: The compatibility of CFS with other agricultural inputs, such as fertilizers and pesticides, is not well understood. CFS may enhance the efficacy of other inputs, while in other cases, it may have an antagonistic effect, reducing the efficacy of other inputs (Kudjordjie et al., 2019).

iv **Concentration and dosage:** The concentration and dosage of CFS required to achieve optimal effects are not well understood. Some studies have reported that lower concentrations of CFS can be more effective than higher concentrations, while others have reported the opposite (Santos et al., 2017).

v **Shelf-life and storage:** The shelf life and storage conditions of CFS can also be a limitation, as the bioactive compounds such as antimicrobial peptides, enzymes, and secondary metabolites in CFS can degrade over time or under inappropriate storage conditions due to factors such as temperature, pH, and UV radiation (Garg et al., 2020). This degradation can reduce the efficacy of CFS and make it difficult to transport and use CFS in remote areas (Borges et al., 2021). Furthermore, compared to chemical agro-inputs, the shelf life of microbial CFS-based formulations is shorter, necessitating frequent application or the use of stabilizing agents (Chowdhury et al., 2015).

vi **Potential phytotoxicity and environmental concerns**: Although CFS is generally considered environmentally friendly, the potential environmental impact of widespread CFS application needs to be carefully assessed. Some microbial metabolites in CFS, when applied in high concentrations, may exhibit phytotoxic effects, which could result in growth inhibition or stress responses in certain crops (Kumar S. et al., 2021). Furthermore, the long-term impact of repeated CFS applications on soil microbial communities and ecosystem balance is not fully understood, warranting further investigation.

vii **Economic and scalability challenges**: The cost of producing CFS at an industrial scale may not be competitive with conventional fertilizers and pesticides which can be cost-prohibitive for some farmers unless optimized biotechnological approaches are adopted (Patel and Saraf, 2017). Additionally, regulatory hurdles in different countries may delay commercialization and adoption. It is crucial to develop cost-effective production and delivery methods for the widespread adoption of CFS technology.

viii **Limited field efficacy**: Although CFS has demonstrated strong efficacy in controlled environments and laboratory settings, its performance in open-field conditions remains unpredictable. Factors such as soil microbiome interactions, climatic variations, and plant genotype can influence the bioactivity of CFS, potentially reducing its effectiveness compared to synthetic agrochemicals (Köhl et al., 2019).

Conclusion

Cell-free supernatant has emerged as a promising tool for sustainable crop production. Sources of microbial CFS used in crop production include bacteria and fungi. Its applications include plant growth promotion, biocontrol of plant diseases, and mitigation of the effects of abiotic stresses, including heavy metal remediation. By harnessing the power of CFS, we can reduce our reliance on synthetic chemicals and promote a more sustainable and environmentally friendly approach to crop production.

Author contributions

EA: Conceptualization, Writing – original draft, Writing – review & editing, Supervision. AO: Writing – original draft. AI: Writing – original draft.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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