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Screening and evaluation of potential microbial bio-activators used in sewage sludge composting

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ABSTRACT

Forty pure microbial cultures have been isolated from sewage sludge to develop biopreparations using the microbiological screening method. Among all studied microorganisms sixteen strains of different taxonomic groups (2 strains of each *Bacillus* and *Streptomyces*, 7 strains of *Pseudomonas*, and 1 strain of each of the following *Acinetobacter*, *Rhizobium*, *Pseudarthrobacter*, *Sphingomonas*, and *Rothia*) were selected providing the best cellulose-degrading ability, as well as having good growth and quick biomass accumulation on various substrates. The isolates were additionally tested for the ability to consume organic and mineral nitrogen species, and to encourage seedlings' growth. Moreover, we have evaluated the nitrogenase activity for some strains. As a result, the following strains of *Bacillus megaterium* № 10, *Rhizobium pusense* № 25, *Sphingomonas paucimobilis* № 49, *Pseudomonas fluorescens* № 83, *Streptomyces albidoflavus* № 81, *Streptomyces graminearus* № 61 demonstrated the potential to be used as bio-activator for sewage sludge composting and as multifunctional biologics in crop production.

Key words: Bio-activator, Growth stimulation, Sequencing, Sewage sludge, Waste treatment.
Article type: Research Article.

INTRODUCTION

Sewage sludge treatment and disposal cause a major problem for municipal governments around the world. Sludge of household waste mainly consists of biodegradable organic materials with a significant amount of inorganic substances (Guven *at al.* 2019). Nevertheless, domestic waste demonstrates great variability in physical, chemical, and biological composition depending on the activities of the given community (Athar *at al.* 2022). Currently, there are several methods for the disposal of sewage sludge ranging from waste dumping in landfill to agricultural use. Although sludge has number applications, there are many concerns about the presence of harmful contaminants including heavy metals, pathogens, and other toxic substances (Singh 2021; Othman *at al.* 2021; Zhang *at al.* 2022). Thus the right disposal method should be identified meeting the efficiency and environmental health criteria. Emerging technologies have been developed for the treatment and disposal of sewage sludge in accordance with strict environmental regulations. The use of effective microorganisms is one of the proposed new methods for wastewater treatment. The technology of effective microorganisms was developed in the 1970s at Ryukyus University, Okinawa, Japan. Studies have shown the great value of effective microorganisms in several fields, including agriculture, animal husbandry, horticulture and landscaping, composting, bioremediation, septic tank cleaning, and algae control. In the 1970s, Teuro Higa reported a combination of about 80 different microorganisms with a positive effect on decaying organic matter, therefore improving soil fertility (Safwat *at al.* 2021). The application of effective microorganisms (EM) can to some extent improve soil and irrigating waters. Additionally, they can be used for seed treatment. Moreover, some find them useful for making an organic spray to improve photosynthesis and as pests and disease control agents. The use of effective microorganisms to reduce

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sewage sludge volumes is often proposed as a feasible plan for both wastewater treatment plants and local wastewater treatment systems (Shalaby 2011). Biological methods of sewage sludge treatment today are the most affordable, efficient, and safe when it comes to local treatment plants used to process household waste. Previously, rather aggressive chemicals were used, which despite the necessary effect, were unsafe for the environment. Recently, environmental biotechnology frequently utilizes microbial preparations as bio-activators used to accelerate the process of biological wastewater treatment. Carefully selected bacterial cultures are the main component of such biological preparations able to decompose various organic and inorganic substances (Zabolotskikh 2019). Modern products for autonomous sewage systems are environmentally friendly biological additives that increase the efficiency of processes occurring in local wastewater treatment plants. The development of locally produced bio-activators is reasonable in terms of import phase-out due to the presence of only foreign preparations on the Kazakhstani market used in septic tanks and autonomous sewers.

MATERIALS AND METHODS

This study aimed to isolate and select promising microorganisms for the development of a bio-activator used in sludge composting. Cumulative cultures were obtained on different nutrient media. Among the studied microorganisms, 40 strains were isolated and further screened to identify the most effective in waste processing and floriculture.

Defining the heterotrophic microbial population

The primary step in the development of biological preparations used for wastewater treatment is to select microorganisms that actively grow on media rich in organic and mineral compounds. Microorganisms able to decompose organic substances found in domestic wastewater were isolated from fresh sludge from urban wastewater treatment plants. Standard complete media (Beef-extract agar and potato agar), and synthetic liquid media (Ashby, Getchenson, Gause, and starch-and-ammonia agar) were used for the cultivation of bacteria that actively consume fiber, fats, and proteins. These nutrient media were used to select bacteria adapted to grow on these substrates. Studied morphological, cultural, physiological, and biochemical properties were compared to the data given in Burgey's Manual to determine the genus of isolated microorganisms. Microorganisms were quantitatively analyzed by inoculating diluted silt suspension on appropriate agar media: beef-extract agar (Accumix, India) used to account for organotrophic bacteria, starch-and-ammonia agar for mineral nitrogen assimilating bacteria, Ashby mannitol agar for atmospheric nitrogen-fixing bacteria (diazotrophs), and Czapek-Dox agar (Himedia, India) for fungi. Nutrient media were prepared by autoclaving in ST-85G Jeiotech autoclave at 121 °C for 20 min, then cooled down to 45-50 °C, mixed well, and poured into petri dishes. Serially diluted sludge suspensions of 10^{-3} and 10^{-5} were inoculated to a corresponding nutrient agar in petri dishes in five replicates. Heterotrophic bacteria were accounted upon 72 h of incubation at 30 °C, and actinomycetes after 5 days at 28 °C.

Defining cellulolytic bacteria

An aqueous extract of sewage sludge in a volume of 100 µL was inoculated onto basal-salt media (2.5 g $NaNO_3$; 2 g KH_2PO_4; 0.2 g $MgSO_4$; 0.2 g NaCl; and 0.1 g $CaCl_2 \cdot 6H_2O$ per liter) containing filter paper for isolation of cellulolytic bacteria. These cultures were then incubated at 37°C for 7 days on a shaker at 100 rpm. Cellulose-consuming bacteria colonies were isolated with the method of serial dilution on a cellulose agar medium containing 0.5 g KH_2PO_4, 0.25 g $MgSO_4$, 2.0 g cellulose, 15 g agar, 2 g gelatin, and 1 liter of distilled water; pH adjusted to 6.8-7.2 (Gupta *at al.* 2012).

Defining nitrogenase activity of bacteria

Nitrogenase activity in bacteria was determined by a chromatographic method through an acetylene reduction procedure (Capone 2018). Each bacterial sample in liquid media (10 mL) was added to 20-mL vials (Agilent, USA) containing 10 µL acetylene (Brand B, first grade 99.1%) and incubated for 2 h at 28 ± 2°C. The amount of the formed ethylene was determined by chromatography using an auto-sampler for paraphrase analysis (Headspace sampler, Agilent 7697A). Pure gases were used to determine the retention times of acetylene and ethylene, and construct a calibration curve for ethylene (purity 99.9%, Sigma-Aldrich, Germany). The amount of acetylene and ethylene was determined by the release time of each gas comparing it with the release time of a known standard gas. The nitrogen-fixing activity was given in nanomoles of ethylene formed in 1 hour of incubation. The nitrogen-fixing activity was calculated using the following formula:

$$Nitrogen\ fixation = \frac{0.0353 \times S \times V}{t}$$ (nanomole, C_2H_4 h^{-1})

where 0.0353 is the ethylene calibration factor, S is the ethylene peak area (mAu), V is the air volume in the vials, and t is the acetylene incubation time (h). A 10.0 µL gas sample was injected using an auto-sampler (Agilent 7697A) into the sample injection device (injector) in splitless mode at 150 °C. Hydrogen carrier gas (99.99%, Hydrogen gas generators, PEAK Scientific, UK) was supplied at a constant flow rate of 29 mL min^{-1}. An HP-PLOT AI203 S column (Agilent, USA) of 50 m long, 0.53 mm in inner diameter, and 0.15 µm thick film was used for separation. The column thermostat temperature was kept at 150 °C for 10 min. The detector temperature was 230 °C. Gas analysis was performed using a gas chromatograph with a flame ionization detector (Agilent 7890B, USA). Each sample was analyzed in triplicates. The retention times of the compounds on the HP-PLOT Al_2O_3 S column are given in Table 1.

Table 1. Compound retention times.

№	Retention time (min)	Compound
1	3.132	Ethylene
2	3.417	Acetylene

Defining microbial resistance to elevated concentrations of organic and mineral nitrogen species

Serially-diluted suspensions of microorganisms (10^{-6}, 10^{-7}, 10^{-8} and 10^{-9}) were plated on a basal-salt media (2.5 g $NaNO_3$, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.2 g NaCl, and 0.1 g $CaCl_2 \cdot 6H_2O$ per liter) in the control, as well as replacing $NaNO_3$ with 5 g NH_4SO_2 and 5 g urea in the test samples. The CFU (colony forming units) of heterotrophic bacteria was accounted upon 45 h, and of actinomycetes after 72 h.

Defining growth-stimulating activities of microorganisms

The seed vigor and viability of lawn seeds and seedlings biometrics were studied to assess the effect of microbial suspension. For germination tests, 25 seeds were used in triplicates. The optimum concentration for promoting plant growth was determined as 0.0001%. The control group was water-treated seeds. Lawn seeds were couched in the thermostat. Germinated lawn seeds were counted on the 2nd-day post-treatment by determining seed vigor, while the viability of lawn seeds on the 5th day. Seedling biometrics were assessed on the 7th day after treatment.

16S gene sequencing

DNA at concentrations of 5.4-74.8 ng μL^{-1} was extracted using bacterial DNA extraction protocol with the GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA). The amplification was carried out using 16S primers (8 Forward 5'-AGAGTTTGATCCTGGCTCAG-3'; 806 Reverse 5'- GGACTACCAGGGTATCTAAT-3'). The PCR mixture contained 25 µL DNA, 1 U DNA polymerase (Thermo Scientific, USA), 0.2 mM of each dNTP, 1× PCR buffer, 2.5 mM $MgCl_2$, and 10 pmol of each primer. The amplification was performed on a SimpliAmp cycler (Thermo Fisher Scientific, USA). The amplified DNA was checked through electrophoresis in a 1.5% agarose gel with 1 × TAE buffer in a Max HU10 horizontal electrophoresis chamber and a Consort EV 243 current source (Hall 1999). The amplified DNA fragments were sequenced using the Sanger method with the BigDye terminator sequencing kit in a total volume of 25 µL containing 18 µL dH_2O, 5 µL 5× buffer, 0.5 µL BigDye, 0.5 µL primer, and 1 µl PCR product. The primer sequences used were the same as for PCR. To ensure sequencing accuracy, the amplified fragments were sequenced with two primers: forward and reverse. The sequencing products were studied on an ABI 3130XL genetic analyzer (Applied Biosystems, USA). Chromatogram analysis and editing were performed using Sequencing Analysis 5.2, Patch 2 (Applied Biosystems, USA). The results were processed in the www.ncbi.com database (Altschul *at al.* 1997).

Statistical analysis

All data are the mean of 3 replicates including standard deviation (SD) values. Differences between variants were assessed by one-way analysis of variance (ANOVA), where p-values less than 0.05 were considered statistically significant. The data obtained were analyzed in the XLSTAT program.

RESULTS

Cellulolytic capacity of bacteria

Toilet paper cellulose is the main component of solid particles in raw municipal wastewater (Singh 2022; Saravanan *at al.* 2022). Potentially harmful to the environment is the accumulation of cellulose fibers and their slow biodegradability. Of the 40 isolated microorganisms from fresh silt sediments, 16 strains were identified as possessing the highest cellulolytic properties (Table 2).

Table 2. The cellulolytic capacity of bacteria isolated from fresh silt sediments.

High degree	Average degree	Low degree	Zero degree
36B, 62B, 64B, 72B, 81B	8B, 10B, 12B, 25B, 48B, 49B, 56B, 61B, 78B, 83B, 88B, 87B	13B, 68B, 74B, 75B, 76B, 77B, 80B, 82B, 85B, 86B, 71B	9B, 11B, 26B, 47B, 63B, 65B, 66B, 67B, 73B, 79B, 84B, 89B

It has been shown that 5 strains isolated from sewage sludge and plant residues possessed a high degree of cellulolytic activity. On average, 90% of the studied strains had an average, low, or zero enzymatic activity.
Only 16 strains with high and average degrees of cellulolytic activity were selected for further investigations. The genus of the selected bacteria was determined based on the gene sequence encoding the 16S rRNA subunit (Table 3).

Table 3. The results of interpreting the nucleotide sequence of bacterial strains.

№ of sample	The closest genus	% of identification	№ of deposition
8	*Bacillus cereus* № 8	100%	OQ472597
10	*Bacillus megaterium* № 10	100%	OQ472997
12	*Acinetobacter radioresistens* № 12	100%	OQ472601
25	*Rhizobium pusense* № 25	100%	OQ472604
36	*Pseudomonas marginalis* № 36	100%	OQ472605
48	*Pseudarthrobacter siccitolerans* № 48	100%	OQ472611
49	*Sphingomonas paucimobilis* № 49	100%	OQ472789
56	*Pseudomonas simiae* № 56	100%	OQ472793
61	*Streptomyces graminearus* № 61	100%	OQ472796
62	*Pseudomonas protegens* № 62	100%	OQ472886
64	*P. marginalis* № 64	99.87%	OQ472890
72	*Rothia mucilaginosa* № 72	100%	OQ472971
81	*Streptomyces albidoflavus* № 81	99.86%	OQ472972
83	*Pseudomonas fluorescens* № 83	95.01%	OQ473037
87	*Pseudomonas putida* № 87	100%	OQ472974
88	*P. ceruminis* № 88	99.86%	OQ472995

Cellulolytic properties of *Streptomyces* species were described in the works of Zhang (2019) and Kocak (2023). There are some successful examples of using *Pseudomonas* and *Bacillus* consortia for sludge and organic waste treatment (Goel *et al.* 2019). Species such as *A. radioresistens* № 12 and *Rothia mucilaginosa* № 72 were excluded from further studies due to their expected pathogenicity.

Defining nitrogenase activity of bacteria

The next step in selection of effective microorganisms was the identification of their nitrogenase activity. Nitrogen fixators were screened and selected among 16 cellulolytic bacteria. All studied isolates possessed a capacity for nitrogen fixation with nitrogenase activity ranging from 36.72 to 59.76 nM C_2H_4/vial/3 days. The results of sample chromatography are shown in Fig. 1. The strains *P. siccitolerans* № 48, *S. albidoflavus* № 81, *P. fluorescens* № 83, *P. putida* № 87, *P. ceruminis* № 88 demonstrated the maximum nitrogenase activity above 50.4 nM C_2H_4/vial/3 days. Earlier, Zhang *at al.* (2018) and Vaz Jauri *at al.* (2019) studied the capacity of *S. albidoflavus* № 81 and *P. fluorescens* № 83 to nitrogen fixation showing their positive effect on plant productivity and antagonism to plant diseases.

Microbial resistance to elevated concentrations of organic and inorganic nitrogen species

The studied crops were tested for resistance to high concentrations of organic and mineral nitrogen species contained in domestic wastewater. Eleven crops were showing a profound tolerance to the main organic and inorganic compounds found in sewage sludge (Table 4). Tested strains assimilated ammonium sulfate less intensively than urea. No bacterial growth was observed when cultivating *B. cereus* 8, *P. siccitolerans* 48, and *P. simiae* 56 on the nutrient media containing 0.5 g dm^{-3} of ammonia sulfate as a nitrogen source. On the contrary, the same strains developed colonies on the second day of incubation on a urea-based nutrient media. Twice as many CFU of *Bacillus megaterium*, *Rhizobium pusense*, *S. graminearus*, and *S. albidoflavus* species were observed on the media containing 0.5 g dm^{-3} of urea when compared to ammonia sulfate.

Assessing the growth-stimulating properties in bacteria

Sewage sludge fertilizer is advised to be used in landscape farming and renovations. For this reason, bioactivators used for sludge composting should contain microorganisms possessing positive effect on the lawn seeds viability, promote the growth and development of seedlings (Table 5).

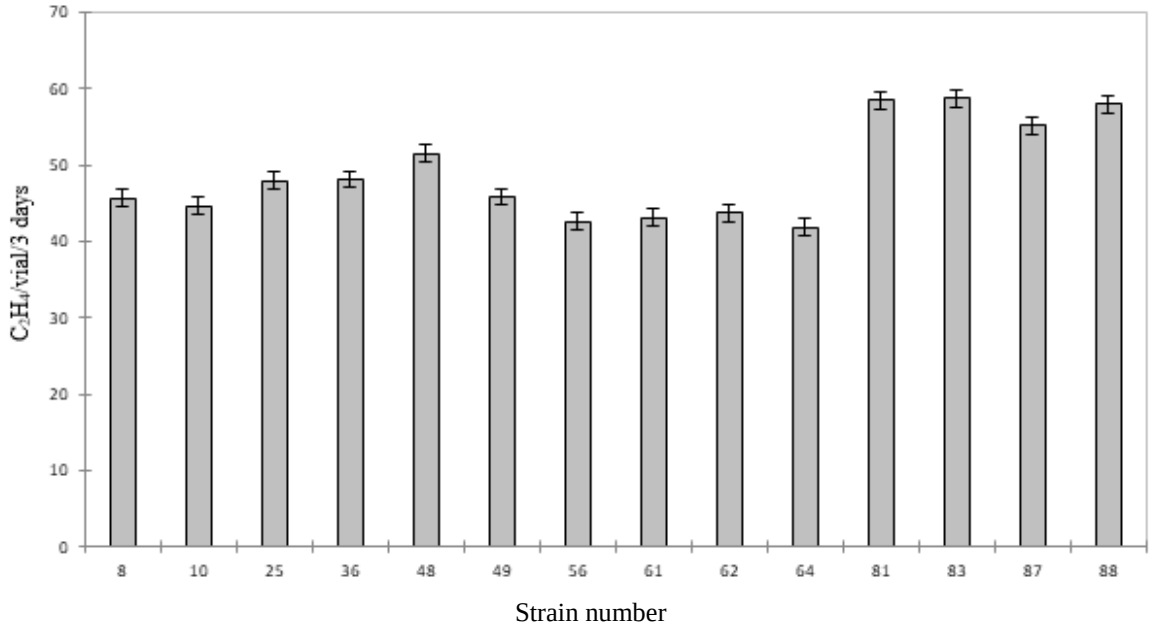

Fig. 1. Nitrogenase activity of liquid cultures.

Table 4. The number of microbial CFU on nutrient media with the elevated amount of organic and mineral nitrogen salts.

№	Strain	NH_4SO_2 CFU	Urea CFU
1	*B. cereus* № 8	0	65.7×10^8
2	*B. megaterium* № 10	10×10^6	40.3×10^6
3	*R. pusense* № 25	15.3×10^8	41.7×10^8
4	*P. marginalis* № 36	10.3×10^6	6×10^8
5	*P. siccitolerans* № 48	0	18.7×10^9
6	*S. paucimobilis* № 49	17×10^9	13.3×10^9
7	*P. simiae* № 56	0	59.7×10^6
8	*P. protegens* № 62	67.3×10^8	22.7×10^9
9	*S. graminearus* № 61	14.7×10^8	19.7×10^9
10	*P. marginalis* № 64	59.7×10^8	15×10^8
11	*P. fluorescens* № 83	70×10^6	7×10^8
12	*S. albidoflavus* № 81	36.7×10^6	43.3×10^6
13	*P. putida* № 87	66×10^8	14.3×10^6
14	*P. ceruminis* № 88	29×10^8	11.7×10^8

The laboratory testing revealed positive effect of *B. megaterium* № 10, *R. pusense* № 25, *S. paucimobilis* № 49, *P. fluorescens* № 83, and *P. putida* № 87 strains on the sowing quality of lawn seeds, where, for example, seed vigor tended to be up to 20% higher for groups treated with these strains when compared to control. In addition, seedlings also demonstrated intensive growth for these species. The highest percentages of lawn seed vigor and germination varied between 85% and 90% for *B. megaterium* № 10, *R. pusense* № 25, *S. paucimobilis* № 49, *P. fluorescens* № 83, *P. marginalis* № 64, and *P. putida* № 87 strains. The difference in seed vigor ($F = 5.447$, $p < 0.001$***) and germination ($F=5.176$, $p < 0.01$**) was found to be significant between control and bacteria treated groups. Inoculation with liquid cultures of *B. megaterium* № 10, *R. pusense* № 25, *S. graminearus* № 61, *S. paucimobilis* № 49, *P. putida* № 87 provided the greatest length of the seedlings which was on average 11% greater than in the control group. Bacteria treatment also contributed to biomass accumulation by seedlings which in turn positively affected plant development. The effect of microbial inoculation on sprout ($F = 3.280$, $p < 0.05$*) and radical ($F = 4.862$, $p < 001$**) lengths was found to be significant. Previously conducted studies have proved modulation of root development, biomass production, lateral root formation, and activation of auxin signaling by strains of *Bacillus megaterium*, *P. putida* and *P. fluorescens* (Ortiz-Castro *at al.* 2020; Wang *at al.* 2021).

Table 5. The effect of microbial liquid cultures on the growth of *Festuca arundinacea* seedlings.

№	Strain	Seed vigor on 3rd day (%)	Germination rate (%)	Sprout length (cm)	Radical length (cm)
1	Control	70	73	2.71 ± 0.03	1 . 42 ± 0 . 05
2	*B. megaterium* № 10	85	88	2.93 ± 0.06	1 . 64 ± 0 . 03
3	*R. pusense* № 25	85	87	3.08 ± 0.14	1 . 55 ± 0 . 08
4	*P. protegens* № 62	75	80	2.7 ± 0.08	1 . 63 ± 0 . 17
5	*S. graminearus* № 61	70	73	3 ± 0.07	1 . 69 ± 0 . 04
6	*S. paucimobilis* № 49	90	94	2.91 ± 0.03	1 . 46 ± 0 . 03
7	*P. fluorescens* № 83	90	90	2.78 ± 0.02	1 . 37 ± 0 . 04
8	*P. marginalis* № 64	85	85	2.74 ± 0.07	1 . 14 ± 0 . 04
9	*P. putida* № 87	85	87	2.94 ± 0.06	1 . 54 ± 0 . 11
10	*S. albidoflavus* № 81	75	75	2.83 ± 0.07	1 . 39 ± 0 . 03
11	*P. marginalis* № 36	76	81	2.89 ± 0.05	1 . 48 ± 0 . 08
12	*P. ceruminis* № 88	81	83	2.85 ± 0.09	1 . 56 ± 0 . 03
Least significant difference				0.2	0.2

DISCUSSION

Microorganisms with the expected efficiency of accelerating the aerobic composting of sewage sludge into organic fertilizer were selected in the course of this study. Microbial degradation of cellulose is known to increase the formation of humic acid (Zhou *at al.* 2022). Stimulation of humic acid formation is a key step in the aerobic composting of organic waste. The elevated content of humic acid can increase the stability of compost products, and reduce the phytotoxicity and bioavailability of heavy metals within (Zheng *et al.* 2022). A high amount of humic acid may be more beneficial for stimulating plant growth by improving the availability of soil nutrients (Holatko *et al.* 2022). Therefore, cellulolytic capacity has been chosen as the main selection criteria for effective strains. This allowed to identify 16 microbial strains possessing high and medium degree of cellulose degrading activity. Further selection included examination of nitrogenease activity, resistance to elevated concentrations of organic and mineral nitrogen species, and the ability to promote plant growth. Sewage sludge is rich in organic nitrogen-containing substances, the microbial transformation of which leads to the release of ammonium nitrogen, which further causes the suppression of nitrogenase activity in the soil (Zhao *et al.* 2020). In this regard, the introduction of microorganisms possessing high nitrogenase activity seems to be legit to prevent the loss of soil nitrogen. Microbial strains with elevated nitrogenase activity above 50.4 nM were identified in this study as *P. siccitolerans* № 48, *S. albidoflavus* № 81, *P. fluorescens* № 83, *P. putida* № 87, *P. ceruminis* № 88. Other strains also possessed nitrogenase capacity to some extent. At the same time, an increased amount of organic and inorganic nitrogen compounds limit the vital microbial activity in sewage sludge. Potential inoculant strains should grow well on nutrient media with nitrogen concentrations higher than normal in order to successfully compete with other microbes. Microbial growth in this study was examined by accounting for CFU, 24 h after inoculating. Tested strains of *Bacillus cereus, Pseudarthrobacter siccitolerans, P. simiae* demonstrated good growth on the urea based nutrient media, but were unable to assimilate ammonium sulfate. However, the ability of strains to use multiple nitrogen sources offers an advantage over other microbes. The following species *Bacillus cereus, Pseudarthrobacter siccitolerans,* and *P. simiae* did not possess this property and thus were excluded from further experiments. The remaining strains developed well on both urea and ammonium sulfate and has been chosen for further screening. Apart from nitrogen fixation, auxin production is crucial for plant growth and development. Auxins are the most numerous and common plant hormones secreted by the majority of plant-associated bacteria (Zinchenko *et al.* 2021; Mohamed *et al.* 2023; Orozco-Mosqueda *et al.* 2023). Microorganisms used in sewage sludge treatment were tested for plant toxicity and growth promotion of *Festuca arundinacea* seedlings. Seed inoculation with *B. megaterium* № 10, *R. pusense* № 25, *S. paucimobilis* № 49, *P. fluorescens* № 83, *S. graminearus* № 61, *P. putida* № 87 improved seed germination, as well as the length of roots and seedlings. Seed viability in tested groups was 21% higher when compared to the control, while roots and seedlings were on

average 11% longer. The results strongly suggest that the above-mentioned microbial strains stimulate plant growth and seed germination. Extensive bacterial screening resulted in the identification of the same genera profoundly used in waste composting, bioremediation, and plant protection. For example, *B. megaterium* is a well-known member of the bacterial consortia used in the processing of sewage sludge under aerobic and anaerobic conditions. It was also found to accelerate decomposition of organophosphorous ether in sewage sludge (Pang *et al.* 2018). *R. pusense* apart from being used in the bioremediation of soils contaminated with heavy metals (Khanna *et al.* 2022), was also useful in agriculture as a plant growth stimulating agent (Khan 2019). *S. paucimobilis* is able to decompose xenobiotics and biodegrade organophosphorous compounds present in soil (Ravintheran, *at al*. 2019). *P. fluorescens*, viable in contaminated and extreme conditions, was found to be successful in cleaning atrazine-, PCBs- and PHAs-contaminated soils. *S. albidoflavus* is known to improve seed germination and early development of corn, wheat, and tomato plants, as well as to restrict the growth of mycelium of various fungal plant pathogens (Kunova *et al.* 2021). *S. graminearus* produce hugerotin, a specific antibiotic of a wide range of biological activities, which mainly inhibit protein synthesis (Bansal *et al.* 2021). The combination of these effective strains offers great potential for accelerating waste composting and in stimulating plant growth. Strains isolated from sewage sludge are expected to survive in waste containing elevated amounts of heavy metals, which are limiting factor for microbial respiration. A number of papers were found to be published regarding the use of microbial consortia for sewage sludge processing (Fan *at al.* 2020). However, only a few publications cover the microbial screening procedures (Parhamfar *et al.* 2020; Buhari *et al.* 2022). We recommend that microbial screening conditions are close to the real application environment.

CONCLUSION

This paper describes the screening procedure of effective microorganisms isolated from fresh sewage sludge known as potential destructors of organic and mineral substances found in domestic wastewater. *B. megaterium* № 10, *R. pusense* № 25, *S. paucimobilis* № 49, *P. fluorescens* № 83, *S. albidoflavus* № 81, *S. graminearus* № 61 demonstrated high efficiency in degrading organic and inorganic compounds found in wastewater suggesting their use as potential bio-activators. The above-listed microbial species possess a set of useful properties making them ideal for the creation of multifunctional biological products consequently used for sludge processing and crop production.

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