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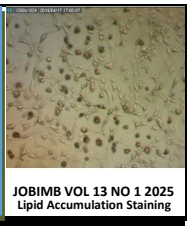

Inorganic Phosphate Solubilizing and Removal activities of *Curtobacteria* and *Bacillus* strains from Sediments of Langat River, Selangor, Malaysia

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ABSTRACT

A critical bioremediation effort is required to restore the phosphorus balance and improve water quality in polluted river sediments. Inorganic phosphate-solubilizing bacteria IPSB) play a vital role by releasing phosphate from organic matter, which helps reduce sediment accumulation and harmful algal blooms. This study aimed to isolate and identify indigenous strains of *Curtobacteria* and *Bacillus* isolated from Langat River sediments to assess their potential for phosphate solubilization and removal. Thirty (30) sediment samples collected between November 2022 and December 2023 at 10 different sampling stations (S1-S10) across the river were screened for phosphate solubilisation, extracellular phosphatase activities, and 16S rDNA gene sequencing. Fifty-eight (58) of the 83 isolated from the initial screening on nutrient agar medium exhibited a significant phosphate solubilization index (PSI), of which 31 isolates were able to decrease acid to release soluble reactive phosphorus (mg/L). S81B and S92G isolates demonstrated significant phosphate removal efficiencies of 64% and 62%, respectively. S81B exhibited higher acid phosphatase activity, while S92G showed more significant alkaline phosphatase activity, suggesting their adaptability to different pH conditions. Phylogenetic analyses revealed that S81B is closely related to *Curtobacteria* species, known for phosphorus mobilization in nutrient-limited environments, while S92G was similar to *Bacillus*, often associated with phosphorus cycling in soil and river ecosystems. These findings highlight the potential of these native strains for bioremediation applications to address phosphorus scarcity and mitigate eutrophication in the Langat River system.

INTRODUCTION

In aquatic ecosystems, phosphorus often acts as a limiting nutrient, controlling primary productivity and the abundance of aquatic organisms [1]. While phosphorus is essential for life, its excessive input into water bodies can have detrimental ecological consequences [2]. Numerous studies have documented significant phosphorus loads entering rivers, leading to eutrophication, algal blooms, and oxygen depletion, which can ultimately result in the degradation of aquatic ecosystems [3]. Malaysian rivers, including the Langat River, are susceptible to phosphorus pollution from various sources. Point sources such as industrial discharges, domestic sewage, and construction activities, as well as non-point sources like agricultural runoff and natural background inputs, contribute to phosphorus loading [4, 5]. This pollution significantly threatens drinking water quality, potentially leading to treatment plant shutdowns and compromised water supply. Additionally, excessive phosphorus can accelerate eutrophication, resulting in algal blooms and oxygen depletion, harming aquatic organisms and disrupting ecosystem balance [6]. Managing phosphorus concentrations in river systems is essential for maintaining ecological balance and preventing them from degradation [7]. In many river systems, the increasing phosphorus inputs from both point sources, such as wastewater treatment plants and non-point sources, like agricultural runoff, highlight the need for effective mitigation strategies [8]. While effective, traditional methods, such as chemical and physical precipitation, often bring significant costs

and generate substantial amounts of sludge, posing disposal challenges [9].

The use of inorganic phosphate-solubilizing bacteria (IPSB) offers an effective bioremediation approach for managing phosphorus pollution in river systems [10]. IPSB species like *Bacillus* and *Pseudomonas* enhance phosphorus availability by effectively solubilizing insoluble phosphate [11]. IPSB facilitates the phosphorus cycling by converting insoluble phosphates into bioavailable forms through organic acid secretion and lowering of environmental pH [12]. This process restores phosphorus balance and promotes phosphate removal in polluted rivers. Making IPSB a valuable tool for sustainable bioremediation techniques [13, 14].

The Langat River, heavily polluted by industrial and agricultural runoffs, presents a prime target for phosphate bioremediation [6]. The river has been identified as the critical source of drinking water for a substantial population in Selangor, indicating the urgent need for ecological restoration [15]. Introducing IPSB offers a promising solution to mitigate phosphate pollution by converting insoluble phosphate into bioavailable forms. However, as noted by [16], implementing bioremediation using IPSB in rivers faces several challenges. The Langat River's contamination with heavy metals like cadmium and lead poses a serious threat to the survival and efficacy of the bacterial strains [4]. Higher metal contaminations can inhibit bacterial growth and activity, diminishing the overall effectiveness of bioremediation [17].

Furthermore, the presence of competing microorganisms and complex ecological interactions within the river can hinder the establishment of IPSB population [5]. In addition, the variability in environmental factors like pH and organic matter content can impact the bio-phosphate removal capabilities of these bacteria, making it essential to select strains not only effective in Phosphate removal but also resilient to the specific conditions of the Langat River [18, 19]. While the potential of using IPSB for bioremediation in the Langat River is promising, a careful assessment of the local environmental context and potential ecological interactions is crucial for successful implementation.

Phosphorus pollution in rivers is commonly addressed through chemical and physical methods; these approaches can be costly and environmentally unsustainable, introducing secondary pollutants [20, 21], especially for dynamic river ecosystems like the Langat River. Consequently, there is an increasing interest in exploring biological approaches like IPSB to assess their feasibility, efficiency, and scalability for real-world applications. Therefore, the study aimed to isolate and identify the potential indigenous IPSB strains capable of removing phosphorus in the Langat River's sediments.

MATERIALS AND METHODS

Study Location

The Langat River is an important water source in Selangor, Malaysia (**Fig. 1**). It originates from the Titiwangsa granite mountain range at Gunung Nuang and flows through several districts, including Hulu Langat, Putrajaya, Sepang, Kuala Langat, and parts of Seremban, before emptying into the Strait of Malacca. The river lies on latitudes 2°40'152" N to 3°16'15" N and longitudes 101°19'20" E to 102°1'10" E., covering a catchment area of approximately 2,360 square kilometers and stretching over 176 kilometers in length. As one of the longest rivers in Selangor, it is essential for about 1.2 million people living in the area and plays a crucial role in the region's water supply [22].

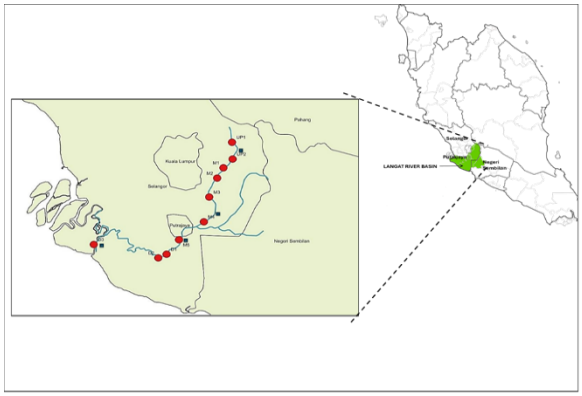

Fig. 1. Map of Peninsular Malaysia showing Selangor and the Langat River

Collection of Sediment

Sediment samples were collected between November 2022 and December 2023 from the Langat River at a depth of approximately 10 centimetres using an Ekman grab sampler. All sediments were handled following the protocol outlined by [23]. Ten sampling stations, labelled S1 to S10, were established along the river (**Table 1**).

Table 1. Description of the sampling locations along the Langat River.

SS	Coordinates	Location	Activity
S1	101.864402° E and 3.2109° N	Pangsun recreational area	Forestry and tourism
S2	101.843163° E and 3.15408° N	Sungai Congkak recreational forest	Government buildings and recreational activities
S3	101.826058° E and 3.125352° N	Dusun Tua	Residential, agricultural and recreational activities
S4	101.800816° E and 3.095793° N	Long quarry road	Mining-related, industrial and commercial activities
S5	101.770172° E and 3.014326° N	Sungai Balak	Industrial, residential and agricultural activities
S6	101.759683° E and 2.916852° N	Bangi	Industries, commerce and urbanization
S7	101.681607° E and 2.855404° N	Denkil	Residential, industrial and palm oil and rubber plantations.
S8	101.642351° E and 2.813239° N	Bukit Changgang	Residential, livestock farming and small-scale industries,
S9	101.632666° E and 2.812698° N	Banting	Small-scale industries and crop farming.
S10	101.408189° E and 2.853165° N	Pulau Carey (Jugra)	Rubber and palm oil plantations and historical tourism activities.

Keys: SS; Sampling station

Isolation of Bacterial Isolates

Sediment samples underwent a series of dilutions, and 0.1 mL of an aliquot was then spread-plated onto a sterile Nutrient Agar plate. Inoculated plates with diluted samples were incubated at 28 ± 02°C for 24 h. After incubation, individual colonies were serially sub-cultured to obtain pure cultures through their physiological morphology as described in the Bergey's Manual of Determinative Bacteriology [24]. These pure cultures of the isolates were then stored in a 40% glycerol stock culture for further analysis.

Screening of Inorganic Phosphate-Solubilizing Bacteria

Qualitative phosphate solubilisation test

The purified bacterial isolates in the glycerol stock cultures were screened for their ability to solubilize inorganic phosphate. A loopful of each bacterial culture was inoculated onto Pikovskaya (PVK) agar plate containing, per litre: 10 g of glucose, 5 g of tricalcium phosphate (TCP), 0.2 g of sodium chloride, 0.2 g of potassium chloride, 0.5 g of ammonium sulfate, 0.1 g of magnesium sulfate, 0.5 g of yeast extract, and trace amounts of ferrous sulfate and manganese sulfate. The inoculated plates were incubated at 28 ± 02°C for 120 h as per the method outlined by Mehata and Nautiyal [25]. Bacterial colonies displaying clear halo zones were identified as potential IPSB capable of solubilizing tricalcium phosphate. The phosphorus solubilization index (PSI) of these isolates was then calculated using the equation (i) described by [26].

$$PSI = \frac{\text{Colony diameter} + \text{Halo diameter}}{\text{Colony diameter}} \quad \text{(Eqn. i)}$$

Quantitative phosphate solubilization test

The bacterial isolates that exhibited qualitative phosphate solubilization potential were grown in Luria Broth (LB) for 24 h, and further evaluated for quantitative phosphate solubilization (mg/L). Subsequently, 50 µL of each culture was transferred into an Erlenmeyer flask containing 20 mL of Pikovskaya broth and incubated for 28 ± 02 ºC with shaking at 140 rpm for 5 days. Every 24 h, 1 mL of each cultured isolate was centrifuged at 40,000 rpm for 20 min to obtain the supernatant. The supernatant was analysed to determine the concentration of soluble reactive phosphorus (SRP) and pH to track the phosphorus release dynamics. The phosphate content of the supernatant was measured using the ascorbic acid method [27] and read at an absorbance of 88nm.

Extracellular Phosphatase Activity of the selected Isolates

Phosphorus acceptability test

To assess the phosphate-reducing abilities of the isolates that exhibited quantitative phosphate solubilisation activity, a series of experiments was conducted. Bacterial cultures were grown in nutrient broth at 28 ± 2° C, for 24 h. After harvesting and resuspending in saline solution, the bacterial concentration was adjusted to an optical density of 0.1. This standard inoculum was then added to PVK broth supplemented with 5% tricalcium phosphate (TCP). Initial measurement of bacterial cell density, pH and SRP content was recorded. The inoculated isolates were then incubated at 28 ± 2 °C for 72 h, with regular measurement of bacterial cell growth, pH, and soluble reactive phosphate level at 24 h intervals. The phosphorus uptake efficiency (PUE) of each isolate was calculated using equation (ii) described by [28].

$$PUE = \frac{\text{Initial concentration} - \text{Final concentration}}{\text{Initial concentration}} \times 100 \quad \text{(Eqn. ii)}$$

Phosphatase (acid and alkaline) activity test

The isolates that displayed significant phosphorus uptake exceeding 60%, were cultivated in PVK broth supplemented with 5 g/L of calcium phosphate as the sole phosphorus source. Following a 72-h incubation period, the cultures were centrifuged, and the resulting supernatants were used to prepare a reaction mixture. This mixture comprised 1 mL buffer pH 5.0 citric acid/sodium-citrate buffer (for acid phosphatase) and 1 mL pH 9.0 Tris-HCl buffer (for alkaline phosphatase), 100 µL p-nitrophenyl phosphate solution, and 3 mL bacterial supernatant. This was incubated at 37 °C for 20 min. The enzymatic reaction was then terminated by adding 1 mL of 3M NaOH, and the released p-nitrophenol was quantified spectrophotometrically at 405 nm, adhering to the procedures outlined by Pantujit and Pongsilp [29]. The amount of enzyme required to liberate 1µmole of p-nitrophenol per minute indicates one unit of enzyme activity, with enzyme activity measured as the micromoles of p-nitrophenol released per minute and specific enzyme activity expressed as micromoles per minute per milligram of enzyme, as described by [30].

Bacteria DNA Barcoding of the Selected Isolate

Genomic DNA (gDNA) of the selected bacterial isolate (S81B) was extracted from the cell cultures using an optimized protocol to ensure high purity and integrity. The extraction process involved cell lysis, protein removal, and DNA precipitation using Bacterial Lysis Buffer from the Bacterial DNA Barcoding Kit (1st BASE, KIT-1100-50). The extracted gDNA was quantified and quality-checked using spectrophotometry (e.g., NanoDrop) and agarose gel electrophoresis, confirming the presence of high-molecular-weight DNA suitable for further analysis.

The full-length 16S rRNA gene (~1500 bp) was amplified using universal primers (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') in a 25 µL PCR reaction [31]. The amplified PCR products were purified using a standard PCR clean-up procedure to remove unincorporated primers, dNTPs, and other reaction components. The purified PCR products were sequenced bidirectionally using the universal internal primers with the BigDye® Terminator v3.1 Cycle Sequencing Kit. The resulting sequences were analysed, aligned, and assembled to generate full-length sequences. These sequences were then compared to reference databases (GenBank) for bacterial strain identification.

Statistical Analysis

All experiments were performed in triplicate, and the resulting data were statistically expressed as means ± standard error at 95% Cl. The analyses were performed using Microsoft Excel.

RESULTS AND DISCUSSIONS

Isolation of Bacterial Isolates

The isolation of 83 distinct bacterial isolates from the 10 sampling stations of the Langat River sediment samples highlights the significant microbial diversity present in this aquatic ecosystem [32]. The variations in colony morphologies, such as small to medium-sized, circular, creamy-yellow colonies with smooth or slightly wrinkled surfaces, suggest that these bacteria may possess specialized metabolic functions. Such morphological diversity often indicates adaptations to specific ecological niches, allowing these isolates to exploit various resources and survive in fluctuating river environmental conditions [33].

This adaptability is crucial for maintaining ecological balance and nutrient cycling within the Langat River ecosystem. The distinction between Gram-positive and Gram-negative bacteria among the isolates further indicates their ecological roles and interactions within the sediment environment [34]. The consistent staining patterns observed indicate fundamental differences in the cell wall structure, which is critical for understanding their functional capabilities [35]. Gram-positive bacteria, characterized by a thick peptidoglycan layer, are generally more resilient to environmental stressors such as osmotic pressure changes due to their robust cell walls [36]. In contrast, Gram-negative bacteria possess an outer membrane that provides additional protection, facilitates nutrient uptake, and resists certain antibiotics [37]. These structural differences can significantly influence how these bacteria respond to environmental challenges and interact with other microorganisms in their habitat [38].

The colonial morphology among the isolates suggests a complex interplay of ecological strategies [39]. For instance, Gram-positive and Gram-negative bacteria may reflect a dynamic community capable of utilizing a wide array of substrates and adapting to varying conditions within the sediment [40]. This functional diversity of the isolates from different stations of Langat River is essential for ecosystem resilience, as different bacterial groups can perform distinct roles in nutrient cycling, organic matter decomposition, and overall ecosystem health [41, 42]. These interactions and the specific functions of different bacterial groups can provide valuable insights into the ecological dynamics of the Langat River and similar environments [43].

Qualitative phosphate solubilization

Fig. 2 shows the variability in colony and halo zone formations across the 58 out of 83 bacterial isolates from different sampling stations. The mean Phosphate Solubilization Index (PSI) values, along with 95% confidence intervals, provide insights into the precision of the measurements at each station. Stations S1 (8 isolates), S2 (4 isolates), and S3 (4 isolates) exhibit relatively consistent colony and halo zone sizes, with mean values between 2.5 and 3.0 cm. However, stations S4 (5 isolates) and S5 (6 isolates) show larger mean values of approximately 4.0 cm, with wider confidence intervals reflecting more significant variability. There is a gradual decline in both colony and halo zone sizes from stations S6 (6 isolates) through S7 (5 isolates), S8 (6 isolates), S9 (7 isolates), to S10 (7 isolates), where the lowest mean values, below 3.0 cm, were recorded. However, the error margins remain relatively consistent across the stations.

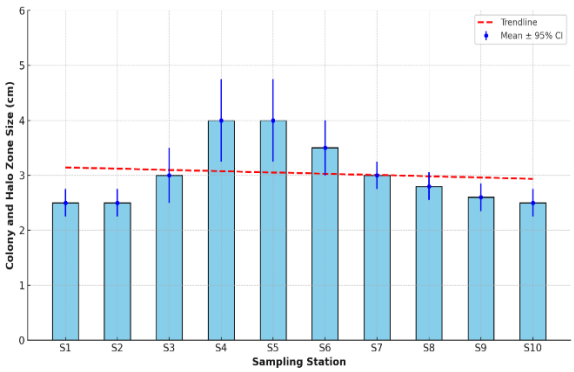

Fig. 2. Phosphate solubilization index (PSI) across different sampling stations with error bars representing the 95% confidence intervals.

As indicated by the colony and halo zones formation, the moderate PSI values observed across the isolates, primarily 2.5 to 3.0 cm, indicate that these isolates can effectively convert insoluble phosphates into bioavailable forms essential for aquatic plant growth [44]. Notably, stations S4 and S5 displayed higher PSI values of approximately 4.0 cm, implying that favourable environmental conditions such as optimal pH or nutrient availability may enhance microbial solubilization potential at these sites [45]. Furthermore, the gradual decrease in PSI from stations S6 through S10 suggests a correlation with nutrient scarcity or other less favourable conditions, as proposed by [46]. This variability in PSI capabilities suggests that environmental conditions significantly influence microbial activity in the Langat River [47].

The absence of specific bacterial isolates from sediment screening on nutrient agar during qualitative phosphate solubilization tests can be attributed to several factors. Genetic and metabolic diversity among bacteria can influence their ability to utilize phosphates effectively [48]. If bacterial isolates are not optimally adapted to the conditions provided during the qualitative analysis, their growth and phosphate solubilization capabilities could be severely impaired [49]. Some strains may lack the necessary enzymes or metabolic pathways for phosphate solubilization, which would prevent them from forming halo zones on PVK agar plates [50].

Environmental conditions, such as pH and temperature, can significantly impact bacterial metabolic activity to solubilize inorganic phosphate compounds in river sediments [51]. [52] investigated the influence of redox conditions in sediments on the mobility and availability of nutrients, particularly phosphorus, and examined solubilization of phosphorus by bacterial isolates in river sediment, suggesting that variations in redox potential across different stations of the Langat River. Additionally, the physical state of the Pikovskaya agar, including nutrient levels and competition with other bacteria, can affect the visibility of halo zones. Clumping behavior among bacterial cells can also lead to uneven distribution on agar plates, hindering the assessment of their phosphate solubilization potential [53]. These factors collectively contribute to the potential absence of bacterial isolates during the qualitative phosphate analysis.

Like many other river ecosystems, the Langat River is susceptible to the detrimental effects of anthropogenic activities [54], which can significantly impact the activity of IPSB and their PSI activities. Agricultural runoff, a common source of pollution in the region, can stimulate IPSB activity and increase PSI due to the influx of nutrients [55]. However, excessive nutrient loading can lead to eutrophication, algal blooms, and shifts in microbial communities, ultimately compromising long-term phosphorus solubilisation efficiency [56]. Industrial activities, particularly those associated with mining and manufacturing, can introduce heavy metals, toxic chemicals, and acidic effluents into the river, further disrupting the ecosystem's delicate balance. These pollutants can directly inhibit IPSB activity, reduce their diversity, and impair their ability to solubilise phosphorus [57].

Urbanisation, with its associated land-use changes and increased impervious surfaces, can exacerbate the problem by increasing runoff and sedimentation. These factors can alter the river's physical and chemical properties, such as water flow, temperature, and nutrient levels, thereby affecting the habitat suitability for IPSB [58].

Climate change, extreme weather events, and altered precipitation patterns can further complicate the situation. Fluctuating temperatures, prolonged droughts, and intense rainfall can stress IPSB populations, reduce their metabolic activity, and limit their ability to maintain a stable PSI [59]. The PSI provides valuable insights into the diversity and abundance of IPSB at different sites [60]. Higher PSI values, along with a greater number of isolates, suggest a more diverse and metabolically active bacterial community capable of effectively solubilizing phosphorus [44]. In rivers like Langat, this diversity plays a critical role in maintaining phosphorus cycling and ecosystem health, as varied IPSB populations offer broader metabolic capacities to sustain phosphorus availability under fluctuating conditions [61, 62]. Thus, PSI acts as an indicator of both nutrient availability and bacterial diversity in river ecosystems [63].

The correlation between PSI and bacterial diversity has been demonstrated by [48], who found that higher diversity enhances nutrient solubilization efficiency. A diverse IPSB community can improve PSI in phosphorus-rich environments, improving the overall health and productivity of aquatic systems [64]. Additionally, the metabolic flexibility of diverse IPSB populations allows adaptation to changing environmental conditions, which is crucial for sustaining phosphorus availability. For instance, in phosphorus-rich settings, different IPSB species may employ distinct pathways for solubilization and mineralization, increasing phosphorus cycling efficiency [65]. This adaptability optimizes nutrient availability and enhances ecosystem resilience in dynamic river systems affected by seasonal changes or anthropogenic activities [66].

The Langat River ecosystem has suffered significantly from human activities, disrupting its nutrient management and ecological balance [67]. Phosphorus cycling has been heavily impacted by agricultural runoff, industrial discharges, and urbanization [68]. These activities often lead to nutrient overload and deteriorating water quality, posing significant challenges to sustainable river management and ecosystem health in the region [69]. The degradation of nutrient dynamics in such environments calls for innovative, sustainable solutions to restore balance and prevent long-term ecological damage [70].

Isolates from S4, S5, and S6, with their significantly higher Phosphorus Solubilization Index (PSI) values, present a promising, environmentally friendly alternative for mitigating phosphorus pollution [71]. These isolates can convert insoluble phosphorus into bioavailable forms, thereby improving phosphorus availability in river systems like the Langat River. Harnessing the solubilization index capabilities of these isolates could provide an effective strategy for reducing phosphorus pollution in water bodies like the Langat River [72]. As Rubin and Görres [73] emphasized, understanding the environmental factors that optimize IPSB growth and their PSI activities is crucial for designing targeted bioremediation interventions that support sustainable phosphorus management and restore ecosystem health.

Quantitative phosphate solubilisation

The analysis of phosphate solubilization of 58 isolates across 10 stations indicated that 31 isolates across 8 stations exhibited distinct trends in the release and reduction of SRP concentration (**Fig. 3**) over a 120-h incubation period, measured at 24-h intervals.

Notably, isolates from Station 1 consistently reduced SRP levels throughout the incubation period. Specifically, isolates S12G, S1MW, and S1MG1 reduced SRP from 5.09-7.28 mg/L to 2.76-3.48 mg/L. Additionally, isolates S12B, S12W, and S1MG2 reduced SRP to approximately 4.49, 4.78, and 4.53 mg/L, respectively. Station 2 isolates demonstrated varying degrees of phosphate reduction. S21G exhibited the most significant reduction, decreasing SRP levels from 4.29 -1.12 mg/L. S22W steadily declined, reducing SRP from 6.50 mg/L to 3.08 mg/L. S22C initially decreased sharply, then stabilized, with SRP levels decreasing from 7.55-4.28 mg/L.

Station 5 isolates exhibited varying SRP trends. S51P and S52P fluctuated, with initial levels around 11.98-12.72 mg/L at 24 h, with an increase to 14.00 mg/L at 96 h and final levels around 7.94-11.97 mg/L. S51C consistently increased from 11.09 -13.95 mg/L. S52B initially fluctuated, then stabilized, with levels ranging from 10.24 to 11.78 mg/L. Station 6 isolates, such as S61Y, S62Y, and S61P, exhibited similar trends, with initial SRP levels around 10.84-12.34 at 48 h, then decreased at final levels to 10.59, 10.08 and 10.95 mg/L, respectively.

S71W, isolated from station 7, showed a significant reduction in SRP, starting from 8.45 mg/L, then increasing to 10.13 at 96 h, and finally decreasing to 1.48 mg/L. S72C exhibited a relatively stable trend, with SRP levels showing inconsistent SRP, with initial levels of 11.88 mg/L at 48 h, then decreasing to 10.79, and at 120 h, increased to 11.25 mg/L. Meanwhile, S72Y demonstrated rapid solubilization followed by a notable decrease, with SRP levels fluctuating between 1.35 and 12.16 mg/L. The isolates S81Y, S81W, and S81B displayed moderate fluctuations in SRP levels. The initial concentrations were around 4.95-6.57 mg/L, then increased from 6.88 to 8.59 mg/L, while the final concentrations fell to approximately 5.73-5.38 mg/L. In contrast, S82P showed a significant reduction in SRP at 24 h from 7.97 mg/L, then increased from 7-9.07 at 72 h, and finally decreased to 4.05 mg/L.

Isolates S91G, S91A, and S92G from station 9 exhibited varying phosphate solubilization activity. The initial SRP levels ranged from 5.24-6.33 mg/L, then increased from 5.24-7.96 at 96 h, while the final concentration ranged from 4.33 to 5.57 mg/L. At station 10, isolates S102B and S102W demonstrated moderate fluctuations and reductions in SRP levels, with initial concentrations between 5.75 and 7.22 mg/L, increasing at 96 h to 6.92 mg/L, and final concentrations between 4.43 and 4.77 mg/L. Isolate S101G consistently decreased SRP from 8.52-5.70 mg/L. In contrast, S10MG showed a limited reduction in SRP, fluctuating between 7.06 mg/L at 24 h, then decreasing at 48 h and 120 h, and increasing to 6.69 mg/L.

However, the pH utilized for the phosphate solubilization by the isolates (**Fig. 4**) indicates that most isolates exhibited moderate ability to decrease pH levels, ranging from 3.0 to 5.0, within the first 24 h. However, specific isolates displayed relatively higher initial pH decreases, such as S12A, S52B, S61P, S91G, and S101G. As incubation progressed to 48 and 74 h, a general decline in pH was observed across most isolates. During this period, many isolates began to diverge, showing gradual increases in pH. In the later stages, particularly at 96 and 120 h, a recovery in pH was noted for most isolates. By 120 h, isolates like S12A, S91G, and S102W reached pH levels approaching 6.0.

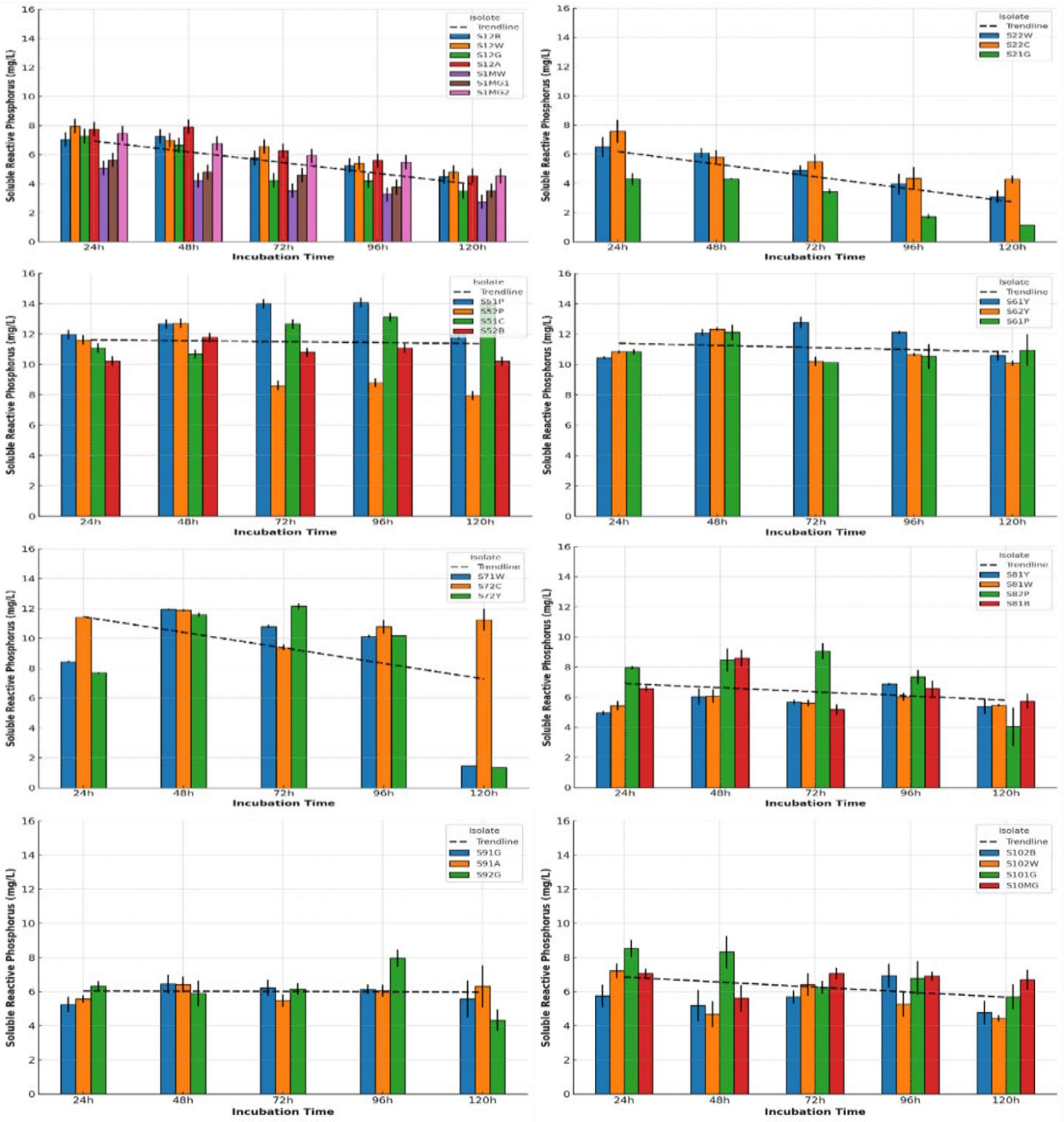

Fig. 3. Concentration of soluble reactive phosphorus (mg/L) across the sampling stations.

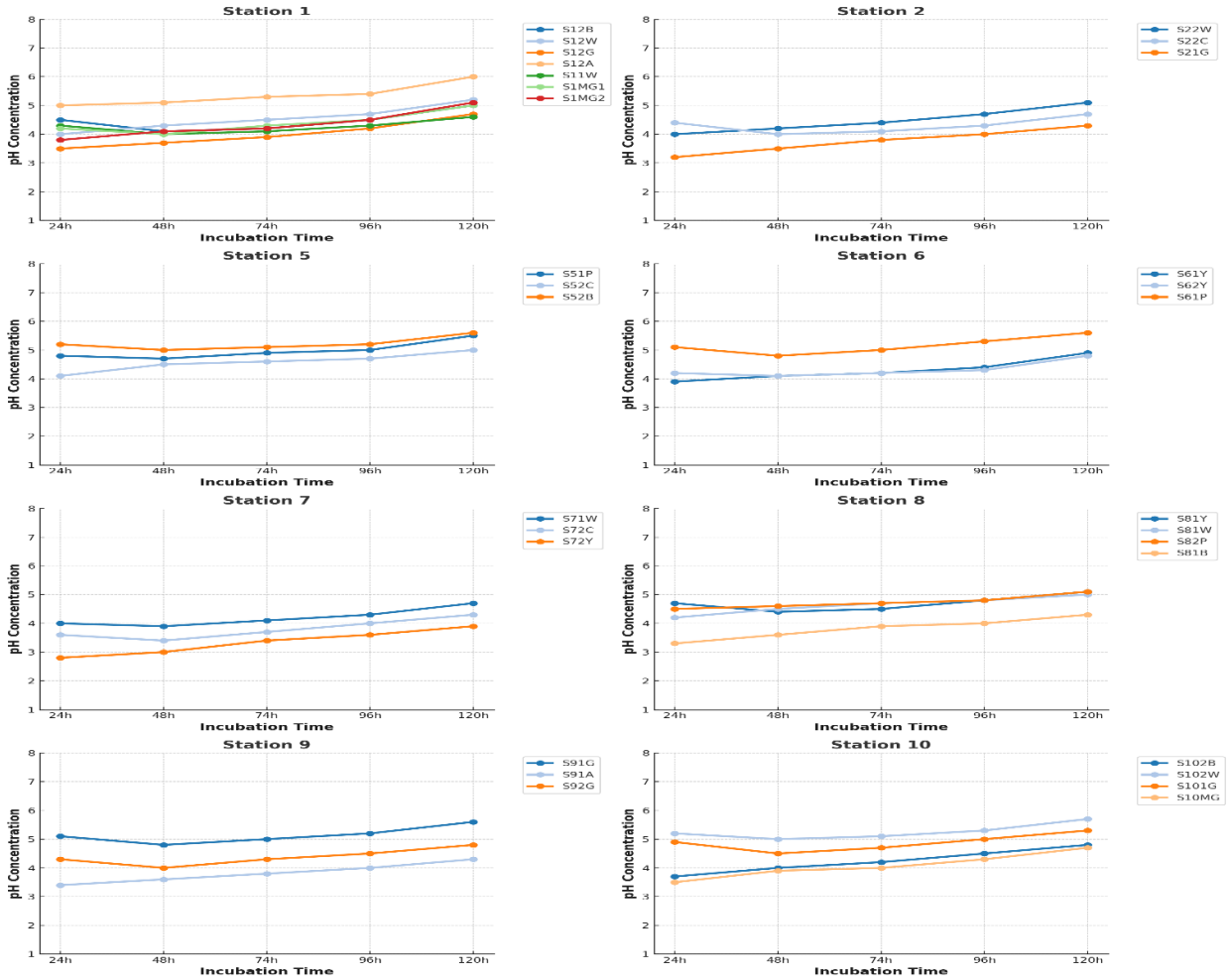

Fig. 4. pH concentration during solubilization of soluble reactive phosphorus (mg/L).

The gradual decline in SRP levels by Station 1 isolates suggests a limited capacity for phosphate solubilization. This may indicate that the microbial isolates from this station are either less efficient at utilizing available phosphorus or are facing nutrient limitations that hinder their metabolic processes [74]. Research shows that microbial phosphate solubilization can be influenced by various factors, including nutrient availability and the composition of microbial communities, which may help explain the observed patterns [75, 76]. In contrast, the initial reduction in SRP at Station 2, followed by stabilization, may reflect shifts in microbial growth phases or changes in nutrient dynamics. Microbial communities often adapt their metabolic strategies in response to fluctuating environmental conditions, and such adaptive responses are critical for maintaining ecological balance and nutrient cycling within aquatic systems [77].

However, the inability of the isolates from stations 3 and 4 to release SRP, despite being subjected to the same experimental conditions as other stations, may be attributed to various ecological and biochemical factors [78, 79]. Microbial community composition at these stations could differ significantly, affecting the phosphate solubilisation of the isolates [80]. IPSB often requires specific environmental conditions like pH, temperature, and nutrient availability to solubilize phosphate [81] effectively. If the isolates from stations 3 and 4 of the Langat River were less adapted to experimental conditions or inherently less efficient phosphate solubilizers, they might not have been able to release SPR during the incubation period [82] significantly.

Similarly, competition among microbial populations for available phosphorus may have hindered SRP reduction at these stations [83]. In environments like the Langat River, where diverse microbial communities coexist, competition for limited resources can restrict the ability of individual strains to solubilize phosphates [84]. If isolates from Stations 2 and 3 encountered intense competition or were outcompeted by more efficient phosphate solubilizers, their phosphate solubilization activity could be diminished. Finally, variations in sediment chemistry and organic matter content across different stations can influence microbial activity and phosphate availability [85]. These factors could have contributed to the observed differences in SRP reduction among the stations.

On the other hand, Stations 5 and 9 maintained higher SRP concentrations, indicating robust solubilization capacity [86]. This enhanced ability may result from favourable metabolic traits among the bacterial isolates or supportive environmental conditions, such as optimal pH and temperature ranges that promote microbial activity [87]. According to [88], various environmental factors, including pH, temperature, nutrient

content, and sediment composition, significantly impact phosphate solubility in river systems. [89] emphasize the crucial role of pH in regulating phosphate dissolution and precipitation. Acidic conditions promote the solubilization of mineral phosphates, while alkaline conditions lead to the precipitation of insoluble phosphate forms, affecting their availability in rivers [90]. Temperature also influences phosphate dynamics by affecting bacterial enzymatic activity. [91] note that optimal temperature ranges enhance the production of organic acids by IPSB, which is essential for mobilizing phosphates from sediments, especially in nutrient-rich environments. The sediment matrix plays a dual role in phosphate dynamics, acting as a sink and a source. High organic matter content in sediments can stimulate IRB activity by providing carbon sources, while minerals like iron and aluminium oxides can bind phosphates tightly, requiring bacterial mediation for their release and bioavailability [92].

The diversity of bacterial taxa in river ecosystems is shaped by factors such as nutrient availability, redox conditions, and external disturbances [93]. Nutrient-rich River systems, often resulting from agricultural runoff, can stimulate microbial activity but may also lead to shifts in community composition towards taxa associated with eutrophication [94, 95]. Redox conditions, determined by dissolved oxygen levels, influence the dominance of aerobic or anaerobic bacteria, which directly impacts phosphorus cycling [96]. Under anaerobic conditions, reducing iron-phosphate complexes can release bound phosphates, creating favourable conditions for facultative anaerobes to utilize these nutrients [97]. Human-induced pressures, such as chemical pollution and sediment disruption, can significantly alter microbial diversity, as the pressures often favour pollutant-tolerant bacteria, potentially reducing overall ecological complexity and resilience in river ecosystems [98].

The variability in SRP reduction observed among the different isolates and stations in the river can be attributed to several factors. Primarily, the diverse bacterial community inhabiting the river, with varying metabolic capabilities, influences phosphate solubilization efficiency [99]. However, environmental conditions coupled with the activities at each sampling station (**Table 1**), such as nutrient levels, pH, and redox potential, also significantly impact bacterial activity and phosphate dynamics [100]. Stations with higher nutrient loads may support increased bacterial growth and activity, leading to more significant phosphate reduction [101]. Additionally, specific minerals or organic matter in the sediment can influence the availability and accessibility of phosphate for bacterial utilization [102].

However, the pH profiles of the isolates across the incubation period (5 days) provide further insights into their phosphate-solubilizing mechanisms. As observed by [103], the initial decrease in pH in most isolates is likely due to the production of organic acids, a common bacterial strategy for solubilizing insoluble phosphate minerals [104]. However, the subsequent increase in pH suggests that the isolates may have depleted their acid-producing capacity or that other metabolic processes, such as alkali production, have become dominant [105]. The variability in pH profiles among the isolates can be attributed to their genetic diversity, which influences their acid-producing capabilities and ability to regulate intracellular pH [106]. Billah et a. [107] reported that bacterial isolates that efficiently utilize available nutrients like phosphorus and maintain optimal growth conditions may exhibit sustained acid production and lower pH levels. Additionally, the type of phosphate mineral present in the medium can influence the effectiveness of acid-mediated solubilization, as some minerals may be more resistant to acid dissolution than others, leading to variations in the extent of pH decrease and phosphate release [108, 109].

Phosphorus acceptability

The 31 isolates with variable phosphate solubilization (mg/L), exposed to the phosphorus acceptability test, resulted in S18B and S92G as the most significant isolates due to their higher phosphorus uptake capacity. Initially, the phosphate levels in the medium with S18B and S92G were recorded at 4.781 ± 0.043 mU/mL and 5.665 ± 0.033 mU/mL, respectively. By the end of the experiment (3 days), S18B had substantially reduced phosphate levels by 64.00%, resulting in a final phosphate concentration of 1.740 ± 0.022 mU/mL. Similarly, S92G showed a significant phosphate uptake, reducing levels by 62.00%, with a final concentration of 2.129 ± 0.102 mU/mL.

The substantial phosphorus uptake efficiency demonstrated by S18B and S92G isolates from Stations 8 and 9 indicates a significant nutrient influx in the lower reaches of the Langat River. This finding aligns with [110], who reported comparable phosphorus uptake capabilities among bacterial species in nutrient-deficient conditions. These bacterial isolates present a promising biological strategy for mitigating inorganic nutrient accumulation, particularly in regions susceptible to nutrient overload [111]. Bacterial isolates, specifically those that can solubilise inorganic phosphate compounds, could have the potential to function as natural bioremediating agents by assimilating and removing excess phosphorus [112], thereby restoring nutrient balance and enhancing overall ecosystem health in river systems [113]. The ability of IPSB to facilitate phosphorus removal in aquatic environments is well-documented [100]. While their phosphorus solubilization capacity may be less pronounced in nutrient-poor systems [114], their remarkable adaptability is evident in the Langat River, a system frequently subjected to nutrient pollution (54).

The uptake of the SRP by S18B and S92G isolates suggests their versatile metabolic strategies, enabling them to thrive in diverse ecological conditions and contribute to phosphorus removal and river ecosystem stability. Their potential applications in bioremediation are promising, particularly in eutrophic water bodies. However, their capacity to solubilize and utilise phosphorus can help mitigate nutrient imbalances [115]. Leveraging these microbial capabilities offers a sustainable approach to reducing environmental impacts, improving water quality, and enhancing the resilience of ecosystems like the Langat River against nutrient-related disturbances [72].

Phosphatase (acid and alkaline) activity

The S18B and S92G showed higher phosphorus uptake activity and significant acid phosphatase activity, with values of 8.49 ± 0.164 mg/L and 7.67 ± 0.228 mg/L, respectively. In contrast, S92G exhibited a higher alkaline phosphatase activity, measured at 9.83 ± 0.679 mg/L, whereas S18B displayed an activity level of 9.17 ± 0.212 mg/L. The observed variability in acid and alkaline phosphatase activities in S81B and S92G isolates provides insights into their extracellular phosphate metabolism under varying pH conditions [116]. The higher acid phosphatase activity demonstrated by S18B compared to S92G suggests its enhanced ability to remove phosphorus in acidic sediments. Conversely, S92G exhibits higher alkaline activity than S81B, reflecting their adaptation to alkaline pH niches within sediment ecosystems [117]. These species-specific enzymatic differences highlight their metabolic versatility and ability to function effectively across the Langat River and diverse environmental

conditions related to water [118]. However, the phosphatase activity levels in S81B and S92G isolates are consistent with observations in other nutrient-rich environments, where phosphatase activities are typically elevated due to extracellular bacterial activities [119]. This suggests that Langat River isolates are well-adapted to utilize enzymatic phosphate removal within their ecological niche, making them potential candidates for phosphorus management strategies in riverine systems [120]. Furthermore, the enzymatic activity of these isolates to diverse pH conditions denotes their potential to enhance phosphorus removal and mitigate ecological impacts, thereby contributing to the long-term sustainability of freshwater ecosystems like the Langat River [121].

Phylogenetic Analysis

Fig. 5 shows the phylogenetic analysis of the 16S rDNA gene sequence of S81B and S92G isolates. S81B demonstrates a close relationship with various *Curtobacteria* species, particularly *Curtobacterium albidum* and *Curtobacterium citreum.* The S81B, with a sequence length of 1453 bp, forms a cohesive branch within the *Curtobacteria* genus, exhibiting high similarity and minimal branch lengths to these species. Further down the phylogenetic tree, the S81B isolates is more distantly related to other *Curtobacteria* species, including *Curtobacterium flaccumfaciens* and *Curtobacterium oceanosedimentum*, which form separate sub-clusters. In contrast, S92G reveals close relationship with several *Bacillus* species, in contrast, S92G reveals close association with several Bacillus species, including *Bacillus tropicus*, *Bacillus albus*, and *Bacillus cereus.* The S92G isolate, with a sequence length of 1480 bp, shares a proximal branch with *Bacillus tropicus*, while being more distantly related to other *Bacillus* species like *Bacillus proteolyticus* and *Bacillus sangunis*, which form separate clusters in the tree.

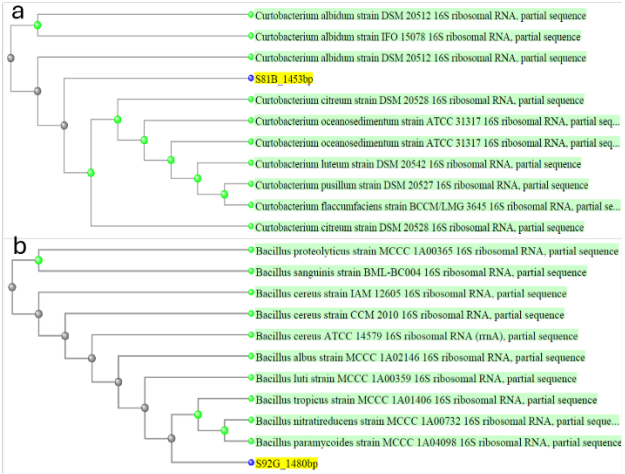

Fig. 5. Phylogenetic trees of (a) S81B and (b) S92G isolates based on amplified 16S rDNA gene sequence with the GenBank nucleotide accession numbers.

The close genetic relationship of S81B isolate with *Curtobacteria* species suggests possession of similar biochemical capabilities, including phosphate solubilization, essential for nutrient removal, especially in phosphorus-limited aquatic environments [122]. However, the clustering of S81B with *Curtobacteria albidum* and *Curtobacteria citreum* with high bootstrap support indicates a shared evolutionary lineage [123]. This shared lineage implies that S81B may utilize organic acids to acidify the environment, enhancing phosphorus solubility, as observed in other *Curtobacteria* species [124].

The potential of IPSB to improve phosphorus availability in various ecosystems, including freshwater environments, has been well-documented [125, 107]. The genetic characterization of these bacteria, including identifying genes responsible for phosphate solubilization, aligns with findings in the *Curtobacteria* lineage [126]. The findings suggest that *Curtobacteria* species could be broadly applied as a biological tool in river ecosystems to regulate phosphorus levels. This could reduce eutrophication in rivers like Langat and enhance water quality and ecosystem stability.

However, the genetic relationship observed in S92G isolate with various *Bacillus* species suggests possession of similar functional traits, such as phosphate solubilization, which is essential for phosphorus removal in river ecosystems [127]. [128] proposed the potential application of *Bacillus* strains in bioremediation strategies for river systems. These bacteria could help mitigate phosphorus scarcity and control eutrophication [129].

Bazinet [130] reported the potential utility of *Bacillus* strains in ecological applications, particularly in river systems. Similarly, [131] have demonstrated the effectiveness of these bacteria in biocontrol and phosphorus management, highlighting their role in sustainable river sediment systems. Therefore, application of the identified *Bacillus* species in river systems could help manage phosphorus levels, thereby addressing eutrophication. This approach aligns with [132], who have emphasized the importance of microbial interventions for sustainable river ecosystems.

The ecological significance of *Curtobacteria* and *Bacillus* species as phosphate solubilizers is well-established, particularly in nutrient-polluted sediment [133]. These bacteria play a crucial role in phosphorus mobilization, a process vital for the health and productivity of river ecosystems [134]. Their ability to enhance phosphorus availability supports the notion that they contribute significantly to nutrient cycling and overall ecosystem function. Furthermore, the phylogenetic clustering of these strains within their respective genera suggests they possess adaptive traits that allow them to thrive in nutrient-polluted river systems [135]. These traits may include efficient phosphorus assimilation and removal mechanisms, which benefit their growth and support the development of surrounding microbial communities by increasing phosphorus availability [136].

CONCLUSION

This study showcases the potential of indigenous *Curtobacteria* and *Bacillus* strains, isolated from Langat River sediment, as effective bioremediation agents, due to their significant inorganic phosphate solubilization and removal efficiency, which is crucial for reducing phosphorus accumulation in the Langat River and similar rivers' sediments. However, the strains' adaptability to varying pH conditions, as indicated by their differential phosphate solubilization activities, broadens their potential for diverse degradation and removal of inorganic phosphate compounds. In addition, their high phosphorus uptake efficiency and tolerance to nutrient fluctuations further support their suitability for phosphate removal in river systems like the Langat River. Future research should focus on field-scale trials to evaluate their efficacy in real-world settings and establish them as a sustainable solution for improving water quality in similar river systems.

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CONFLICT OF INTEREST

No conflict of interest

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