

ACCELERATION OF PARAQUAT BIODEGRADATION  
BY ISOLATED SOIL BACTERIA

*PENINGKATAN BIODEGRADASI PARAKUAT  
OLEH BAKTERI YANG DIISOLASI DARI TANAH*

Erni Martani<sup>†</sup>)

*Faculty of Agriculture, Gadjah Mada University*

Nur Arfa Yanti

*Faculty of Education, Haluoleo University, Kendari*

Sebastian Margino

*Faculty of Agriculture, Gadjah Mada University*

Sri Noegrohati

*Faculty of Pharmacy, Gadjah Mada University*

<sup>†</sup>)Corresponding author, E-mail : martani@faperta.ugm.ac.id

**INTISARI**

Penggunaan herbisida yang tidak tepat dapat mengakibatkan gangguan terhadap ekosistem, dan dampak negatif ini akan berkurang bila degradasi dipercepat. Herbisida parakuat terdegradasi secara lambat di dalam tanah gambut. Penelitian ini dilakukan untuk mengkaji peran bakteri dalam mendegradasi parakuat dalam tanah gambut. Bakteri diisolasi dari berbagai jenis tanah di Indonesia dengan menggunakan teknik pengayaan (enrichment) dalam medium bebas N. Parakuat ditambahkan ke dalam medium ini pada konsentrasi yang ditingkatkan secara bertahap dari 10, 20 hingga 40 ppm. Pengujian kemampuan degradasi dilakukan dalam medium sintetik serta ekstrak tanah gambut fibrik dan saphrik.

Hasil pengamatan menunjukkan bahwa beberapa isolat bakteri memiliki kemampuan mendegradasi parakuat di dalam medium sintetik. Namun tidak ada satupun isolat yang mampu mendegradasi parakuat dalam medium ekstrak tanah gambut. Diduga beberapa faktor lingkungan merupakan penyebab kegagalan degradasi parakuat tersebut. Aktivitas degradasi parakuat meningkat bila medium ekstrak tanah ditingkatkan pH-nya dari 3,5 menjadi sekitar 5,5. Bakteri SM1, yang diisolasi dari tanah sulfat masam Kalimantan Tengah, mampu mendegradasi parakuat dalam ekstrak tanah gambut fibrik sebanyak 30% selama 15 hari. Saat ini sedang diteliti peningkatan degradasi parakuat di tanah gambut menggunakan inokulum campuran yang terdiri atas beberapa isolat bakteri unggul.

Kata-kata kunci : parakuat, degradasi, isolat bakteri.

**ABSTRACT**

Herbicides residues were reported to have impact on the ecosystem. It was thought that acceleration of paraquat degradation would minimize these impacts. Paraquat was persistent in peat soil due to low pH. This study was done to investigate the role of bacterial isolates on the acceleration of paraquat degradation, especially in peat soil. The bacteria were isolated from several kinds of Indonesian soils using enrichment culture technique in a modified N-free medium. The medium was added with paraquat at gradually increase-concentration from 10, 20 and 40 ppm (w/w). Examinations in paraquat degradation were done in two levels. The first was in a synthetic medium (N-free medium); the second was in soil extract medium. Two kinds of peat were used to make the soil extract media, i.e. fibric and saphric peat soils.

Several bacterial isolates were able to degrade paraquat in N-free medium. However, the degradation mode was different with those in peat soil extract media. None of them degraded paraquat in fibric and saphric soil extract media. It was suggested that the

environmental limiting factors were responsible to the failure of paraquat degradation. Two selected isolates were able to degrade paraquat when the pH value of the extract medium was enhanced to around 5.5. Bacterial isolate of SM1, which was isolated from acid sulfate soil of Central Kalimantan, was the best isolate which was able to degrade paraquat in synthetic medium and peat soil extract media, especially in fibric extract medium. It degraded around 30% of paraquat within 15 day. Experiments are being done to enhance paraquat degradation by inoculation of mixed cultures of selected bacterial isolates.

Key words: paraquat, degradation, bacterial isolates

## INTRODUCTION

Weeds are the most severe and widespread biological constraint to agriculture, especially on rice (Naylor, 1996). Herbicides were used to control the growth of these weeds. However, misuse of these agrochemicals influenced the ecosystems and nontarget organisms (Anderson, 1978; Naylor, 1996; Roger & Simpson, 1996).

Paraquat (N, N'-dimethyl bipyridylium dichloride) is an active agent of herbicides widely used in peat land. No degradation of paraquat in peat soil at least for more than 2 months was observed (Margino *et al.*, 2000), as paraquat degradation needs six years in soil (Alexander, 1994). Its persistence in peat might be due to its chemical stability in low pH environments (Anonymous, 1984) and/or ecological factors of peat soil that did not support the growth of paraquat-degrading microorganisms. It is widely known that pH value and nutrient status of peat soil was low. Peat soil also contained some heavy metals and microbial toxic substances, such as phenolic compounds (Sabiham *et al.*, 1997). Persistence of paraquat influenced the population dynamics of soil microorganisms (Katayama & Kuwatsuka, 1992; Margino *et al.*, 2000; Setyaningsih *et al.*, 2001) or inhibited the growth of soybean (Martani *et al.*, 2001) and corn (Martani *et al.*, 2000). It was suggested that acceleration of paraquat degradation, especially in peat soil, would reduce these ecological impacts.

Biodegradation of xenobiotic depends on the density and activity of the xenobiotic

degrading microorganisms. Unfortunately, in some natural environments, xenobiotic degrader was in very low density (Martani & Seto, 1990). Paraquat can be degraded by *Achromobacter* sp., *Clostridium pasteurianum* and *Corynebacterium fascians* (Cripps & Roberts, 1978), *Corynebacterium* sp. (WHO, 1984), *Streptomyces* sp. and *Nocardia* sp. (Cripps & Roberts, 1978; Carr *et al.*, 1985), or soil yeast *Lypomyces starkeyi* (Hatta *et al.*, 1984; Katayama & Kuwatsuka, 1992).

Inoculation of a degrader accelerates xenobiotic degradation (Martani, 1996). In some cases, however, microbial inoculation failed to enhance xenobiotics degradation due to ecological limiting factors, such as pH value and temperature (Alexander, 1999) anti-microbial substances (Goldstein *et al.*, 1985; Martani & Seto, 1991b), or limiting carbon, nitrogen and phosphorous (Martani & Seto, 1990).

This study was conducted to obtain paraquat degrading soil bacterial isolates. These isolates were expected to serve as microbial inoculums to accelerate paraquat degradation, especially in peat soil.

## MATERIALS AND METHODS

**Bacterial isolation.** Six kinds of soils were used as bacterial sources, i.e. three kinds of peat soils (fibric, hemic and saphric), which were obtained from the Province of Central Kalimantan, peat soil from Central Java, acid sulfate soil (from Central Kalimantan) and vertisol from Lombok Island. Paraquat

degrading bacteria were isolated using Enrichment Culture Technique in modified-N-free medium (Katayama & Kuwatsuka, 1992). Modification was done by adjusting glucose and paraquat concentration. Concentration of added paraquat was enhanced gradually from 10, 20 up to 40 ppm. At the same time, glucose concentration was gradually reduced. The growing colonies were transferred to 50%-diluted nutrient agar slant containing 20 ppm paraquat.

**Degradation experiments.** Degradation observations were done in two levels. The first was in synthetic medium (N-free medium) (Katayama & Kuwatsuka, 1992); and the second was in peat soil extract media. Fibric and saphric peat soil were used for making these extract media. The isolate was inoculated at the density of  $10^6$  cell/mL. The initial paraquat concentration was 20 ppm. Incubation was conducted on a 125 rpm shaker at room temperature (29 – 31 °C). Capability to degrade paraquat was measured based on paraquat residue and bacterial growth. Cell number was measured using Total Plate Count on Modified Nutrient Agar.

**Paraquat and residual analyses.** Gramoxone® (Zeneca Ltd; active agent 200 mg of paraquat di chloride/L) was used for bacterial isolation and degradation experiment. For analyses of paraquat residue in degradation experiments, paraquat standard was used (99.5%). Paraquat residue was analyzed periodically using UV-spectrophotometric measurement (Anderson & Drew, 1971).

## RESULTS AND DISCUSSION

**Bacterial isolation.** First, bacterial isolation was focused on the paraquat degrader found in peat soils obtained from Central Kalimantan. Three kinds of Kalimantan peat soil were used, i.e. fibric, hemic and saphric peat soils. Saphric is the most advanced decomposed peat soil, followed by hemic and fibric. Another peat soil from Central Java was used also. However, the numbers of isolates from these soils were only nine (Table 1). Therefore, other soils were also selected as source of isolates. Acid sulfate soil was chosen due to its similar pH value with peat soil, which was around three. Vertisol from Lombok Island was chosen due to the wide use of paraquat in this province. All of isolates were kept on 50%-diluted nutrient agar added with paraquat to keep their degradation activity. Viability examination shown that two isolates (F2 and VLT4) could not grow well after transferred several times into new medium. Therefore, only 12 of the isolates were used for degradation experiment.

Due to the environmental limiting factors, scarcity of paraquat degrading bacteria in peat soil has been expected. The low number of paraquat degrading bacteria might be responsible to the persistence of paraquat in peat soil (Martani & Seto, 1991b). Degradation of 2,4-dichlorophenol in groundwater was very slow when the initial number of degrader was  $10^4$  cell/mL, and very fast when the degrader density was inoculated at  $10^6$  cell/mL. As reported by Margino *et al.* (2000), no paraquat degradation was detected in peat soil within 2 months.

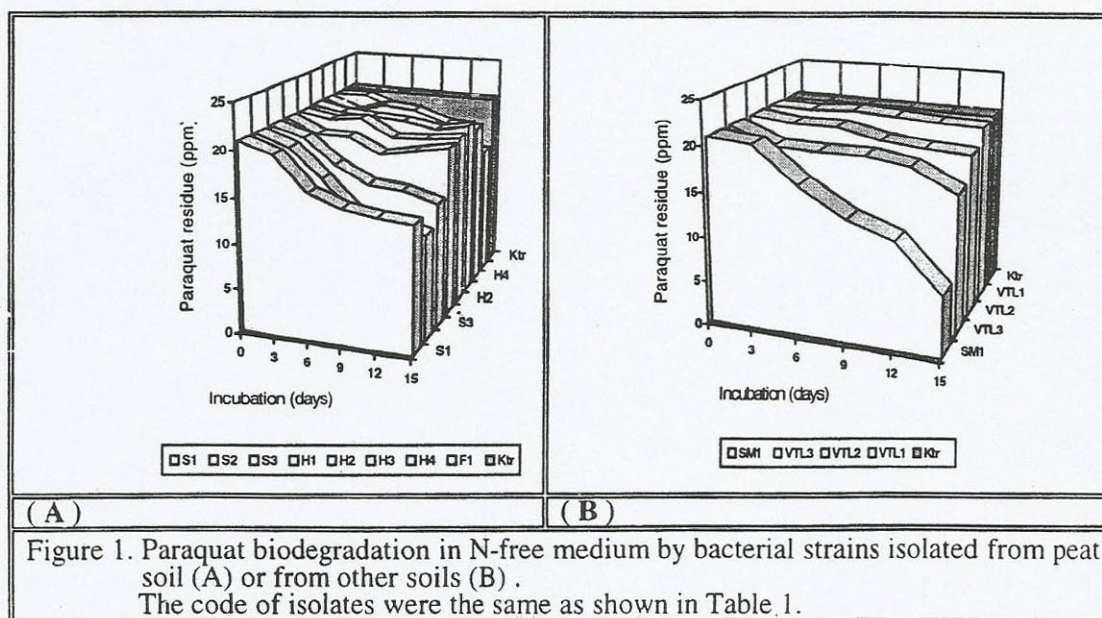
Table 1. Soil Bacterial Isolates from Kalimantan, Java and Lombok

No.	Soil Samples	Locations	Code	Number of Isolates
1.	Fibric Peat Soil	Pangkoh, Central Kalimantan	F	2
2.	Hemic Peat Soil	Pangkoh, Central Kalimantan	H	4
3.	Saphric Peat Soil	Pangkoh, Central Kalimantan	S	3
4.	Peat Soil	Rawa Pening, Central Java	--	0
5.	Acid Sulfate	Palingkau, Central Kalimantan	SM	1
6.	Vertisol	Pujut, Lombok	VTL	4
Total Bacterial Isolates				14

**Paraquat biodegradation experiment in modified N-free medium.** The first degradation experiment was done in modified N-free medium. This medium was used to examine the ability of isolate to use paraquat as carbon and/ or nitrogen source (Katayama & Kuwatsuka, 1992). The results showed that paraquat did not degrade in non-inoculated medium. Bacteria isolated from saphric peat soil have the highest ability to degrade paraquat compared to other peat soil isolates. The isolates of S2 and S3 degraded 44% and 33% of paraquat, respectively (Fig. 1 A). All of the hemic soil isolates (H1, H2,

H3 and H4), and also VTL1 which was isolated from vertisol, could not degrade paraquat. The F1 isolate degraded 28.5% of paraquat. An acid sulfate isolate, SM1, degraded 67.7% of the herbicide (Fig. 1 B). VTL2 and VTL3 degraded paraquat 11% and 24.5%, respectively.

Katayama & Kuwatsuka (1992) isolated a soil yeast of *Lypomyces starkeyi* which degraded paraquat as it was the sole nitrogen source. This paraquat degrading yeast was extensively studied (Carr *et al.*, 1985; Hatta *et al.*, 1986). In this study, four soil paraquat degrading bacterial isolates, i.e. S2, S3, F1, and SM1; were selected out from 12 isolates (Fig. 1).



**Paraquat biodegradation in peat soil extract media.** In the second step, these selected strains were examined on their ability to degrade paraquat in peat soil extract medium. Two kinds of media were used, there were fibric and saphric peat soil extract media. The medium decomposed peat soil, hemic, was used only as isolate source, and not for degradation experiment. The selected isolates in these extract media were examined their ability in adaptation to peat soil conditions, such as pH value and nutrient status. Characteristics of the media were shown in Table 2. The pH value of fibric and saphric peat soil extract media were 3.5 and 3.7, respectively. The ratio of C-N of fibric was much higher than those of saphric peat soil due to the higher concentration of total N.

Isolates of SM1, F1, S2 and S3 were inoculated separately into the media at initial density of  $10^6$  cell/mL. Paraquat degradation was detected neither in inoculated nor in non-inoculated media. Its means that the selected isolates could not degrade paraquat in fibric and saphric peat soil extract medium (Fig. 2). It was suggested that low pH value was responsible for the failure of paraquat degradation. Alexander (1999) reported that biodegradation of xenobiotic compounds were influenced by the environmental pH value. Paraquat is stable in acid environments (Anonymous, 1984), due to the chemical reaction between positive charges of paraquat with negatively charged of organic matters in peat soil. As shown in Table 2, pH values of fibric and saphric extract media were 3.5 and 3.7, respectively.

Table 2. Characteristics of soil extract media

Parameter	Fibric	Saphric
pH value	3.50	3.70
C-organic (%)	68.53	63.00
Total N	1.99	3.98
C-N ratio	34.44	15.83

Based on those considerations, we adjusted pH value of media up to around 5 by adding lime  $\{Ca(OH)_2\}$  to the fibric and saphric soil extract media. Figure 3 shows that microbial inoculation accelerated paraquat degradation. SM1 and S2 degraded paraquat partially, especially in fibric peat soil. Its means that increasing pH value in the media enhancing paraquat degradation. Liming treatment enhanced pH value, and indirectly influenced the availability of nutrients (Subiksa, *et al.*, 1997). Liming also reduced the concentration of toxic substances, such as phenolic compounds (Sabiham *et al.*, 1997). These conditions should support the growth and activities of microorganisms.

Margino *et al.* (2000) reported that the increase of pH value supported the growth of microorganisms in peat soil. It was suggested that enhancement of microbial growth was caused by supporting conditions in their micro-environments. The increase of microbial growth, especially the paraquat degrader, would enhance paraquat degradation. In spite of that, instability of paraquat in relatively high pH value may also responsible to the acceleration of paraquat degradation in soil extract medium added with NaOH.

Comparing the paraquat degradation in N-free medium (Fig. 1), it might be seen that the ability of paraquat biodegradation in soil extract medium was much lower, especially by the isolates of SM1 and S2. In N-free-medium, SM1 and S2 degraded paraquat as high as 67.7 and 44%, respectively (Fig. 1). However, in peat soil extract media, they only degraded a little amount of paraquat. These data reflect that environmental conditions in soil extract influenced the ability of isolates to degrade paraquat. Nutrient status in N-free medium was better than those in soil extract medium. Research on 2,4-DCP degradation reported by Martani and Seto (1991a), supported the data obtained in this study. Xenobiotics degradation is depends highly

on the environmental condition where the chemical exposure happened (Alexander, 1999; Goldstein *et al.*, 1985). Therefore, xenobiotics degrader should show its ability in adapting natural conditions before it was inoculated into environments. Without this ability, acceleration of degradation would not be detected.

The growth of these selected isolates in limed soil extract media were shown in Fig. 4. They grew from  $10^6$  level to  $10^{15}$  within 15 days. During this period, however, degraded paraquat was less than 30% (Fig. 3). These data showed that isolates grew especially by using C sources other than paraquat. As shown in table 2, the fibric and saphric extract media consist of C organic as high as 68.53 and 63.00%, respectively.

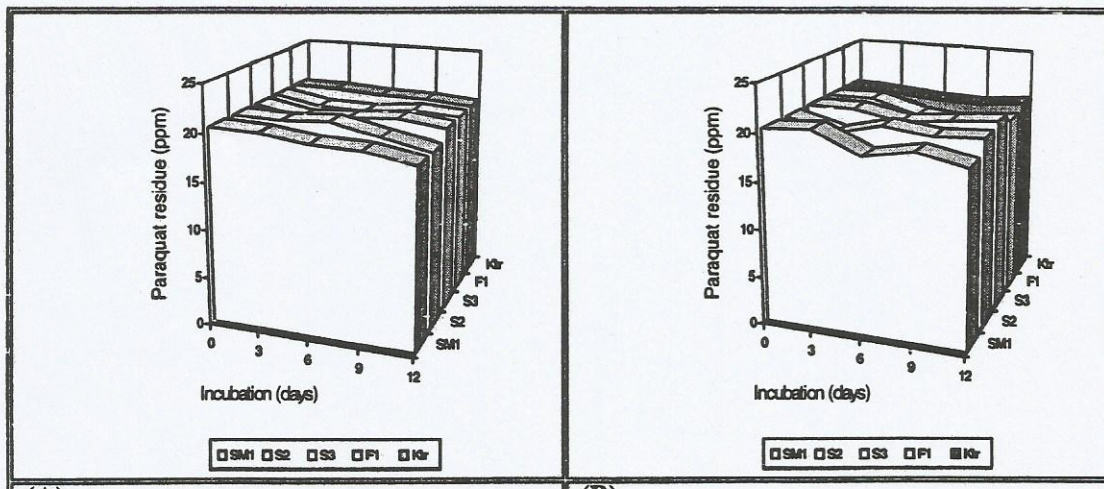


Figure 2. Biodegradation of paraquat in fibric (A) and saphric(B) extract media without liming treatment.

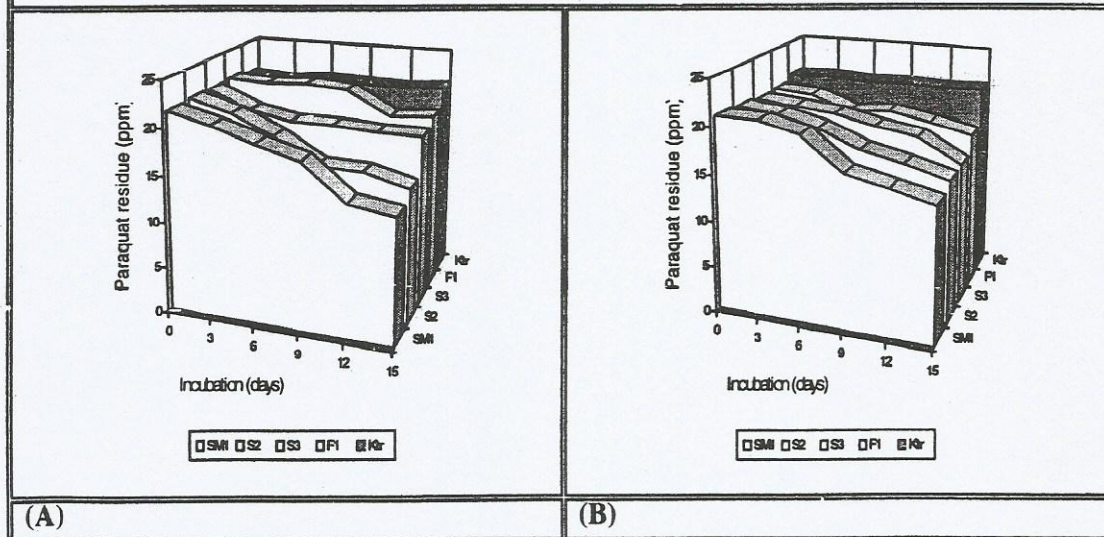


Figure 3. Biodegradation of paraquat in fibric (A) and saphric(B) extract media with liming treatment.

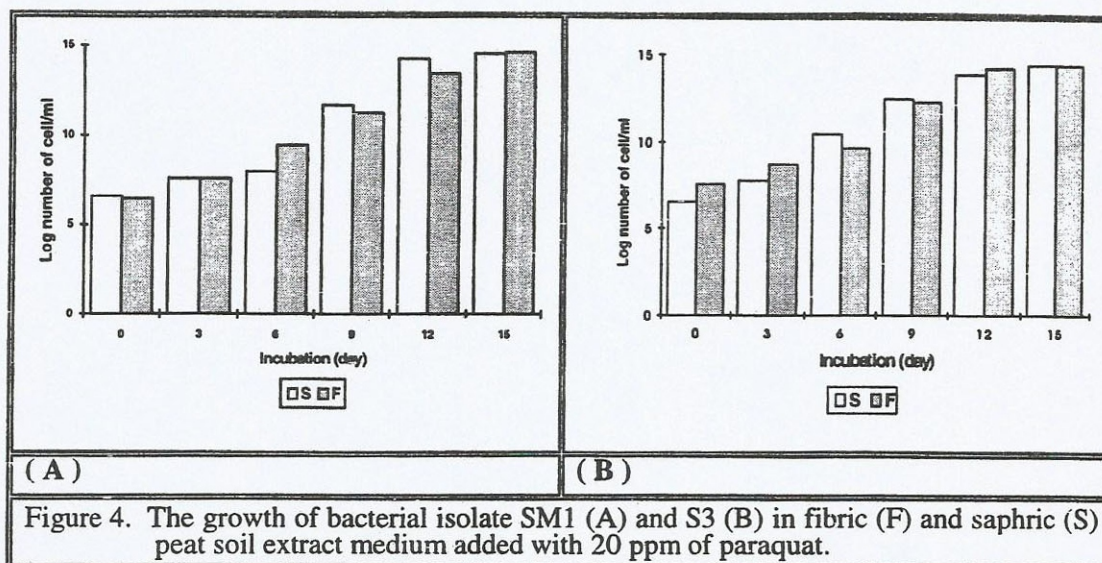


Figure 4. The growth of bacterial isolate SM1 (A) and S3 (B) in fibric (F) and saphric (S) peat soil extract medium added with 20 ppm of paraquat.

Some xenobiotics were degraded by means of preference or by co-metabolism. In these mechanisms, availability of alternative carbon sources other than the xenobiotic itself is obligatory. Several researches showed that paraquat could be degraded by microorganisms and used as N source (Katayama & Kuwatsuka, 1992; Carr *et al.*, 1985; Hatta *et al.*, 1986). As shown in Table 2, soil extracts media containing carbon and nitrogen in a high enough concentration for microbial growth. Due to these data, it was suggested that the isolates grew especially by using carbon and/or nitrogen sources other than paraquat.

Although these isolates could not degrade paraquat as the sole of C and/or N sources, the decrease of paraquat residue is one of advantages in reducing the negative impacts of paraquat to environmental biota.

The isolates of SM1 and S2 were able to reduce paraquat residue in peat soil extract media treated with lime. Microscopic examination showed that SM1 is a gram-negative rod shape bacteria, while S2 is gram-negative coccus bacteria. With some additional technologies, they have the potency as microbial inoculum, singular or in mixed cultures forms, to accelerate

paraquat degradation. To minimize environmental impacts of paraquat, currently we are trying to accelerate paraquat degradation in peat soil by using these two isolates as inoculum.

## CONCLUSIONS

1. Paraquat degrading bacteria could be isolated from many kinds of soils. Some of them degraded paraquat only in synthetic medium, but lost their ability in peat soil extract media.
2. Degradation of paraquat by isolates was influenced by pH value of the medium. The pH value below 4 was the limiting factor for paraquat degradation.
3. An acid sulfate bacterial isolate, SM1, has the highest ability to degrade paraquat in synthetic medium and peat soil extract media. Another prospective isolate was S2, which was isolated from saphric peat soil.

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