SELECTION OF BACTERIAL CONSORTIUM FOR THE DEGRADATION OF HEXACHLOROBENZENE

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ABSTRACT

The degradation of hexachlorobenzene (HCB) poses a significant environmental challenge due to its persistence and toxicity. Bacterial consortia, comprised of multiple microbial species, offer a promising solution for HCB biodegradation. Effective strains are combined to create consortia that work synergistically. In this sense, this study aimed to construct and select bacterial consortia with the potential to degrade hexachlorobenzene, by bacterium growth in a pesticide-containing medium, biochemical tests (catalase and oxidase), consortium antagonism tests, and analysis of the consortium growth in HCB-containing medium. Based on the results, two bacterial consortia (C6 and C7) with efficient HCB degradation capacity were selected. Applying bacterial consortia can mitigate the environmental impacts of HCB, promoting the bioremediation of areas contaminated by this organochloride.

Keywords: Hexachlorobenzene. Degradation. Bacterial consortium.

1 INTRODUCTION

Hexachlorobenzene (HCB) is an organochlorine pesticide class that promotes a significant environmental impact. This compound is classified as a persistent organic pollutant (POP) and was included by the Stockholm Convention in 2001 among the 12 recalcitrant compounds[1]. HCB has a half-life of approximately six years in sediments and water. The poor disposal management, slow degradation, and residual effect of HCB leads to contamination of aquatic ecosystems, affecting various life forms. This impact makes exploring diverse HCB treatment strategies a priority [2].

Bioremediation is considered an accessible and environmentally friendly strategy, by utilizing microorganisms as biological agents to metabolically degrade toxic compounds to complete mineralization, thereby reducing the emergence of toxic by-products [3,4]. In this context, consortia are considered efficient bioremediation tools.

The term "consortium" usually refers to a group of diverse microorganisms that can act synergistically without antagonism in a community. The use of microorganisms in the form of consortia represents a viable, efficient, and controlled alternative for degrading pesticides, considering the protein/enzymatic roles and activity of each microorganism [5]. Therefore, bacterial consortia enhance bioremediation, minimizing pollutant toxicity, making it a promising, cost-effective, and accessible method for treating compounds like HCB.

2 MATERIALS & METHODS

Activation of Microorganisms: Growth in HCB and Biochemical Tests

Three bacterial strains, *Bacillus sp.* (M1 and M4), *Bacillus cereus* (M6), and *Pseudomonas sp.* (P. sp.), previously isolated and cultivated in LB medium, were activated in LB and minimal salts medium (MSM) at 28°C for 24 h. The bacterium growth in HCB was assessed on Petri dishes with MSM agar medium supplemented with an ethanolic solution of 4 µM HCB as the sole carbon source and incubated at 28°C for 72 h. The cultures were maintained at -20°C in glycerol medium.

To detect catalase activity in the isolates, a bacterial colony was placed on a microscope slide (2.6 × 7.6 cm) using a bacteriological loop, and a drop of 3% (v/v) hydrogen peroxide (H_2O_2) was added. The formation of bubbles, indicating oxygen release, was considered a positive test [6]. For the oxidase test, a colony from each isolate was placed on the reactive surface of an oxidase test strip for 2 min, following the manufacturer's instructions (Brazil's Probac, São Paulo). A bluish-violet color indicated a positive reaction for oxidase [7].

Construction of Bacterial Consortium: Antagonism Test and Growth Analysis in the Presence of HCB

For the antagonism test, bacteria selected for their HCB-degrading capacity were inoculated in duplicate on MSM-agar medium supplemented with 4 µM HCB, while cultivation in MSM + LB was used as a positive control [8]. Specifically, 10^4 CFU of each microorganism were inoculated into MSM + HCB-agar and MSM + LB-agar and incubated (28°C/ 24 h). Agar blocks (Ø = 6 mm)

containing the microorganism were then transferred and placed inverted at equidistant positions on the test plates. The plates were incubated at 28°C for 24, 48, and 72 h to determine the presence or absence of inhibitory halos around each block, indicative of antagonism.

Bacterial consortia were formed from individual cultures of bacterial isolates in LB medium (28°C/24h) to obtain a pre-inoculum with 1,5 x10^8 in sterile saline solution [9]. Seven consortia were prepared in MSM with 4 µM HCB and grown at 28°C for 72 hours at 200 rpm. To construct double consortia, 500 µL of each isolate (0.75 × 10^8 cells/mL) were inoculated into 9 mL of MSM + HCB. For the consortium of four isolates, 250 µL of each isolate (0.375 × 10^8 cells/mL) were used. Bacterial growth was monitored by turbidimetry at 540 nm every 24 hours [10]. Data were expressed as mean ± SD and analyzed using the Tukey-Kramer post hoc test, with p values ≤ 0.05 considered statistically significant.

3 RESULTS & DISCUSSION

Growth of Microorganisms in Hexachlorobenzene

Figure 1A shows the activation of the bacterial isolates, and Figure 1B presents the growth capacity of these isolates in medium supplemented with 4 µM HCB. All microorganisms exhibited the ability to grow in a medium with HCB. These results are consistent with the study of [9] which highlights the metabolic capacity of these bacteria *(Bacillus sp., B. cereus* and *Pseudomonas sp.)* to use HCB as a carbon source.

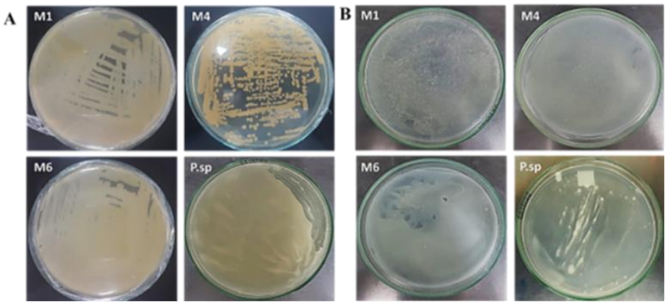

Figure 1 Bacterial isolates with HCB-degrading capacity after 72 h of growth. **A:** MSM+LB; **B:** MSM+HCB. **M1:** *Bacillus* sp.; **M4:** *Bacillus* sp.; **M6:** *Bacillus cereus*; **P. sp:** *Pseudomonas* sp.

Biochemical Oxidase and Catalase Tests

Qualitative enzymatic tests showed that only isolate M1 (*Bacillus sp*.) was oxidase-negative, while all isolates exhibited a catalase-positive reaction. Positive reactions for these enzymes indicate bacteria with capacity to produce oxidoreductases, involved in degradative mechanisms of aromatic rings present in several recalcitrant compounds, such as HCB. This oxidation-reduction generates simple molecules that can be metabolized by bacteria to ATP, CO_2 and water [11,12]. Table 1 shows the results of the qualitative assessment of catalase and oxidase tests, as well as the gram type of each isolate.

Table 1 Gram stain and enzymatic oxidase and catalase tests of isolates with HCB degrading capacity.

Tests	*Bacillus* sp.	*Bacillus* sp.	*Bacillus cereus*	*Pseudomonas* sp.
Gram stain	+	+	+	-
Oxidase	–	+	+	+
Catalase	+	+	+	+

Construction of Bacterial Consortium

No antagonistic activity was observed between the bacteria, enabling the construction of effective, diverse, and synergistic consortia to HCB degrading. Table 2 shows the result of the consortia composition and antagonism assay.

Table 2 Construction of bacterial consortium and antagonism assay after 72 h of growth. (-) indicates non-formation of inhibitory halos between microorganisms. **M1:** *Bacillus sp*.; **M4:** *Bacillus sp*.; **M6:** *Bacillus cereus*; **P. sp:** *Pseudomonas sp*.

Bacterial Consortia	Consortia composition	Antagonistic activity
C1	P. sp + M1	-
C2	P. sp + M4	-
C3	P. sp + M6	-
C4	M1+M4	-
C5	M1+M6	-
C6	M4+M6	-
C7	P. sp+M1+M4+M6	-

The growth of isolates and consortia is shown in Figure 2. The bacterial consortia at 72 h showed higher optical density (OD) values, indicating high growth than bacterium isolates, except for the isolated P. sp. (*Pseudomonas sp*.). Although P. sp. isolate has shown low growth in the first hours of cultivation, in 72 hours it showed greater growth, indicating high potential to degrade HCB. These results confirm that microbial communities constructed present functional and robust microorganisms with efficient metabolic to degradate HCB.

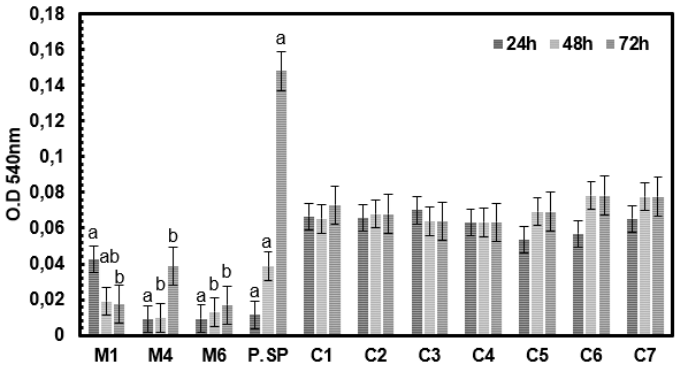

Figure 2 Turbidimetric determination showing the growth of bacterial isolates and bacterial consortia at intervals of 24h, 48h, 72h. Different letters denote significant differences in growth between microbial isolates for each time interval. p>0,05.

4 CONCLUSION

All selected isolates showed the ability to use HCB as a carbon source, probably due to the action of oxidative enzymes, which was qualitatively evidenced in biochemical oxidase and catalase assays. No antagonistic activity was observed in the constructed consortia. Consortia C6 (*Bacillus sp*. + *B. cereus*) and C7 (*Bacillus sp*. + *B. cereus* + *Pseudomonas sp*.) showed the greatest growth in 48 h of cultivation. Thus, the results of this work demonstrate the efficiency of microbial consortiums for treating organochlorine pesticides such as HCB.

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