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Biodegradation of commercial cypermethrin by microorganisms isolated from agricultural soils exposed to pyrethroid pesticides

Biodegradación de cipermetrina comercial por microorganismos aislados de suelos agrícolas expuestos a pesticidas piretroides

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ABSTRACT

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The increasing use of pesticides in agricultural systems is a major contributor to water body pollution. Cypermethrin, a highly toxic insecticide for aquatic organisms, is one of the most commonly sprayed pesticides in small agricultural towns in Bolivian valleys. Therefore, eco-friendly strategies are required to degrade this pesticide. Microorganisms capable of degrading cypermethrin could be used for the remediation of soil and water systems. In this work, fungal and bacterial strains from agricultural soils (Tahuapalca, Bolivia) with the ability to grow with cypermethrin as their sole carbon source were isolated. Their ability to degrade pesticides under static and agitated conditions was studied. Three microbial isolates successfully degraded 74.5% of cypermethrin (1.5 g L^{-1}) in 41 days under static conditions. Agitation during incubation significantly enhanced degradation activity by fungal isolates, achieving 95% degradation of cypermethrin within 10 days. The presence of sucrose as an extra carbon source did not improve cypermethrin degradation under agitated conditions.

RESUMEN

Palabras clave:
Bacteria
Bioremediación
Cinética de degradación
Hongos
Piretroide

El uso creciente de pesticidas en los sistemas agrícolas es una de las principales fuentes de contaminación de los cuerpos de agua. La cipermetrina, un insecticida altamente tóxico para los organismos acuáticos, es uno de los pesticidas más comúnmente aplicados en pequeñas localidades agrícolas de los valles bolivianos. Por lo tanto, se necesita una solución ecológica para degradar este pesticida. Los microorganismos capaces de degradar este compuesto podrían ser una alternativa para biorremediar sistemas de agua y suelos. En este trabajo, se aislaron cepas fúngicas y bacterianas de suelos agrícolas (Tahuapalca, Bolivia) con la capacidad de crecer con este pesticida como su única fuente de carbono. Se determinó su capacidad para degradar el pesticida en condiciones estáticas y con agitación. Tres de los aislados microbianos degradaron con éxito el 74,5% de la cipermetrina (1,5 g L^{-1}) en 41 días en condiciones estáticas. La agitación durante la incubación mejoró significativamente la actividad fúngica de degradación, logrando una degradación de hasta el 95% de la cipermetrina en 10 días. Por otro lado, se observó que la presencia de sacarosa como fuente adicional de carbono no mejoró significativamente la degradación de este pesticida en condiciones de agitación.

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The economy of most small towns and villages in Bolivia relies entirely on agriculture. Therefore, when an insect infestation emerges, many farmers immediately respond with the application of pesticides regardless of their toxicity, dispersion and pollution capacity (Barrón Cuenca et al. 2020; Tirado 2018). Small villages in the La Paz valley benefit from warm climates and an adequate water supply through irrigation grooves, which allows the production of vegetables all year long (Copa Bobarin 2011). However, since crops are grown on plots with moderate to strong slopes, these irrigation practices could cause carriage of pesticides down the hill towards rivers, creating water pollution.

Cypermethrin, a pyrethroid pesticide, is one of the most used agrochemicals in the region to control insect plagues. Cypermethrin is highly toxic for several aquatic life forms; concentrations lower than 1 ppm can be lethal (Farag et al. 2021; Velisek et al. 2006), especially for microcrustaceans such as the freshwater prawn (*Palaemonetes argentinus*) (LD_{50}=3.1x10^{-6} ppm) (Collins and Cappello 2006; Domagalski et al. 2010). Furthermore, cypermethrin has a remarkable persistence (1 to 30.8 days) in water and river currents (Kansal et al. 2023; Mugni et al. 2011).

Water and soil polluted with pesticides like cypermethrin can be bioremediate through microbial biodegradation (Malla et al. 2022; Singh et al. 1999). This can be achieved because microorganisms have the capacity to feed on pesticides as their only carbon source (Tallur et al. 2008; Wang et al. 2010). Certain microorganisms, such as *Streptomyces aureus* and *Bacillus cereus,* can completely degrade pyrethroid pesticides such as deltamethrin (Chen et al. 2011) and cypermethrin (Narayanan et al. 2020) under controlled conditions. Microorganisms native to polluted soils have being in contact with pollutants like pesticides and maybe already tolerant to pollutants (Gangola et al. 2023). They have also the advantage to be adapted to the soil conditions where they have been found and when isolated can perform better as bioremediation agents.

Because of the polluting effects of cypermethrin in the La Paz valley and its surroundings, research on native microorganisms that can degrade this agrochemical in agricultural soils is needed. Therefore, as a first step towards cypermethrin bioremediation, the discovery of cypermethrin-resistant microorganisms is needed. Thus, the aim of the present study is to isolate cypermethrin-resistant microorganisms from agricultural soils and to evaluate their ability to degrade cypermethrin under different conditions such as agitation and alternative source of carbon supplementation.

MATERIALS AND METHODS

Chemicals and reagents

Sodium hydroxide, potassium diacid phosphate, ammonium nitrate and magnesium sulphate were purchased from Scharlau (Scharlab, Barcelona, Spain). Potassium chloride, iron sulfate, picric acid and sodium carbonate were purchased from BioPack (Zárate, Bs. As., Argentina). Sucrose and dextrose were purchased from Sigma Aldrich (St. Louis, MO, USA). Cypermetrhin (200 g L^{-1}) (Cypertrin 200, TQC, Peru) was purchased from a local agricultural store.

Isolation of microorganisms

Soil samples (5 g) were taken from agricultural soils in Tahuapalca (La Paz, Bolivia). Thirty-eight fields were sampled from three agricultural zones within the Tahuapalca community (16°43'02.3"S, 67°52'28.6"W). One gram (1 g) of every soil sample was used to inoculate 20 mL of minimal culture medium (MCM; KH_2PO_4, 1.4 g L^{-1}; NH_4NO_3, 10 g L^{-1}; $MgSO_4 7H_2O$, 0.1 g L^{-1}; KCl, 0.5 g L^{-1} and $FeSO_4$*$7H_2O$, 0.01 g L^{-1}) supplemented with cypermethrin (1.5 g L^{-1}) as the sole carbon source. The flasks were incubated at 25 °C without shaking. After one week of incubation, these cultures were used as inoculum for flasks containing 20 mL of MCM supplemented with cypermethrin (1.5 g L^{-1}), again, as the sole carbon source. Incubation of this new group of flasks was performed for one additional week under the same conditions. Then, 200 µL from each culture were used as inoculates for potato dextrose agar (PDA) (Made in-house, Potato broth 200 g L^{-1}, Dextrose 20 g L^{-1}, Agar 15 g L^{-1}) plates that contained either the antibiotic ampicillin (1 g L^{-1}) (Laboratorios Bago, La Paz, Bolivia) or the antifungal fluconazole (1 g L^{-1}) (La Santé, Bogotá, Colombia). The plates were incubated at 25 °C for a week for bacteria and two weeks for fungi. Representative isolates were selected for further analysis. Methodology was adapted from R Foster et al. (2004).

Quantification of cypermethrin

Cypermethrin quantification was done with a method

newly developed here. The method aims to detect the cyanide group of Cypermethrin by its hydrolysis and later a colorimetric reaction that can be analysed by spectrophotometry. Thus, one milliliter (1 mL) of each MCM supplemented with cypermethrin was mixed with 1 mL of 5 M sodium hydroxide, and the mixture was left to react for 15 minutes in order to achieve complete hydrolysis. The resulting mixture was centrifuged at 4,500 rpm for 10 minutes. The supernatant was carefully extracted and transferred to a new tube, to which 1 mL of sodium picrate was added. Sodium picrate was prepared by mixing picric acid [0.05 M] with sodium carbonate [0.5 M] in a 1:1 proportion (pH=11.2) (Oliveros-Bastidas et al. 2009). The reaction was left to develop for 120 min. Immediately after the reaction time ended, the absorbance of the sample was read at 555 nm with a UV-Vis Spectrophotometer (Quimis, Q798U, São Paulo, Brazil). The timing of this last step is key because the color is stable over a short period of time (Between 110 to 140 min after the beginning of the reaction). Quantification was performed with a six-point standard curve which had a reliable correlation coefficient (r=0.9993). The cypermethrin concentrations applied for development of the standard curve were 100, 250, 500, 750, 1,000 and 1,250 g L^{-1}. The limit of detection was 50 g L^{-1}.

Microbial degradation of cypermethrin under static conditions

Fungal isolates UBAF-004 and UBAF-005, as well as bacterial isolates BBAC-001 and BBAC-002, were inoculated with a sterile inoculation loop to flasks that contained 20 mL of MCM supplemented with cypermethrin (1.5 g L^{-1}) as the sole carbon source. The samples were incubated at 25 °C on a static platform in a shadowed area under the lab bench. For cypermethrin quantification, MCM samples were extracted from each flask after 1, 2, 5, 9, 13, 17, 20, 29, 34, and 41 days post-inoculation (dpi). At least three flasks were evaluated for each time point simultaneously. Two non-inoculated control flasks were tested: Control B was opened every time the inoculated flasks were sampled, and Control A was opened only on the last sampling day (41 dpi).

Microbial degradation of cypermethrin with agitation

Fungal isolates UBAF-004 and UBAF-005, as well as bacterial isolate BBAC-002, were inoculated to three flasks each. The flasks contained 20 mL of MCM with cypermethrin (1 g L^{-1}) as a sole carbon source. They were incubated at 25 °C on an orbital shaker (150 rpm) with a transparent plastic cover. Samples of liquid growth culture were extracted after 1, 5, 10, 20 days after inoculation to examine the cypermethrin concentration. At least 3 flasks were evaluated for each time point simultaneously; the control had the same treatment without inoculation.

Microbial degradation of cypermethrin in culture media containing a supplementary carbon source

Fungal isolates UBAF-004 and UBAF-005 were tested under similar conditions as section microbial degradation of cypermethrin under static conditions as described above, with the exception that MCM was supplemented with sucrose (1 g L^{-1}) in addition to cypermethrin. The bacterial isolate BBAC-002 was also tested, but the MCM was supplemented with dextrose (2 g L^{-1}) in addition to cypermethrin. At least three flasks were evaluated for each time point simultaneously; the control had the same treatment without inoculation.

Data analysis

The effect of different strains on cypermethrin degradation was assessed using a one-way ANOVA. The response variable was the cypermethrin concentration in liquid culture media measured on the final sampling day. Tukey's HSD post hoc test identified differences among treatments. Analyses were conducted in R version 4.4 (R Core Team 2023) and RStudio 2024 (Posit Software, PBC). Images were generated using the plyr, forcats, multcomp, cowplot, and ggplot2 packages (Wickham 2009).

RESULTS AND DISCUSSION

Screening and isolation of cypermethrin-degrading microorganisms

The growth of at least one fungal colony in 10 out of 36 plaques that contained cypermethrin as the sole carbon source and ampicillin was observed. Bacterial colonies were observed in 13 out of 36 plaques that contained cypermethrin and fluconazole. After subculturing the colonies that were observed in the initial screening, two bacterial (BBAC-001 and BBAC-002) and two fungal (UBAF-004 and UBAF-005) isolates were obtained that were used for the following assays (Figure 1). The screening results showed that several microbial strains

isolated from Tahuapalca agricultural soils could survive in the presence of high cypermethrin concentrations and use it as the sole carbon source in the growth media as described before (Kansal et al. 2023). The difficulties for taxonomic identification relying on macro and microscopic pictures raises the need to perform molecular taxonomic identification (e.g., 16S DNA sequencing). Molecular identification could greatly enhance the impact of this research by comparing degradation kinetics data to similar species found elsewhere and revealing the novelty of the strains isolated in this study.

According to available information, the present study used one of the highest concentrations of cypermethrin tested so far (1.5 g L^{-1},1,500 ppm, 3.6 mM) (Huang et al. 2018). This concentration was chosen because it is the dose recommended by the manufacturer for agricultural application.

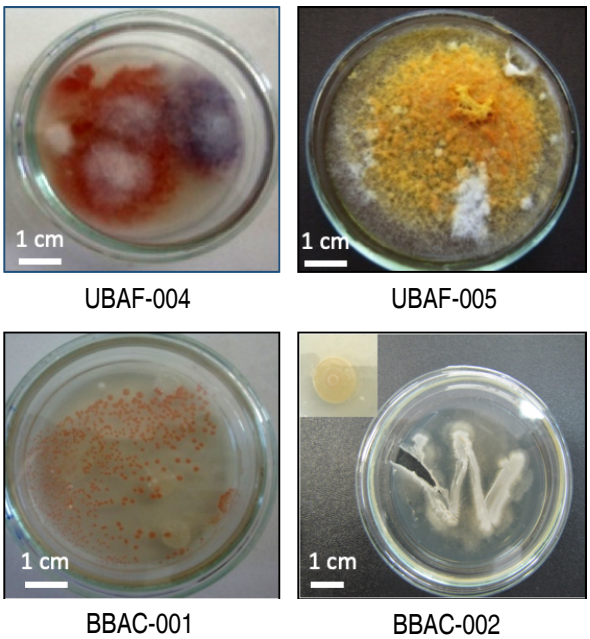


Figure 1. Fungal (UBAF-004 and UBAF-005) and bacterial (BBAC-001 and BBAC-002) isolates obtained after initial screening with cypermethrin as the sole carbon source.

Degradation of cypermethrin by microorganisms in static growth

Under static conditions, one isolate (BBAC-002) did not degrade cypermethrin significantly and three isolates were able to significantly reduce cypermethrin concentration at 41 dpi. Isolates BBAC-001 (77%, $P<0.05$), UBAF-004 (76%, $P<0.05$) and UBAF-005 (71%, $P<0.05$) were able to achieve a greater reduction of cypermethrin compared to control A (45%) (Figure 2). However, when inoculated flasks were compared to control B, which had been equally perturbed (flasks opened every sampling time instead of only once), the cypermethrin concentration was not significantly reduced (Figure 2). Although not statistically significant, control B (64%) showed a greater cypermethrin degradation than control A (45%), which was less disturbed during sampling. Cypermethrin concentration during the 41 days of analysis showed a similar trend across all flasks inoculated with fungal and bacterial strains, as well as uninoculated flasks opened at every sampling. The cypermethrin degradation time by fungal and bacterial isolates in this study took much longer compared to other cypermethrin degradation studies, which usually report degradation within 5 days (Chen et al. 2012; Sundaram et al. 2013). This study was performed under static conditions and high cypermethrin concentrations (1-1.5 g L^{-1}), which are 10 times higher (0.05 g L^{-1} or below) than other studies that are normally incubated in a rotary shaker (Lawan et al. 2021; Sundaram et al. 2013). This could be the explanation behind the lower degradation rates observed in these experiments.

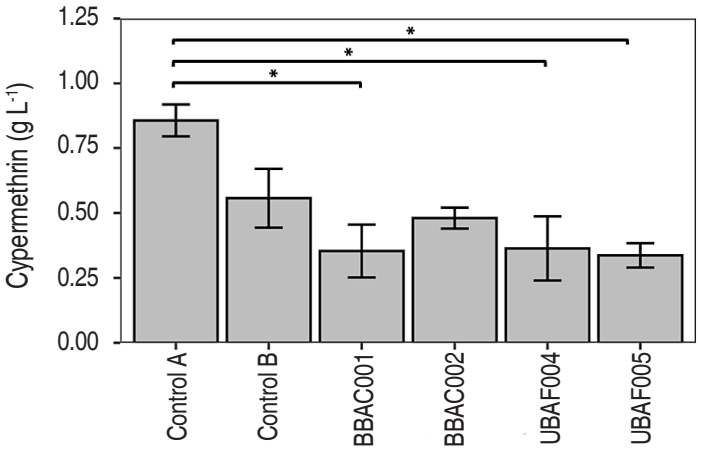


Figure 2. Degradation of cypermethrin by fungal (UBAF-004 and UBAF-005) and bacterial (BBAC-001 and BBAC-002) isolates at 41 dpi. Statistically significant differences are denoted with an * (P<0.05). Control A flasks were opened only once, and Control B flasks were opened 10 times. Data show means ± SE per treatment.

Cypermethrin degradation in non-inoculated minimal culture media was observed in this study. This has previously been reported by Grant et al. (2002), who observed that around 50% of cypermethrin degraded within 21 days in the absence of microorganisms in the culture media. This might be due to the presence of transition metals such as iron (Fe) in the culture media, which might form chemical coordination compounds with cypermethrin cyanide group or its aromatic groups (Rafique and Tariq 2015; Wadepohl 1992).

Temperature might have been another factor that affected the biodegradation rate. The temperatures in this study varied between 20 and 25 °C while other biodegradation studies used higher temperatures between 32 and 45 °C (Bhatt et al. 2020; Liang et al. 2005; Maloney et al. 1993). These temperatures are considered optimal for enzymatic activities such as the biodegradation of pyrethroid pesticides. The absence of Molybdenum (Mb) and magnesium (Mg) in this MCM might have also contributed to the reduced enzymatic activity shown by these isolates, given that Mb and Mg can increase up to 17% the degradation of other pyrethroids such as cyhalotrin (Zhai et al. 2012). Nevertheless, it has been reported that most of the carboxylesterases that degrade pyrethroids that might be present in these isolates do not need co-factors (Li et al. 2008; Wu et al. 2006; Zhan et al. 2020).

Cypermethrin degradation might vary from species to species; however, the most common degradation pathway occurs through enzymatic hydrolysis (Kansal et al. 2023). Nevertheless, adsorption of cypermethrin by *Lactiplantibacillus plantarum* RS60 (Zhang et al. 2023) and *Pediococcus acidilactici* were reported (Zhang et al. 2025). These can be alternative pathways to reduce cypermethrin pollution in the environment.

Degradation of cypermethrin by microorganisms on growth with agitation

Agitation (150 rpm) during incubation significantly increased cypermethrin removal by the fungal isolates (UBAF-004 and UBAF-005) (Figure 3). However, it had no significant effect on the bacterial strain BBAC-002. Under agitation conditions, the strain UBAF-004 achieved a 95% degradation of cypermethrin within 10 days. The high cypermethrin degradation rate obtained by strain UBAF-004 within 10 days with agitation is similar to results previously reported with the bacterial strain *Micrococcus* sp. *CPN1*, which degraded 90% of the pyrethroid cypermethrin (1 g L^{-1}) after 8 days (Tallur et al. 2008). Furthermore, it has been widely described that biodegradation, enzymatic activity and biomass production are correlated to the increase of agitation velocity up to a maximum of 750 rpm (Bhatt et al. 2020; Hussain et al. 2006; Mahendra et al. 2010).

Degradation of cypermethrin in culture media that contain supplementary carbon source

The addition of sucrose as a supplementary carbon source to MCM enhanced significantly the degradation of cypermethrin by fungal strain UBAF-004 (Figure 4).

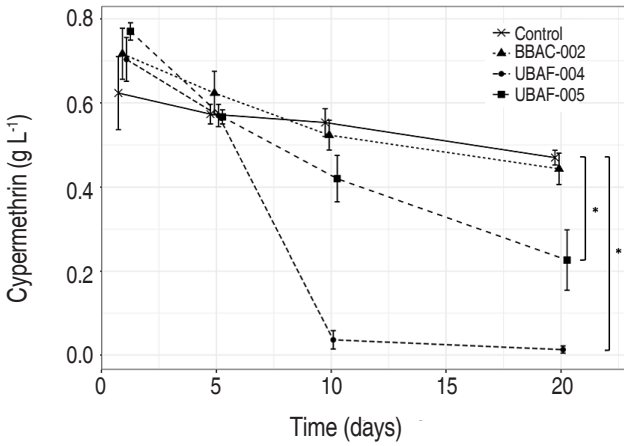


Figure 3. Degradation kinetics of cypermethrin as a sole carbon source by fungal strains UBAF-004, UBAF-005 and bacterial strain BBAC-002 under agitation during 20 days of incubation. Statistically significant differences are denoted with an * (P<0.05). Data shows means ± SE per treatment.

No significant improvement of degradation was observed for UBAF-005 when supplementary carbon source was provided. For bacterial strain BBAC-002 dextrose and sucrose were tested but no improvement of degradation was observed. The minimal culture medium supplemented with sucrose as a complementary carbon source increased cypermethrin degradation significantly in one fungal isolate (UBAF-004). The enhanced degradation of pyrethroids by supplementary carbon source is in agreement with previous studies that used sucrose as carbon source apart from the pesticide and showed better pesticide degradation (Chen et al. 2011; Cycoń and Piotrowska-Seget 2016).

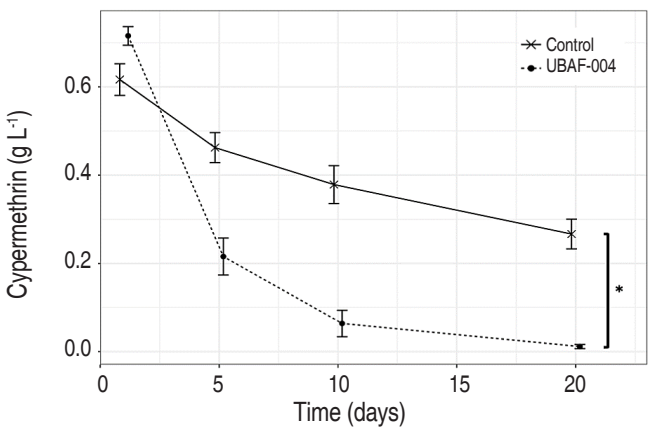


Figure 4. Degradation of cypermethrin with sucrose as an alternative carbon source on MMC along 20 days for strain UBAF-004 under agitation. Statistically significant differences are denoted with an * (P<0.05). Data shows means ± SE per treatment.

CONCLUSION

Fungal and bacterial strains that can use cypermethrin as the sole carbon source could be isolated from agricultural soils of Tahuapalca. The tested strains degraded cypermethrin at concentrations normally applied by agricultural workers. Agitation might be necessary to achieve efficient cypermethrin degradation with the fungal isolates. The fungal isolates, particularly UBAF-004, can be a potential microbe to be employed in bioremediation systems such as bioreactors for water drainages. In fact, its potential to be applied in bioreactors is currently under investigation. This microbe can also be a potential bioaugmentation agent to bioremediate agricultural soils and it should be further studied.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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