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“Selection of an actinobacteria consortium for enhancing diazinon degradation”

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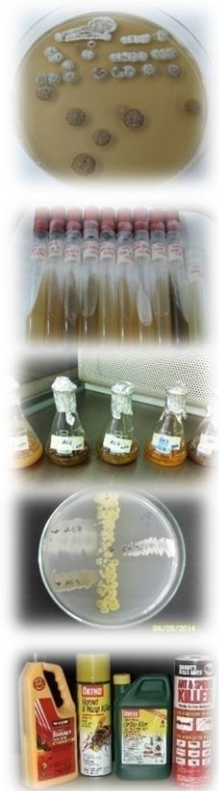
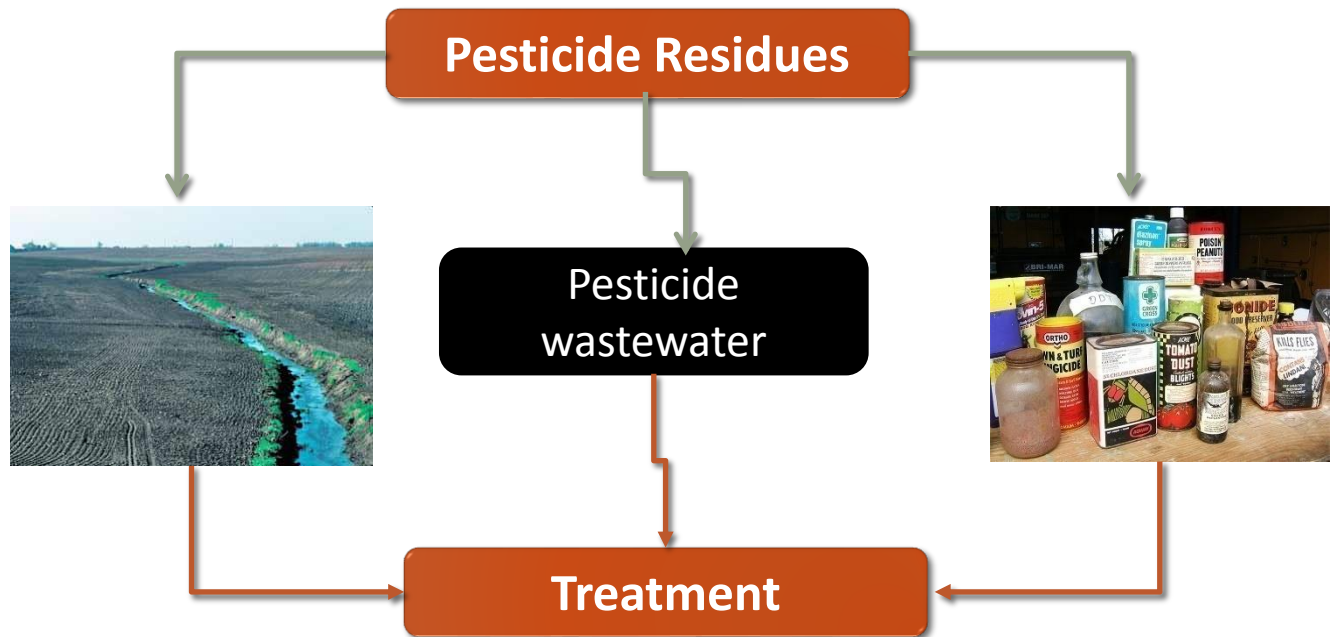
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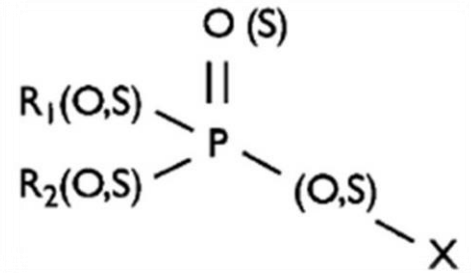
04/08/2014

Introduction

Pesticides are a very important group of environmental pollutants used in intensive agriculture for protection against diseases and pests.



Introduction



Organophosphorus pesticides (OPs)

Diazinon

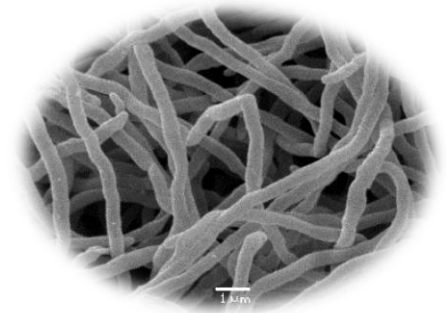
(*O,O*-diethyl-*O*-[2-isopropyl-4-methyl-6-pyrimidinyl] phosphorothioate)

OPs are:

- The most widely used pesticide worldwide
- Inhibitors of the enzyme acetylcholinesterase
- Moderate to high toxicity
- Carcinogenic and mutagenic
- Contaminants of soil and water



Introduction



Actinobacteria or Actinomycete



Physiological and biochemical characteristic

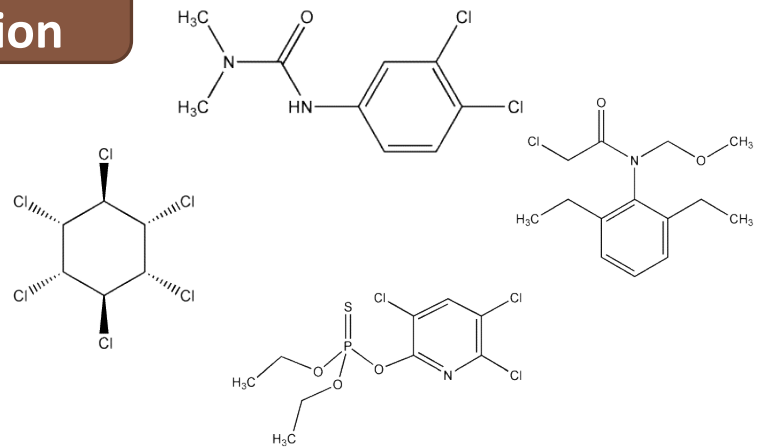
Bioremediation

Medicine

Agriculture

Environmental Science

Pesticide degradation



Objective

To select an actinobacteria consortium to enhance the diazinon degradation

Materials and Methods (1)

1

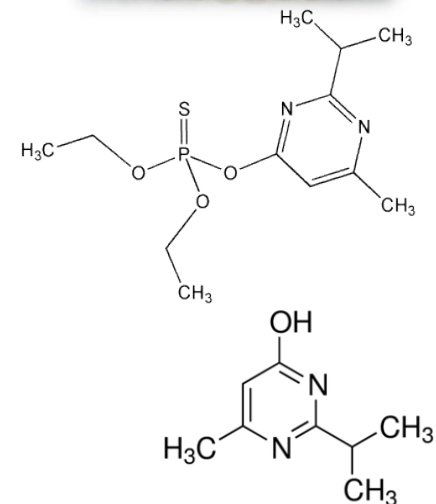
Organophosphorus-degrading actinobacteria identified as *Streptomyces* sp. strains AC5, AC6, AC7, AC9, GA3, GA11, ISP4 and ISP13 were used

2

Diazinon (*O,O*-diethyl-*O*-[2-isopropyl-4-methyl-6-pyrimidinyl] phosphorothioate)

3

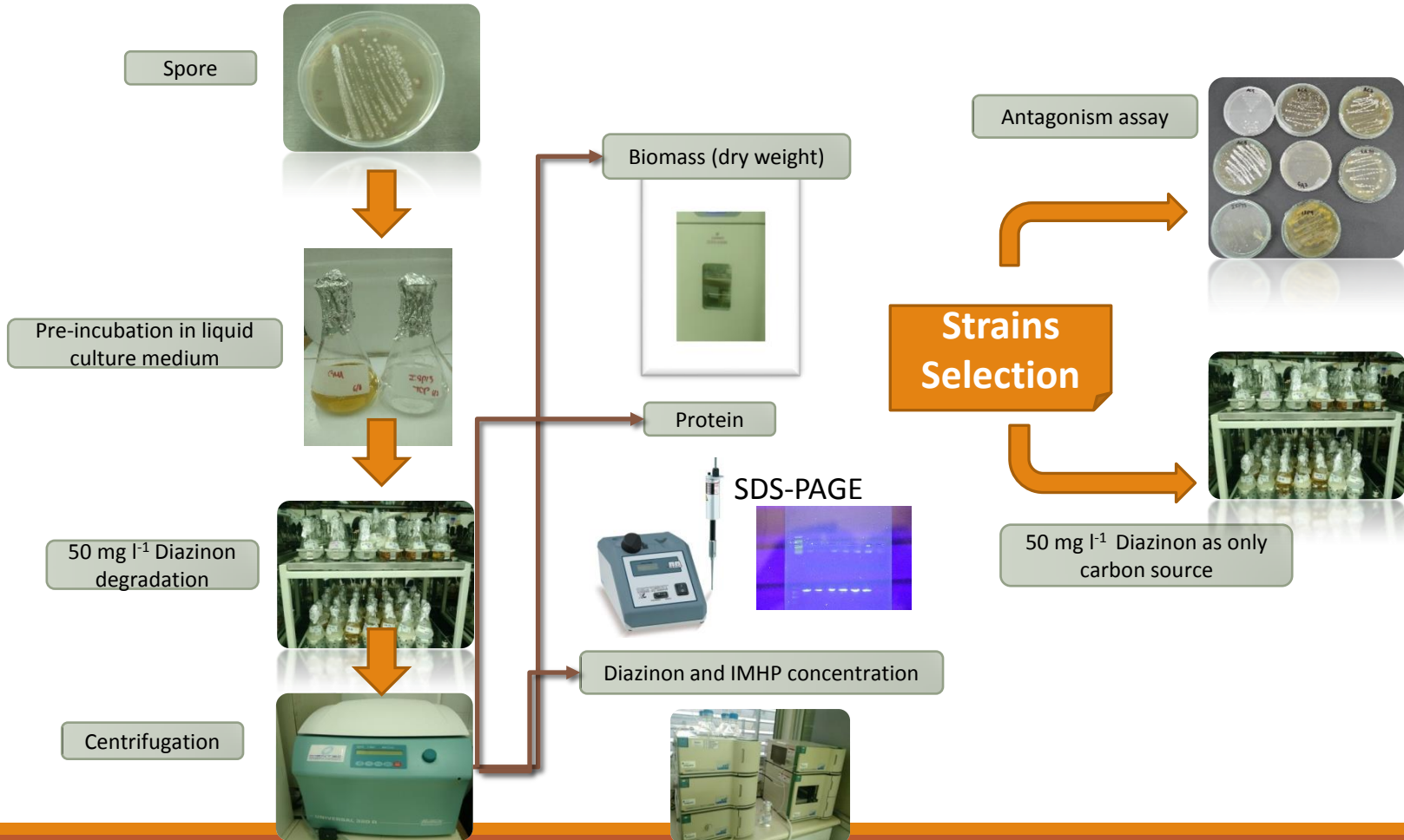
2-isopropyl-6-methyl-4-pyrimidinol (IMHP)



Materials and Methods (2)

Study with single culture

Study with mixed culture



Results and discussion

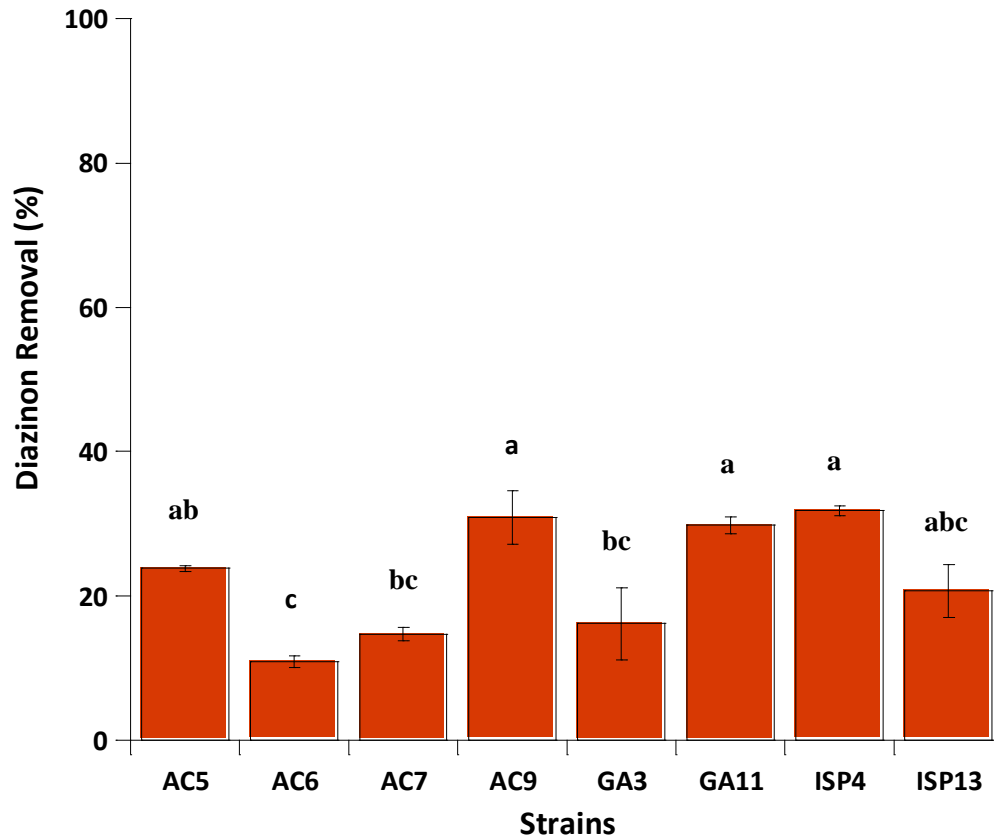


Figure 1. Diazinon removal from liquid medium by pure cultures of *Streptomyces* spp. strains AC5, AC6, AC7, AC9, GA3, GA11, ISP4 and ISP13 after 96 h of incubation. The error bars represent the standard error of the means of three replicates. The values with different letters indicate significant differences ($p \leq 0.05$, Tukey test).

Results and discussion

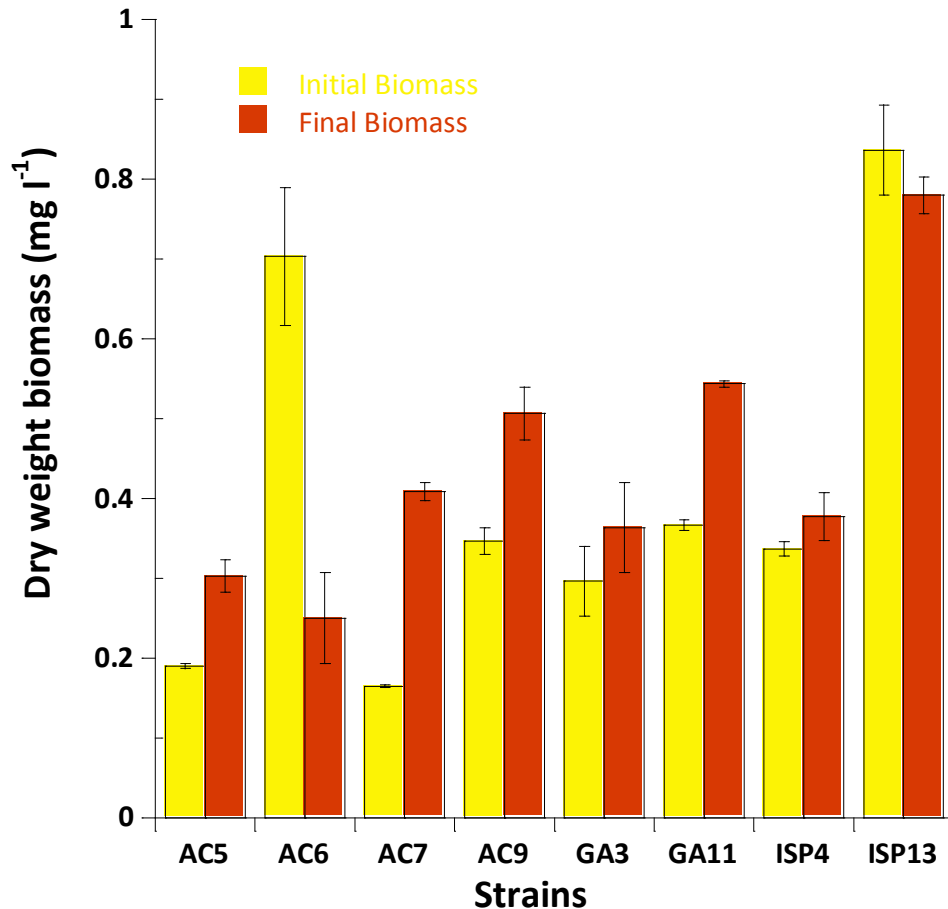


Figure 2. Initial biomass and final biomass of *Streptomyces* spp. strains AC5, AC6, AC7, AC9, GA3, GA11, ISP4 and ISP13 after 96 h of incubation. The errors bars represent the standard error of the means of three replicates.

Results and discussion

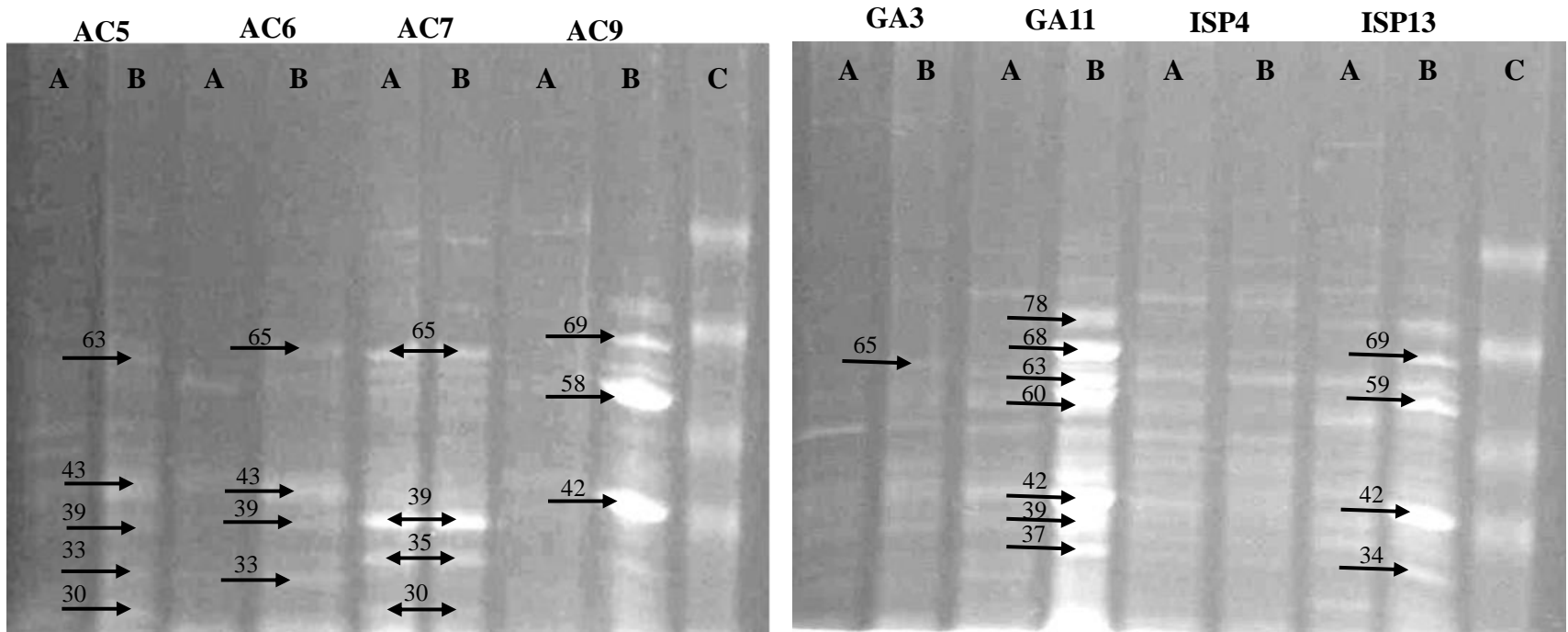


Figure 3. Denaturing PAGE analysis of proteins from *Streptomyces* spp. strains AC5, AC6, AC7, AC9, GA3, GA11, ISP4 and ISP13. The cells were cultured in MM medium with 50 mg l⁻¹ diazinon. Lane A, without diazinon; lane B, with diazinon and lane C, molecular weight marker. The new protein bands or prominent bands are indicated by the arrows.

Results and discussion

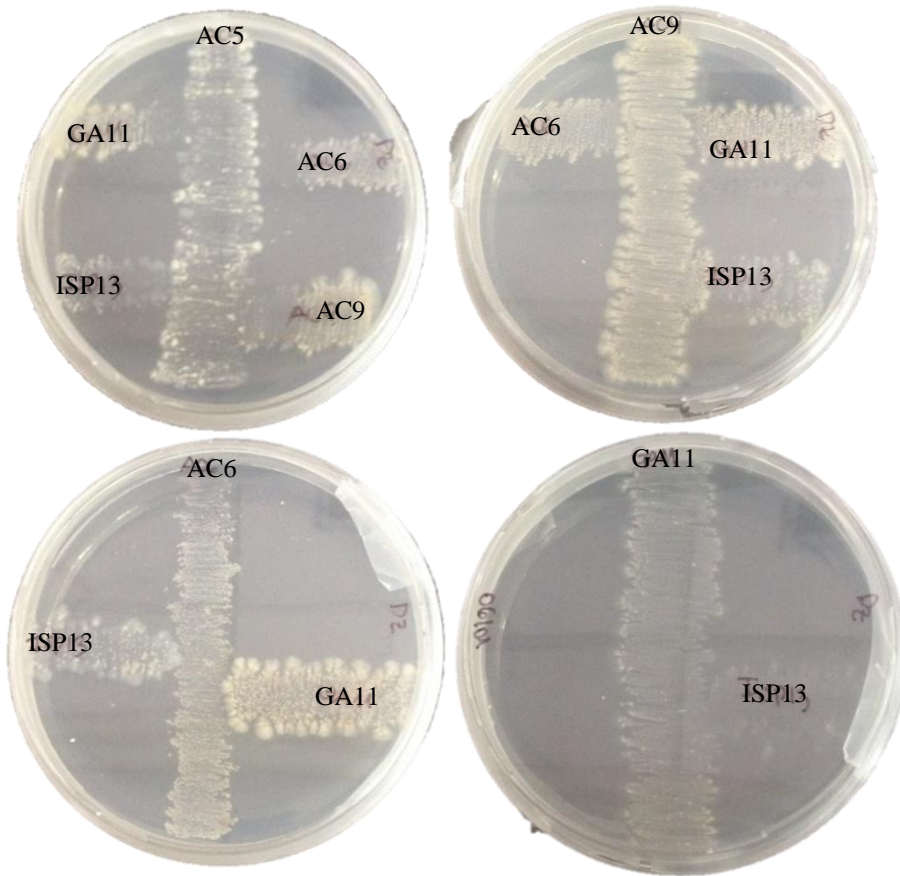


Figure 4. Antagonism assay among: (a) *Streptomyces* spp. strains AC5, AC6, AC9, GA11 and ISP13, (b) *Streptomyces* spp. strains AC6, AC9, GA11 and ISP13, (c) *Streptomyces* spp. strains AC6, GA11 and ISP13, and (d) *Streptomyces* spp. strains GA11 and ISP13.

Results and discussion

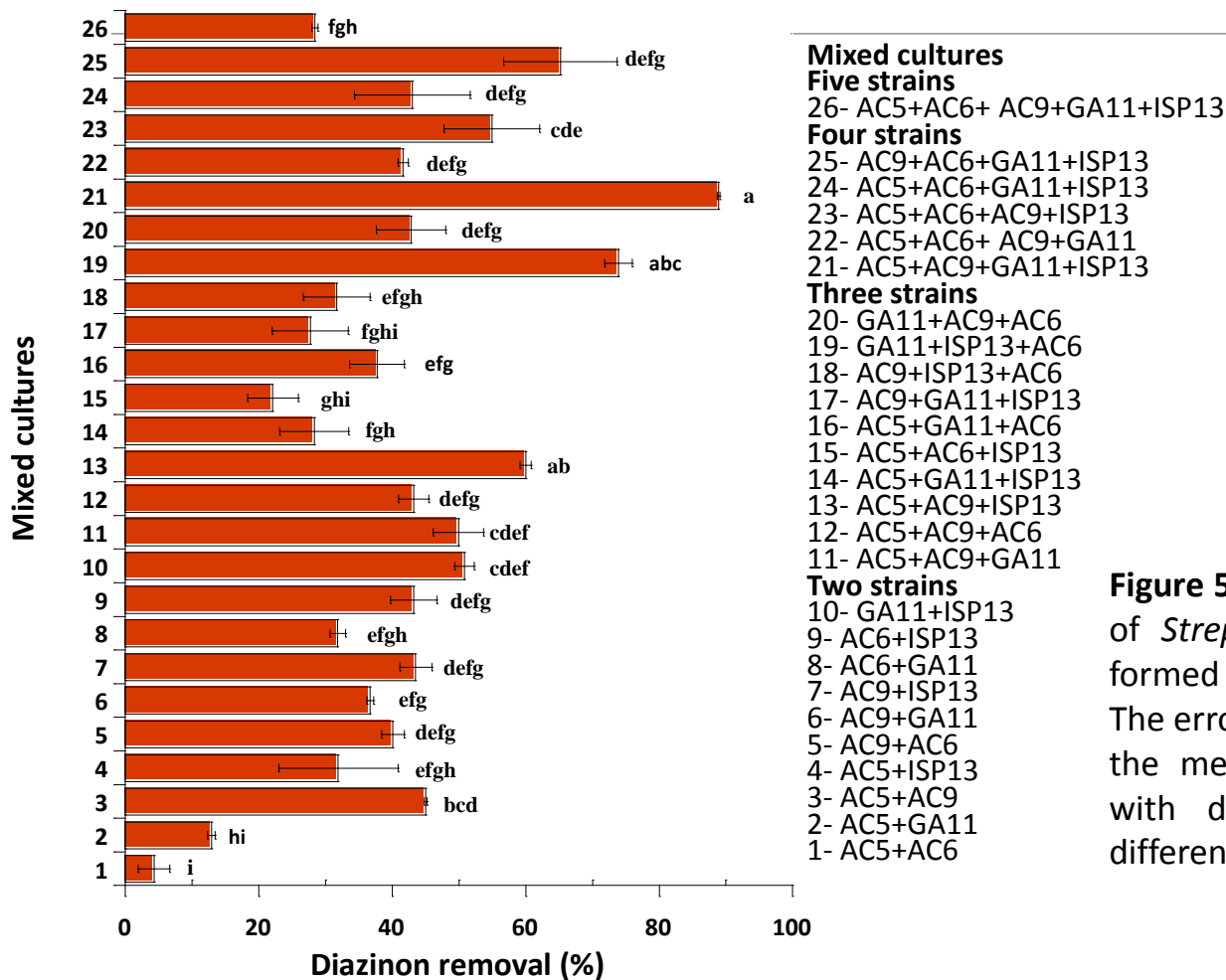


Figure 5. Diazinon removal by mixed cultures of *Streptomyces*. The mixed cultures were formed with two, three, four and five strains. The error bars represent the standard error of the means of three replicates. The values with different letters indicate significant differences ($p \leq 0.05$, Tukey test).

Results and discussion

Table 1. IMHP production by mixed cultures of actinobacteria

Mixed culture	IMHP production (mg l ⁻¹)	Mixed culture	IMHP production (mg l ⁻¹)
Two strains		Four strains	
1- AC5+AC6	0.361 ± 0.010 d	21- AC5+AC9+GA11+ISP13	0.380 ± 0.027 d
2- AC5+GA11	0.242 ± 0.001 d	22- AC5+AC6+AC9+GA11	0.253 ± 0.013 d
3- AC5+AC9	0.176 ± 0.027 d	23- AC5+AC6+AC9+ISP13	1.587 ± 0.137 c
4- AC5+ISP13	0.170 ± 0.023 d	24- AC5+AC6+GA11+ISP13	0.315 ± 0.027 d
5- AC9+AC6	0.258 ± 0.028 d	25- AC9+AC6+GA11+ISP13	2.196 ± 0.136bc
6- AC9+GA11	0.256 ± 0.013 d	Five strains	
7- AC9+ISP13	0.232 ± 0.009 d	26- AC5+AC9+GA11+ISP4+ISP13	2.196 ± 0.001 d
8- AC6+GA11	0.341 ± 0.013 d		
9- AC6+ISP13	0.216 ± 0.002 d		
10- GA11+ISP13	0.239 ± 0.007 d		
Three strains			
11- AC5+AC9+GA11	0.160 ± 0.008 d		
12- AC5+AC9+AC6	4.057 ± 0.012 a		
13- AC5+AC9+ISP13	1.758 ± 0.088 c		
14- AC5+GA11+ISP13	0.172 ± 0.010 d		
15- AC5+AC6+ISP13	0.195 ± 0.008 d		
16- AC5+GA11+AC6	0.193 ± 0.008 d		
17- AC9+GA11+ISP13	0.207 ± 0.002 d		
18- AC9+ISP13+AC6	0.211 ± 0.016 d		
19- GA11+ISP13+AC6	3.774 ± 0.102 a		
20- GA11+AC9+AC6	3.954 ± 0.107 a		

Results and discussion

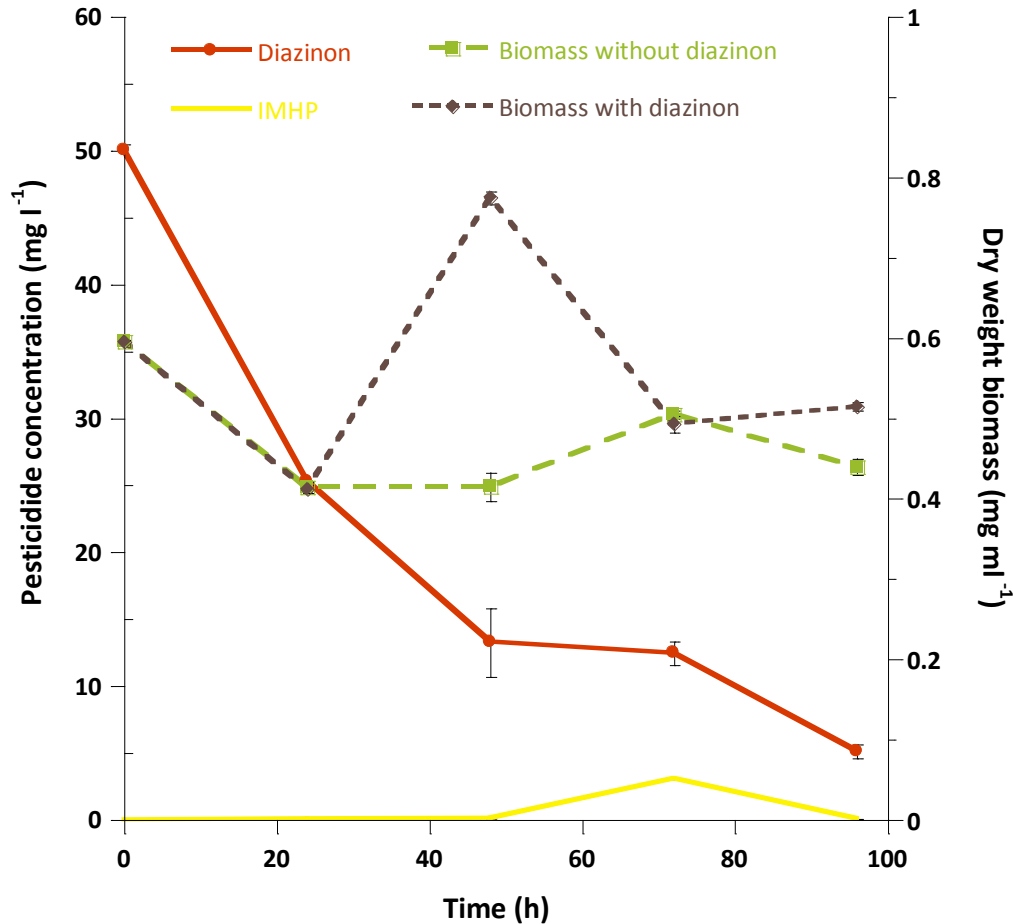


Figure 6. Concentration of diazinon and IMHP, and microbial growth of the mixed culture of actinobacteria formed by the *Streptomyces* spp. strains AC5, AC9, GA11 and ISP13. The error bars represent the standard error of the means of three replicates.

Conclusion

- ❖ Mixed cultures of *Streptomyces* spp. increase the diazinon removal
- ❖ Diazinon-degrading enzymes are expressed by some strains of *Streptomyces*
- ❖ Diazinon is used as an only carbon source for growth by studied *Streptomyces*
- ❖ Formation of the diazinon metabolite 2-isopropyl-6-methyl-4-pyrimidinol was observed



FONDECYT

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Thank you! Danke sehr!