Isolation of a bacterial species in the insecticide polluted areas

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- Different areas of Bangalore was studied.
- An agricultural farm, R.K farms was the area selected and screened for insecticide pollutant.

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- Chlorpyriphos-insecticide most abundantly found in the soil.
- Studies were further extended to fresh water bodies
- Yellanallapachetty lake in the bangalore-chennai road was selected for studies.

- The same insecticidal residue was screened along with the other residues.
- Chlorpyrifos is one of the about 100 organophosphate insecticides available in market today
- This insecticide is in common use

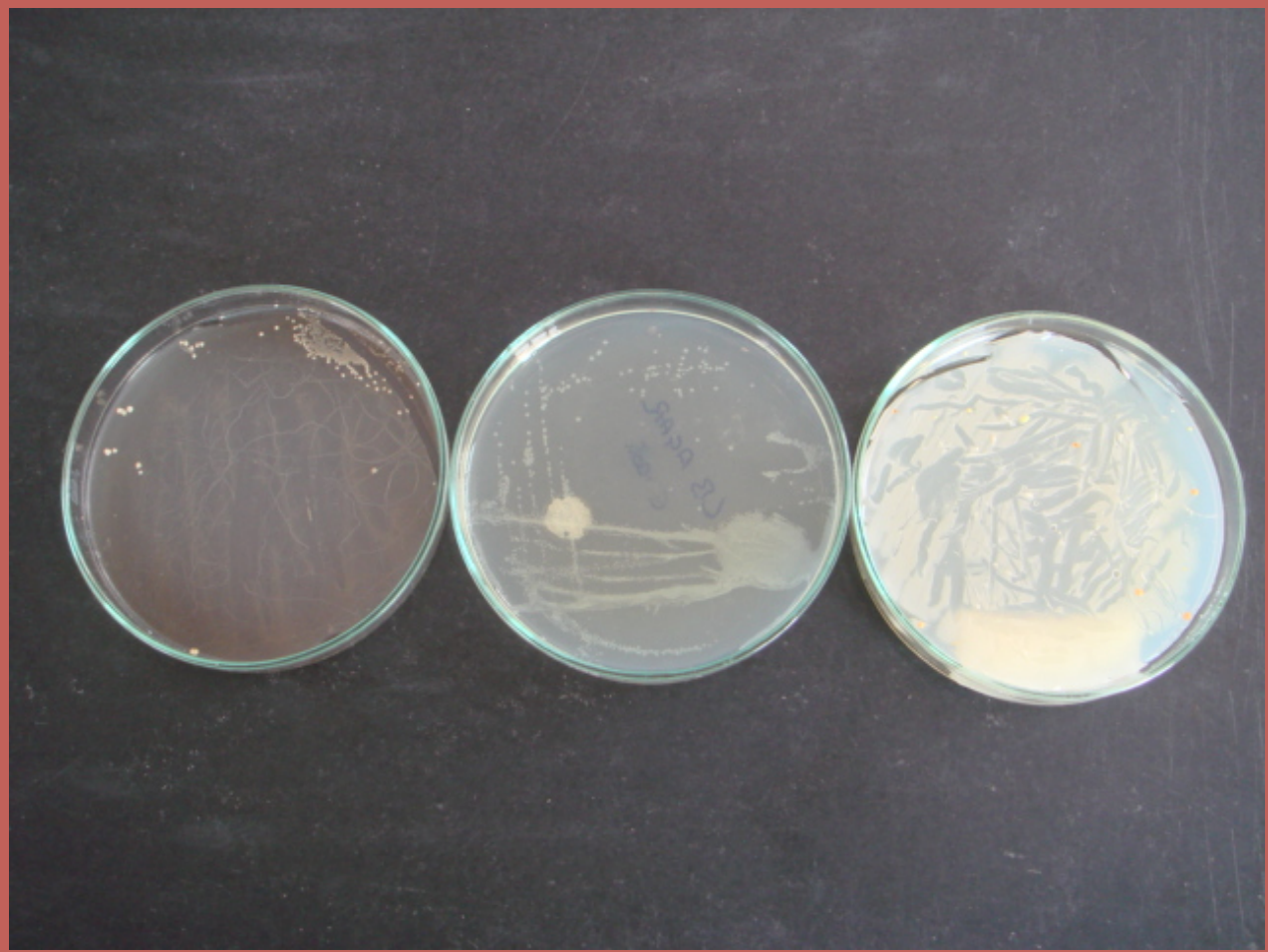
- Both the soil and water samples were screened for microflora.
- Cultural, microscopical and biochemical studies were conducted

Table 1: cultural characteristics of water microflora								
Plate dilution	**Size**	**Shape**	**Margin**	**Elevation**	**Pigmentation**	**Opacity**	**Texture**	**Gram's nature**
10^{-1}	**Pin point**	**Circular**	**Irregular**	**Raised**	**White**	**Opaque**	**Smooth**	**- rods**
	Medium	**Circular**	**Entire**	**Raised**	**Pinkish**	**Translucent**	**Smooth**	**+ rods**
	Medium	**Circular**	**Entire**	**Convex**	**yellow**	**Opaque**	**Smooth**	**+ cocci**
	Large	**Irregular**	**Serrate**	**Raised**	**White**	**Opaque**	**smooth**	**+ rods**
	Small	**Circular**	**Wavy**	**Umbonate**	**White**	**Translucent**	**smooth**	**+ rods**
10^{-2}	**Pinpoint**	**Circular**	**Irregular**	**Raised**	**White**	**Opaque**	**Smooth**	**+ cocci**
	Small	**Circular**	**Wavy**	**Umbonate**	**White**	**Translucent**	**Smooth**	**+ rods**
	Large	**Circular**	**Entire**	**Convex**	**White**	**Opaque**	**Smooth**	**+ rods**
10^{-3}	**Pinpoint**	**Circular**	**Irregular**	**Raised**	**White**	**Opaque**	**smooth**	**- rods**
	Large	**Circular**	**Entire**	**Convex**	**White**	**Opaque**	**Smooth**	**+ rods**

Table 2: cultural characteristics of soil microflora

Plate dilution	Size	Shape	Margin	Elevation	Pigmentation	Opacity	Texture	Gram's nature	
10^{-1}	Small	Circular	Wavy	Umbonate	White	Translucent	smooth	+ rods	
	Medium	Circular	Entire	Raised	Pinkish	Translucent	Smooth	+ rods	
	Medium	Circular	Entire	Convex	White	Opaque	Smooth	+ rods	
	Large	Irregular	Serrate	Raised	White	Opaque	smooth	+ rods	
	Large	Circular	Entire	Convex	White	Opaque	Smooth	+ rods	
10^{-2}	Pinpoint	Circular	Irregular	Raised	White	Opaque	Smooth	+ rods	
	Small	Circular	Wavy	Umbonate	White	Translucent	Smooth	+ rods	
	Pinpoint	Circular	Irregular	Raised	White	Opaque	smooth	- rods	
10^{-3}	Large	Circular	Entire	Convex	Off white	Opaque	Smooth	+ rods	
	Pin point	Circular	Irregular	Raised	White	Opaque	Smooth	- rods	

FIGURE:1 Bacterial colonies of soil microflora



- Gram positive rods were selected for further morphological studies.

Gram’s reaction	**Gram positive**
Morphology	**Rods**
Motility	**Motile**
Endospore	**terminal**

- The morphological studies confirmed the genus of the bacterial form as *Bacillus*

- The species of this *Bacillus* was detected by a set of biochemical tests

Table 4: Biochemical tests	
Test	**Result**
Indole production	+
Methyl red	+++
Voges-proskauer	-
pH in VP medium <6	+
Citrate utilization	-
Nitrate reduction	+
Urease	-
Sugar fermentation –Glucose	++
Lactose	-
Mannitol	-
Catalase	+++
Oxidase	++
Amylase	+++

Table 5: Studies on temperature tolerance profile of the isolate					
Temperature	Incubation period (Hrs)	Growth on agar	Degree of growth	Growth on broth	Degree of growth
37^0c	24	+	3+	+	3+
45^0c	24	+	3+	+	3+
55^0c	24	+	3+	+	3+
60^0c	24	+	3+	+	3+
65^0c	24	+	+	+	+

FIGURE:2 BACTERIAL COLONIES AT VARYING TEMPARATURES

- The bacterial form isolated is thus confirmed to be *Bacillus stearothermophilus.*
- This strain is capable of growing at temperature as high as 65^0c
- This is however a useful diagnostic character.

- This strain was further tested for salinity tolerance and tolerance to the selected insecticide-chlorpyriphos

Table 6: Results of turbidometric studies on stress tolerance profile			
Stress agent	**Concentration**	**Incubation temperature**	**O.D. at 660nm**
Sodium chloride	**2%**	**55^0c**	**0.05(±0.01)**
	6%	**55^0c**	**0.09(±0.03)**
	8%	**55^0c**	**0.01(±0.02)**
	10%	**55^0c**	**0.01(±0.02)**
chlorpyrifos	**0.001%**	**55^0c**	**0.03(±0.03)**
	0.005%	**55^0c**	**0.05(±0.02)**
control	**2%v/v**	**37^0c**	**0.05(±0.01)**
	2%v/v	**55^0c**	**0.09(±0.03)**

- A thermostable bacterium was isolated from agricultural soil and lake.
- This strain was classified into genus *Bacillus* and species *stearothermophilus*
- The strain was found to show tolerance and growth at temperature as high as 65^0c

- The strain was also found to show tolerance to various other stress factors such as salinity and insecticide.
- The preliminary investigations have shown that the isolated bacterium can take pollutants-insecticides (chlorpyriphos)
- Further studies are in progress with reference to the uptake and degradation of pollutants

Thank you!!!