Probiotics Cultures: A new alternative for the control of *Aedes aegypti*, *Anopheles albimanus* and *Culex quinquefasciatus* (Diptera: Culicidae)

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PECET
UNIVERSIDAD DE ANTIOQUIA

Program For The Study And Control Of Tropical Diseases

Control and Vectors

A. albimanus

A. aegypti

C. quinquefasciatus

Insect of blood feeding

↓

Malaria, Dengue, Encephalitis, West Nile, Filariasis etc.

↓

Prevention and control of mosquitoes population: urgent and immediate demand

↓

Convencional control strategies: application of a wide spectrum of chemicals and pesticides that produce adverse effects

- High risk in humans and environment
- Appearance of a new generation of mosquito with resistant
- Increment in the price on new pesticides

Research on new Eco-friendly tools for the control of mosquito vectors

↓

Biocontrol

K. Das, A.K. Mukherjee / Acta Tropica 97 (2006) 168–173
Mittal, P.K., 2003. J. Vect. Borne Dis. 40, 20–32.
http://www.edicionesmedicas.com.ar/Actualidad/Ultimas_noticias/Culex_quinquefasciatus

Control by Biological Agents

- *Bacillus thuringenesis* var. *israelensis* (Bti) and *B. sphaericus* (Bs): high effectiveness with low concentrations and safe for no blank organisms.
- Resistance has been registered with Bs in mosquito population (Nielsen-Leroux et al.,1995; Poopathi et al., 1999; Su and Mulla, 2004),

↓

Mix of toxins act on different targets of the insect

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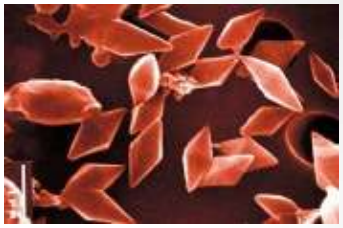
- *Bacillus subtilis* / lipopeptides with huge biotechnology potential and biopharmaceutical application
- Several isoforms of surfactins, fengycin and iturin.

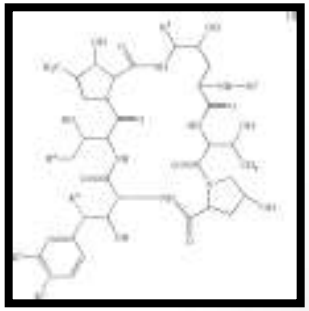
↓

Microorganisms Consortia



Bacillus thuringiensis : spore and protein crystals

Bacillus subtillis



Cycle Lipopetides

http://anupriti.blogspot.com/2009/07/data-storage-in-bacteria-astonishing.html
http://www.learner.org/courses/biology/archive/images/1006.html
http://www.freepatentsonline.com/6825003.html

Knight, R.L., et al, 2003. Ecol. Eng. 21, 211–232. / Lacey, L.A., Undeen, A.H., 1986. Annu. Rev. Entomol. 31, 265–296./ Mittal, P.K., 2003. J. Vect. Borne Dis. 40, 20–32. / Wirth, MC., et al, 2005. J. Invertebr. Pathol. 88, 154–162./ Vater, J., et al., 2002. Appl. Environ. Microbiol. 68, 6210–6219.
Cooper, D.G., et al., 1989. J. Ferment. Technol. 59, 97–101. Assie, L.K., et al., 2002. Meded Rijksuniv Gent Fak Landbouwkd Toegep Biol Wet. 67, 647–655.

Worldwide Applications

PROBIOTICS

Probiotics are live microorganisms which, when applied in adequate amounts deliver a health benefit to the host.

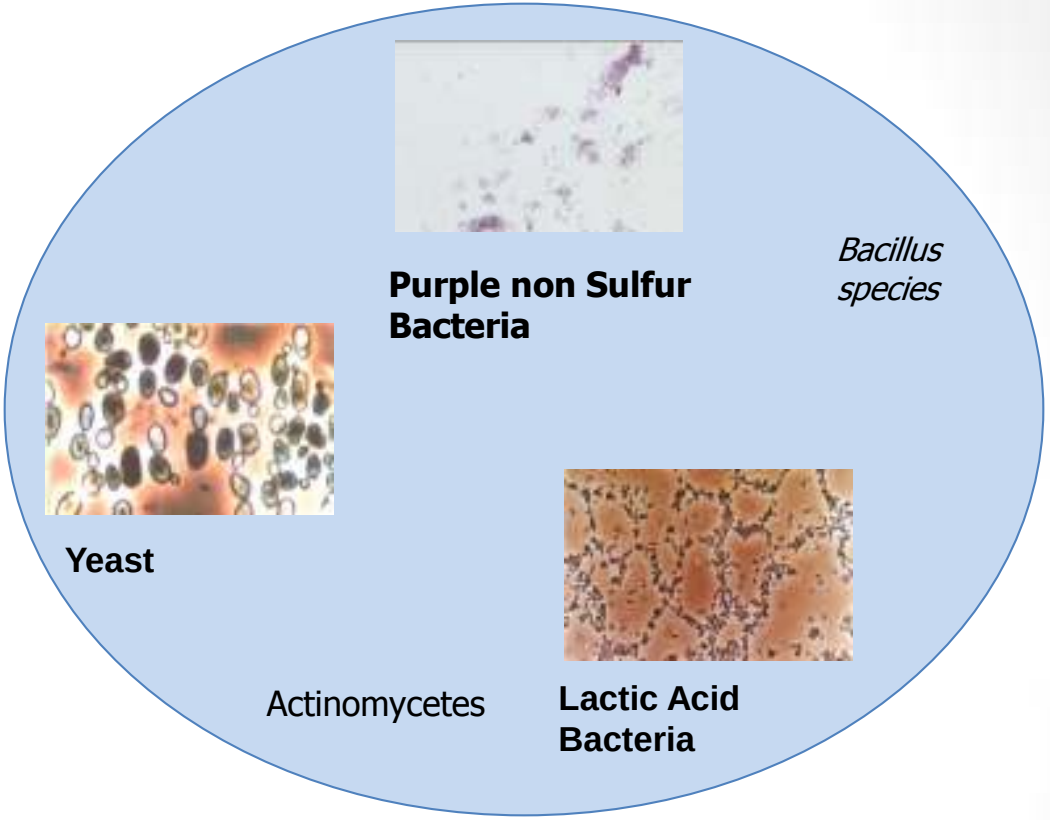
USE

- Animal Health: Regulation flora intestinal
- Rehabilitation on waste areas: Solid Waste Management
- Odor control, composting, animal farming
- Agriculture, bioremediation, soil fertility, yield
- Disasters: Tsunamis and earthquakes
- Waste water treatments
- Pathogen control in water, soil, food.

Fuller, R. 1989. J. Appl. Bacteriol. **66:**365–378

Probiotic Species List

Bacillus subtilis var "natto"
Bifidobacterium animalis
Bifidobacterium bifidum
Bifidobacterium longum
Lactobacillus acidophilus
Lactobacillus bulgaricus
Lactobacillus casei
Lactobacillus delbrueckii
Lactobacillus fermentum
Lactobacillus plantarum
Lactococcus lactis
Lactococcus lactis subsp. Diacetylactis
Rhodopseudomonas palustris
Rhodopseudomonas spheroides
Saccharomyces cerevisae
Saccharomyces thermophilus



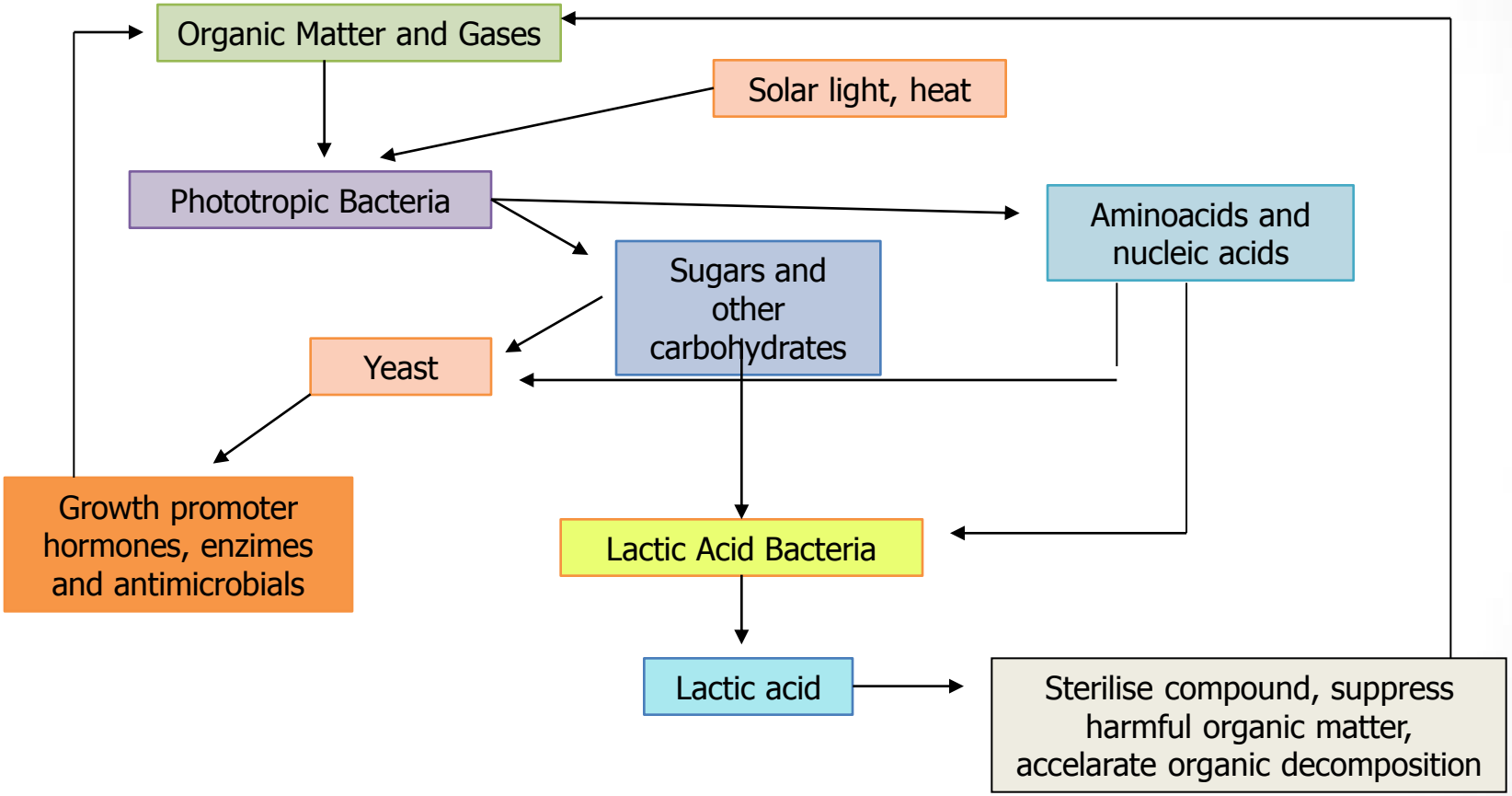
Probiotics Consortia

- Grown in multi-strain bacteria
- Very genetically diverse
- More resilient in the "real world"

Pure Culture

- Laboratory grown as single-strains
- Limited genetic diversity
- More vulnerable in the "real world"

Compounds production path



Initiative on the use of Probiotics

"Micro organismos for macro problems»

Year	Sample People	% Incidence	Total Cost Medicine
2003	2,609	12.23	US $5,500
2004 start Probiotics	657	2.40	US $1,200
2005	391	2.26	US $841
2006	640	0.68	US $617
2007	280	0.95	US $237

India Tea Gardens ↓ *Helopeltis theivora*

Was decided to extend the research to Arthropods of medical importance

General Objetive

To evaluate the efficacy of Probiotic cultures over immatures phases of mosquito *Aedes aegypti, Anopheles albimanus* and *Culex quinquefasciatus* in laboratory conditions

Specific Objetives

- To establish the susceptibility in immatures phases of *A. albimanus, Ae. aegypti* and *Cx. quinquefasciatus* in different probiotic concentrations

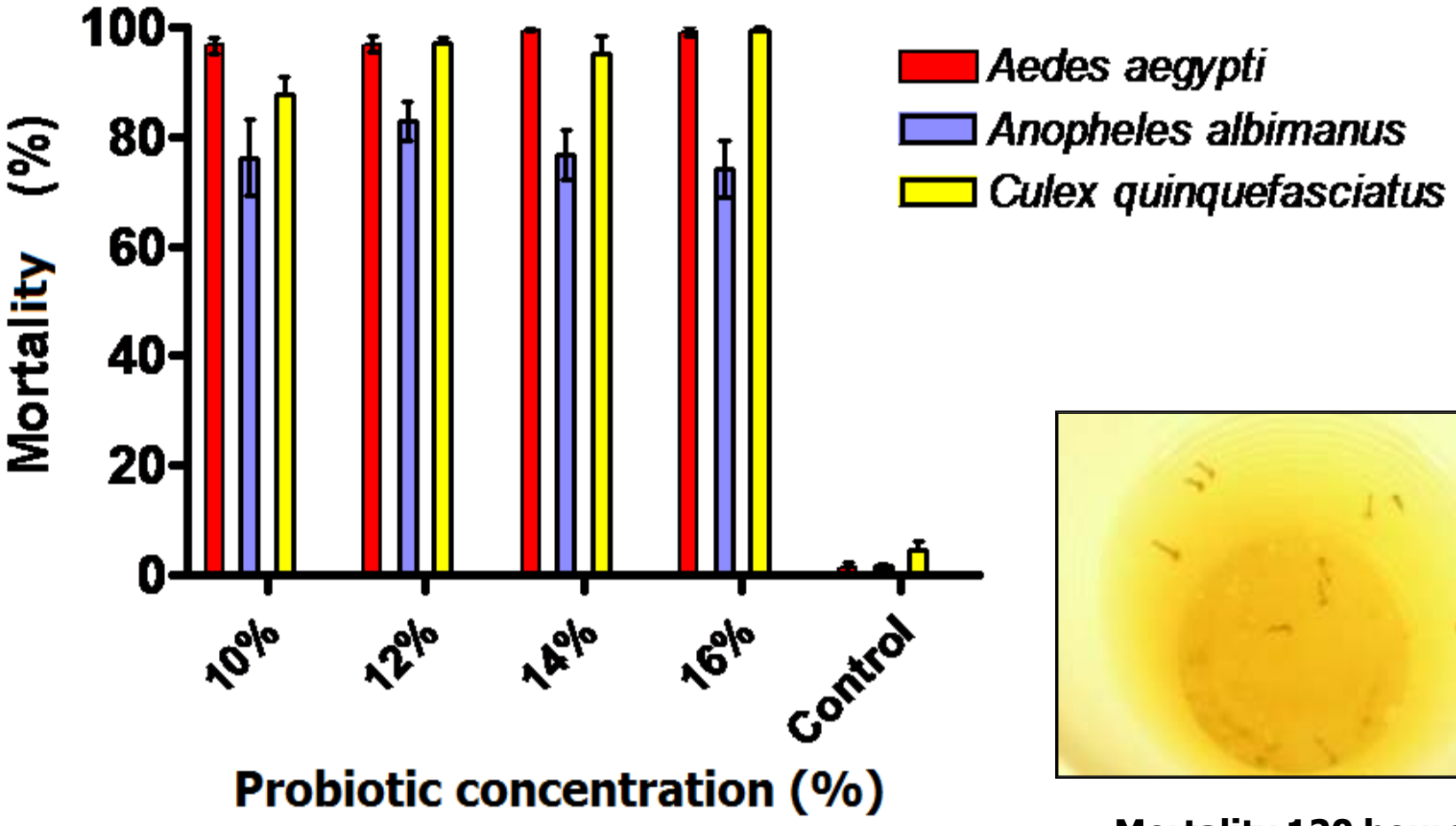
- To determine the Lethal Concentration fifty (LC_{50}) and ninety (LC_{90}) of Probiotic cultures for larvae of *A. albimanus, A. Aegypti* and *Cx. Quinquefasciatus*

- To demostrate the reproducibility of a product with low cost and eco friendly as biological control alternative

Methodology

1. • Breed immature forms
   • Larvae separation and account
   • Probiotic dilutions preparation
2. • Probiotic applications
   • Measurement (1) of physicochemical factors at the beginning of the experiments
3. • Follow up and registration of mortality (24, 48, 72 and 120 hours
   • Measurement (2) of physicochemical factors at the end of the experiments
4. • Experiment conditions: average temperature: 28 ºC ± 2 and Relative humidity 70 ±5 %
   • Experiement repetitions / specie: 10 (4000 larvae / test)

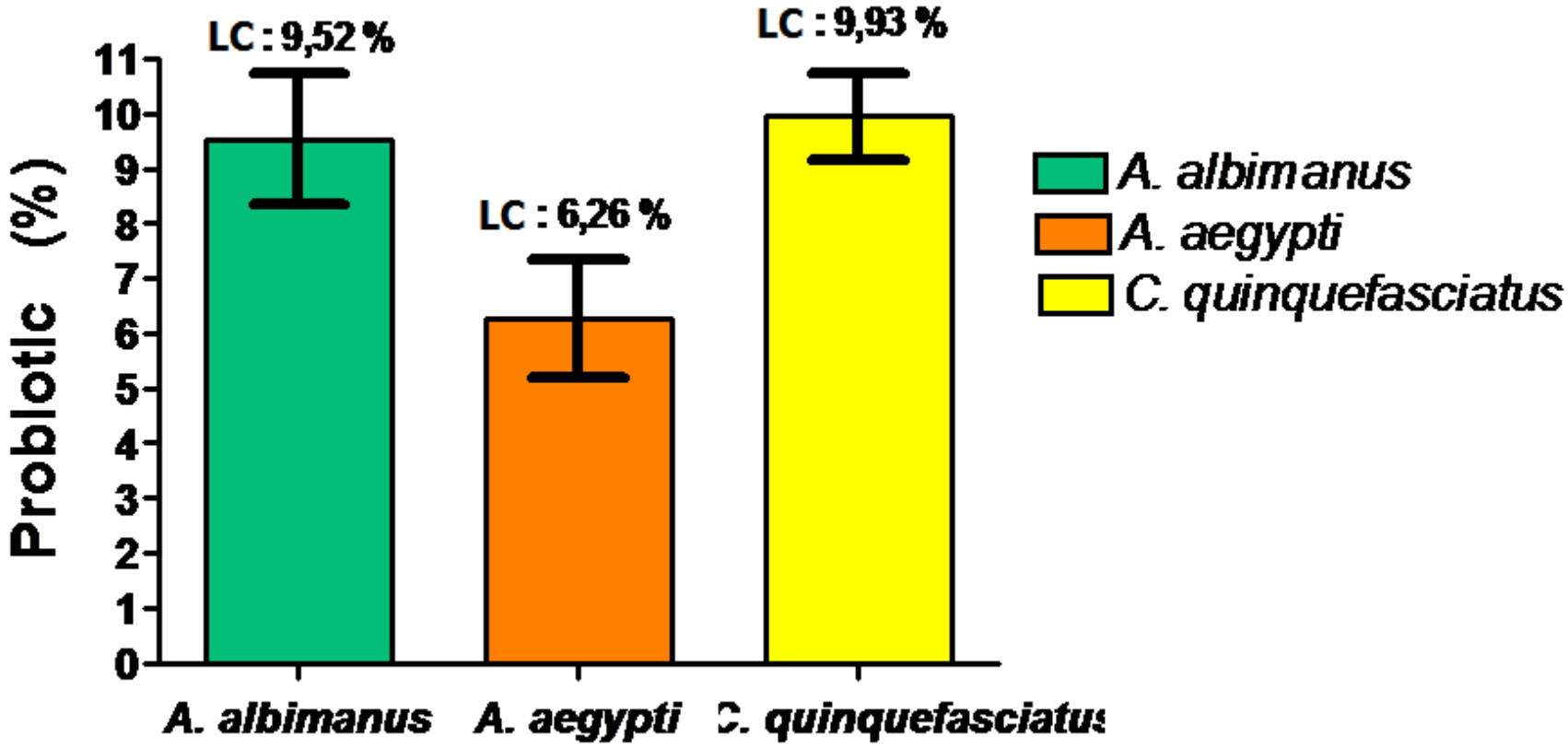
Mortality (%) vs Concentration



Mortality 120 hours

- Susceptibility *A. aegypti* < *C. quinquefasciatus* < *A. albimanus* after 120 hours of application.

Lethality (LC_{50}) after 120 hours



- *A. aegypti* more susceptible with less probiotic concentration
- Geetha et al., 2007: Bti *C. quinquefasciatus* more susceptible (4 ng/ml) and *Anopheles stephensi less* susceptible (18 ng/ml)

Physicochemical factors *Aedes aegypti*

Concentration	Measurement	pH	O_2 (%)	Conductivity (uS/m)
10 %	1	3,22 **a**	68,06 **c**	578,68 **e**
	2	3,22 **a**	39,06 **d**	623,68 **e**
	C -	7,03 **b**	66,87 **c**	66,93 **f**
12 %	1	3,21 **a**	64,62 **c**	660,06 **e**
	2	3,18 **a**	28,87 **d**	641,43 **e**
	C -	7,13 **b**	70,12 **c**	66,66 **f**
14 %	1	3,20 **a**	65,50 **c**	718,18 **e**
	2	3,18 **a**	27,31 **d**	704,18 **e**
	C -	7,05 **b**	69,50 **c**	65,73 **f**
16 %	1	3,20 **a**	68,93 **c**	785,18 **e**
	2	3,17 **a**	28,62 **d**	751,75 **e**
	C -	7,22 **b**	69,12 **c**	65,91 **f**

Different letters indicate significative statistic differences $p < 0,0001$. Test of Tukey. 1: Measurement of the factor at the beginning, 2: Measurement of the factor at the end of the experiment, C -: Control without application of probiotics.

Physicochemical factors *Anopheles albimanus*

Concentration	Measurement	pH	O_2 (%)	Conductivity (uS/m)
10 %	1	3,23 **a**	50,18 **d**	582,31 **g**
	2	3,06 **b**	41,56 **e**	619,62 **g**
	C -	6,60 **c**	63,25 **f**	65,58 **h**
12 %	1	3,26 **a**	51,93 **d**	666,75 **g**
	2	3,03 **b**	40,62 **e**	713,81 **g**
	C -	6,63 **c**	66,00 **f**	65,61 **h**
14 %	1	3,26 **a**	51,62 **d**	709,43 **g**
	2	3,02 **b**	40,93 **e**	849,50 **g**
	C -	6,70 **c**	65,25 **f**	65,32 **h**
16 %	1	3,23 **a**	52,81 **d**	766,12 **g**
	2	3,00 **b**	37,50 **e**	848,93 **g**
	C -	6,71 **c**	64,87 **f**	63,53 **h**

Different letters indicate significative statistic differences $p < 0,0001$. Test of Tukey. 1: Measurement of the factor at the beginning, 2: Measurement of the factor at the end of the experiment, C -: Control without application of probiotics.

Discussion

- Susceptibility varies according to the species. A. aegypti more susceptible than A. albimanus.
- LC_{50} of the probiotic is high. In *B. subtillis* (5-25 ul/ml) (Geetha et al. , 2007)
- Cultivation of probiotic: synergistic effect and offers less option to resistance of mosquito populations to the biolarvicide, genetically more diverse
- Physico-chemical factors (pH, temperature, solar exposure and age of the larvae) influence the effectiveness of the formulations with bacteria or toxins against mosquito larvae (Mulla, 1985; Becker et al. , 1992; Mittal et al. , 1995; Nayar et al. , 1999).
- Lipopeptids of *B. subtilis* : insensitive to sunlight and equally effective to kill larvae. Advantage over the conventional biolarvicides *Bti*, and *Bs*.
- Ecofriendly alternative and without lethal effects compared with pesticides.

Discussion

- The effectiveness *Bs.* and *Bti.* against larvae of anopheline mosquitoes is reduced about 10 times in laboratory tests to 21°C compared with tests, carried out at 31 °C (Mittal, 2003).

- Studies with raw lipopeptids indicated a reduction in the larvicidal power around 4% by 23°C , on its original activity that occurs at 35°C (K. Das & A. K. Mukherjee, 2006).

- Strains of *B. subtillis* high thermostable power between two strains evaluated after periods of warming 100°C by 60 minutes (K. Das & A. K. Mukherjee, 2006).

- Toxins of proteins of *Bti* and *Bs* are highly sensitive to sunlight (UV radiation). Exposure to sunlight 6 hours reduces the strength larvicide near the 50 and 75% respectively (Mittal, 2003).

- The probiotic effect up to a month and shelf life about a year

Explanation for the Mechanism of Action

Microorganisms

Other effects NO known

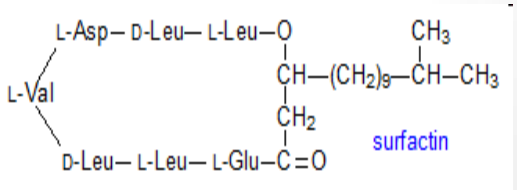
Production Alcohols, Esters and other metabolites

Esterilization effect (OH+)

Digestive system of the larvae is disturbed

Disminution of the pH

Death



Cycle Lipopeptides : *B. subtillis*

Conclusions

- Susceptibility presented variations according to the species (*A. aegypti* < *C. quinquefasciatus* < *A. albimanus*) after 120 hours of application.
- *Aedes aegypti* more susceptible to the probiotic, mortality > 90 %; *A. albimanus* mortality ~ 80% and less susceptible. Only *C. quinquefasciatus* effect depending on dose.
- LC_{50} for *Aedes aegypti* was the lowest (6.26 %) and statistically different to that obtained for the other species.
- *Aedes aegypti* , presented less variation in physico-chemical factors indicating greater effectiveness to the larvicide.

Perspectives

- Studies in no target organisms: fishes and insects
- Studies on the mechanism of action and the synergist effect
- Ecofriendly alternative
- More field studies that will bring more information in the behaviour of the natural probiotics and larvae/pupa control on wild mosquitoe.

Acknowledgement

- Medical and Molecular Enthomology Unit (UEMM)
- Dra. Margarita Correa (Auroville)
- To PECET for allow the research and development of the different graduate theses