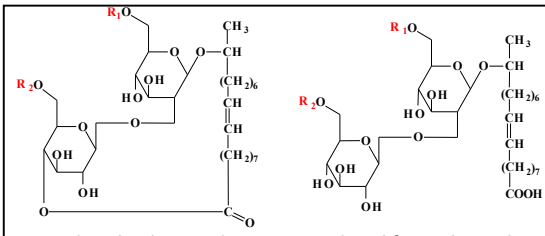
SYNTHEZYME'S FUNGICIDES AND ANTIMICROBIALS

SyntheZyme™ is a green chemistry company developing bio-based chemical building blocks, bio-plastics and biologically active biosurfactants/antimicrobials from renewable sources using proprietary engineered yeasts.



Sophorolipid natural mixture produced from plant oils and sugars in >300 g/L. Acetylation (R1 and R2 groups) can occur at none, one or both primary hydroxyl groups.

Antimicrobial technology: They are compounds used in a wide range of applications varying from architectural coatings and agricultural products to water treatment. These compounds control unwanted bacteria, fungi, mold and other organisms. As such, they enhance the durability, performance, and safety of products for consumers and workers. An increasingly strong regulatory and legislative environment is driving industries to seek out less toxic, naturally derived solutions. In response to this need, SyntheZyme has developed a family of plant-based modified biosurfactants (sophorolipids).

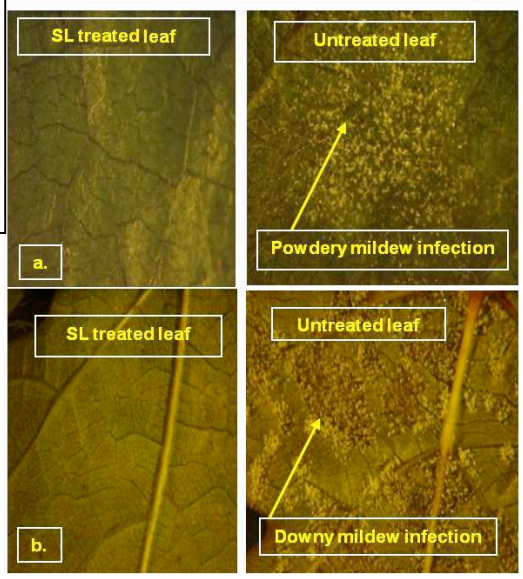
Sophorolipid production via fermentation (left) and phase separated sophorolipids (right) after letting the culture broth settle

Sophorolipids (SLs) are produced efficiently via fermentation from sugars and plant oils giving volumetric yields of natural SLs >300 g/L. Furthermore, SLs are easily isolated from fermentations since they are excreted from the yeast and then self-assemble by forming a viscous brown liquid at the bottom of the fermentor. The natural SLs themselves can be used as actives or ingredients for cleaning, antimicrobial and cosmetic applications. However, the problem encountered is they are not as active as synthetic materials and, therefore, have limited market usage in these applications. SyntheZyme has successful designed and synthesized modified sophorolipids that meet and exceed the performance of competitive products. SyntheZyme's technology of modified sophorolipids to enhance their performance in a range of applications is IP protected and selected product examples are described below.

Spinach plant unprotected

SyntheZyme's biopesticide effectively protected spinach plants.

Effect of SL-derivatives on grape-leaves infected with (a) powdery mildew and (b) downy mildew pathogen



Proprietary modified sophorolipid compositions have been developed that are highly active against a broad spectrum of plant pathogens. These compounds have activities of 2-16 folds higher than natural (unmodified) sophorolipids. They provide *i*) activity that is 2 to 30 times higher (0.1 to 10mgthan leading competitive chemical and bio-pesticide products e.g., Quadris (Syngenta), Serenade (Agraquest) and the antibiotic cycloheximide (actidione) and *ii*) proven broad spectrum activity against plant pathogens (bacteria, and fungi) such as *Phytophthora infestans*, *P. capsici*, *Alternaria solani*, *A. tomatophila*, *A. alternata*, *Botrytis cinerea*, *Ustilago maydis*, *Fusarium asiaticum*, *F. austroamericanum*, *F. cerealis*, *F. graminearum*, *Penicillium chrysogenum*, *P. digitatum*, *P. funiculosum*, *Aspergillus niger* and

Aureobasidium pullulans. Results of zoospore lysis by SL-derivatives showed that spore viability was completely lost with lysis of spore cell wall at concentrations of 500-50µg/ml. The

OPTIMIZED ANTIFUNGAL ACTIVITY OF LEAD SL COMPOUNDS

Pathogen	SL-AM-3	SL-AM-4	SL-AM-7	SL-AM-8	SL-AM-18	SL-AM-19	SL-AM-20	Quadris	Serenade	Antibiotic
	MIC values in mg/ml									
Alternaria tomatophilia	0.3	0.15	0.3	0.6	1.25	2.5	5	0.3	2.5	0.3
A. solani	0.15	0.6	0.31	0.31	1.25	2.5	2.5	1.25	2.5	0.15
A. alternata	1.25	1.25	1.25	1.25	2.5	2.5	10	2.5	2.5	0.15
Fusarim oxysporum	-	5	5	2.5	1.25	2.5	2.5	1.25	2.5	0.3
Botrytis cinerea	0.6	0.6	0.6	0.6	5	-	-	0.6	1.25	2.5
Phytophthora infestans	5	5	10	10	-	-	-	0.6	2.5	2.5
Ustilago maydis	-	5	2.5	2.5	1.25	2.5	5	2.5	2.5	2.5
Phytophthora capsici	10	10	0.6	0.6	-	-	-	0.3	0.6	2.5
Fusarium asiaticum	-	0.6	2.5	2.5	1.25	2.5	5	5	10	-
F. austroamericanum	0.6	0.6	1.25	1.25	-	-	-	5	10	5
F. cerealis	0.6	-	1.25	1.25	2.5	5	5	5	10	5
F. graminearum	0.6	0.6	5	10	5	2.5	5	5	10	5
Penicillium chrysogenum	-	2.5	5	10	-	-	-	5	2.5	5
P. digitatum	0.3	0.3	0.6	0.6	-	-	-	0.6	0.6	0.6
P. funiculosum	2.5	2.5	1.25	2.5	5	2.5	2.5	5	10	-
Aspergillus niger	5	5	10	10	-	5	-	5	10	5
Aureobasidium pullulans	1.25	1.25	5	5	-	5	-	0.6	0.6	5
Chaetomium globosum	5	5	0.6	0.6	2.5	2.5	10	-	-	-

Values in red highlighted with yellow	Promising results
Values highlighted with green	Lead compounds

SL-derivatives, SL-AM-3,4,7,8,18,19 & 20 active at 0.15 to 10mg/ml minimum inhibition concentrations (MIC) against 14,17,18,11,12 & 11 different fungal pathogens, respectively.

modified sophorolipid is the active ingredient and not a microbe, as is the case in some competitor products. Microbes may not survive in all environments where they are applied as biopesticides. Im portant attributes of these compounds is, they are produced from readily renewable feedstocks, highly active, non-toxic to non-target organisms (i.e. microbes, plants and animals), fully biodegradable, safe-to-use from handling to application and, disposal.

SyntheZyme has also developed proprietary anti-microbial modified sophorolipid compositions that are highly active against a broad spectrum of human pathogens have also been developed. SyntheZyme's modified sophorolipid anti-microbials have a *100 to 1000* folds higher activity than natural (unmodified) sophorolipids. They provide: *i*) activity that is competitive to standard antibiotics such as Streptomycin, *ii*) wide capability with proven effectiveness against *Bacillus sp*., *Streptococcus agalactiae, Moraxella* sp., and *Rhodococcus erythropolis.*

Antimicrobial activity of SLs against human pathogens

Strains	SL-A1	SL-A2	SL–A3	SL–A4	SL-A5	SL-A6
	Minimum inhibitory concentration$_{100}$ (mg/ml)					
Bacillus NRRL	1.67	N/A	6.17×10^{-2}	6.17×10^{-2}	0.56	5
Streptococcus agolatiae	1.67	5	6.86×10^{-3}	2.05×10^{-2}	6.17×10^{-2}	5
Moraxella	1.67	5	2.05×10^{-2}	6.17×10^{-2}	6.17×10^{-2}	5
Rhodoccocus erythropolis	N/A	0.56	6.86×10^{-3}	5	5	5