



Application of biosurfactants as antimicrobial additive in food and beverages

SuperBIO Workshop Biosurfactants, Bio Base Europe Pilot Plant, Gent

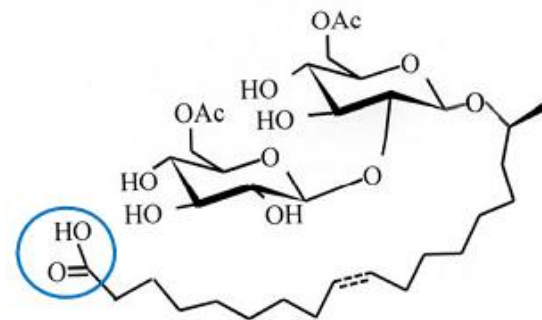
Dr. Thomas Henkel, Managing Director, April 3, 2017

Glycolipids: microbially derived Bio-Surfactants

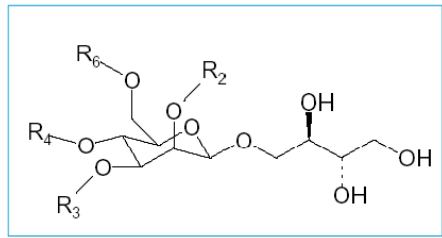
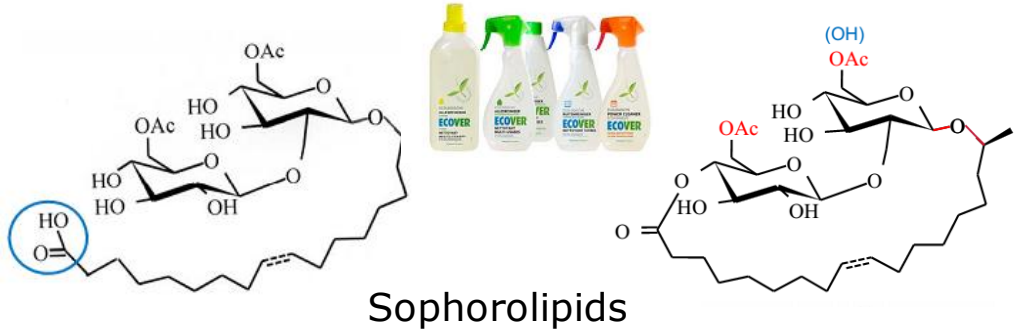
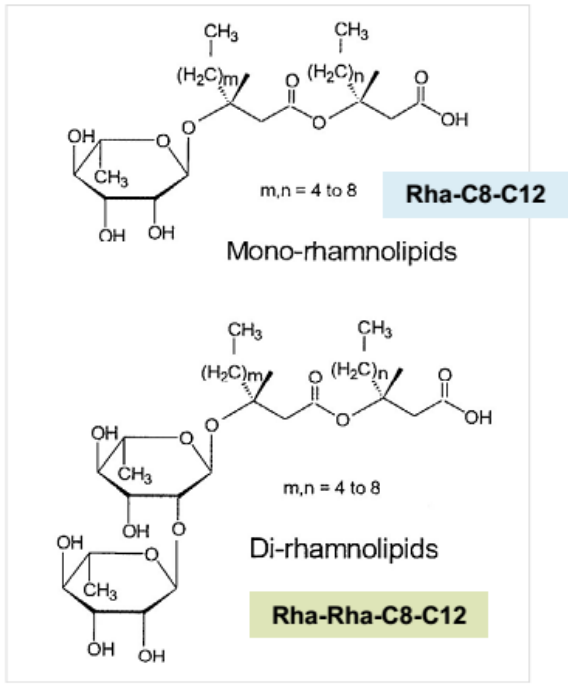
- Amphiphilic agents with surfactant properties
- Produced by a variety of microorganisms with various structures
 - Rhamnolipids, Sophorolipids, etc.
- Broadly researched for their production and commercial use
- Multiple biological activities reported
- Multiple use advantages discussed: lower tox, biodegradable, environmental compatible, sustainably produced,

... candidates for “Bio-Based” products
 (Bioeconomy)

S. Lang | Workshop “Biosurfactants Challenges and Perspectives”, Frankfurt/M.; 05/2013

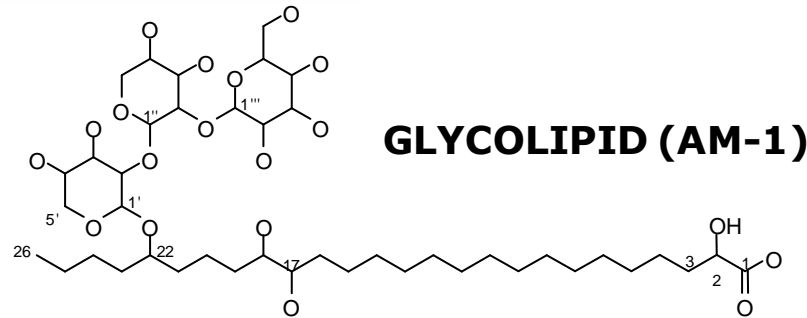


Glycolipids represent prominent Bio-Surfactants with known but weak anti-microbial activity

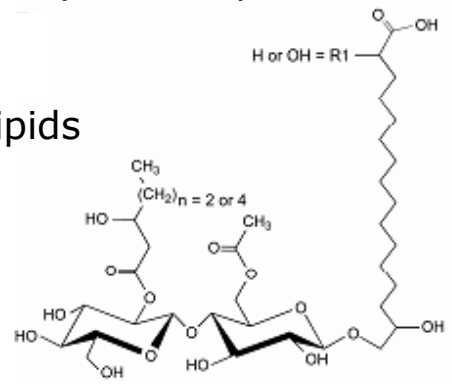


MEL A-C: R₂, R₃ = fatty acids C₇-C₁₄
 MEL A: R₄, R₆ = acetyl
 MEL B: R₄ = acetyl, R₆ = H
 MEL C: R₄ = H, R₆ = acetyl

Mannosylerythritol Lipids



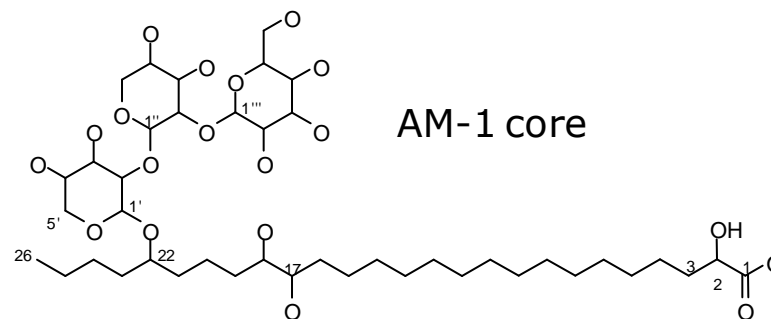
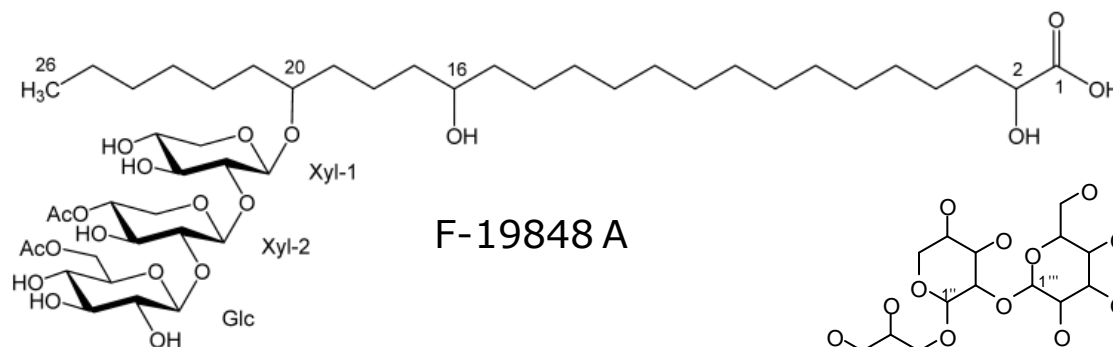
Cellobiose Lipids



GLYCOLIPID AM-1 (“Glykenins, F-19848”)

- product of *Dacrymyces* sp. (Basidiomycetes)
- weak inhibitor of hyaluronic acid (HA) binding to CD44 (F-19848 A)
- inhibitory activity against gram-positive bacteria, polio and Herpes virus

... no commercial use yet reported!



Fumiko NISHIDA et al., J. MassSpectrom.Soc.Jpn. Vol.43, No.1, 1995

Yuki Hirota-Takahata et al., J.Antibiot. 60(10): 633-639, 2007

Glycolipids from Yeast: anti-microbial activities

| Type | yeast/mold (MIC) | gram+ (MIC) |
|-----------------------------------|------------------|------------------|
| • Sophorolipids | na | 100ppm/no act.** |
| • Mannosylerythritol Lipids (MEL) | na | na |
| • Ustilagic acids | 8-600ppm* | na |
| • Glycolipid AM-1 | 5-25ppm*** | 50-100ppm*** |

... only limited data available in comparison, but

... AM-1 appears far superior in MIC levels vs. other Glycolipids

na = not available

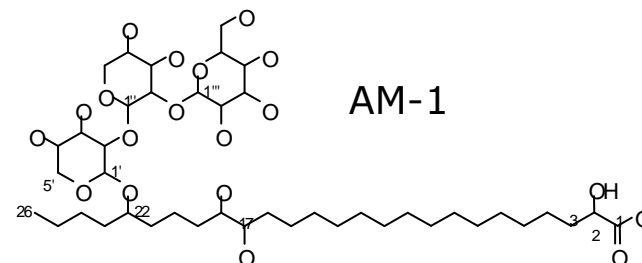
*Wacker Chemie WO2013/037818 A2

**Dissertation Beate Teichmann, Marburg/Lahn, Februar 2009

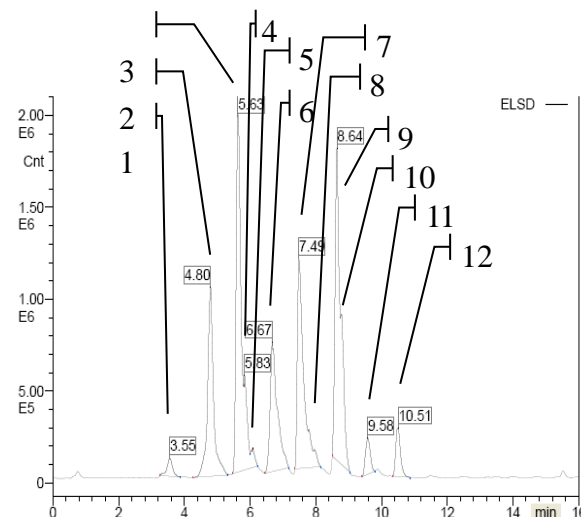
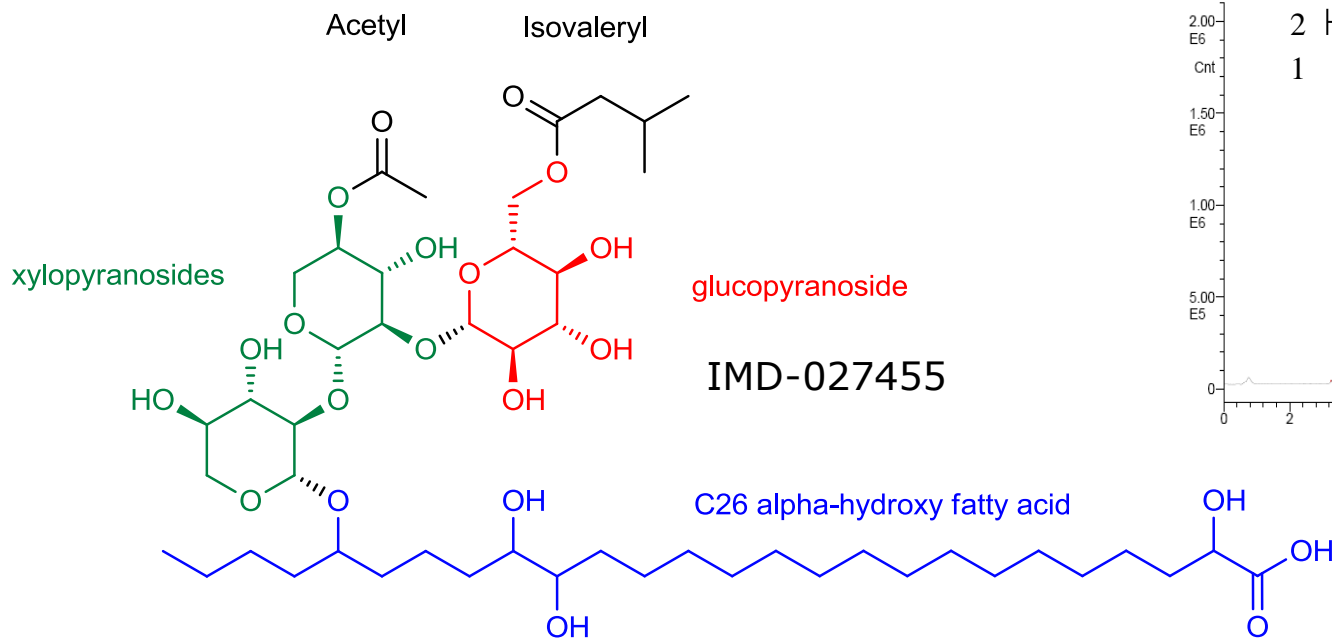
***Vishal Shah et al., ANTIMICROBIALAGENTS AND CHEMOTHERAPY, Jan. 2007, p. 397–400

***Joseph N. Sleiman et al., Annals of Clinical & Laboratory Science, vol. 39, no. 1, 2009

*** INS data



Glycolipid AM-1 with distinct anti-microbial properties

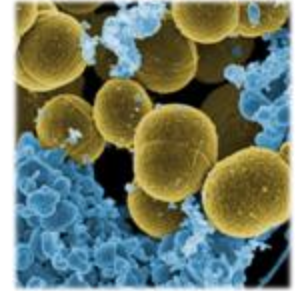


- AM-1 represents a mixture of various congeners
- Variations are mainly due to number, type and positioning of acylation

AM-1 perspective in applications

Cosmetics/personal care

- Prevent spoilage during storage (e.g. by *Aspergillum*, *Candida albicans*)



Foods/Beverage

- Prevent food spoilage during processing and storage (e.g. by yeasts)
- Prevent contamination with pathogens (e.g. by *Bacillus cereus*)

Medical Use

- Coating of catheter (biofilm); Support wound healing

... broad application spectrum foreseeable and in testing

Application Example: Carbonated soft drinks

Preservation of Granini die Limo Limette + Zitrone

AM-1 dissolved at **15 ppm** use level

No microbial growth after **3 months** despite inoculation with mold or yeast mixtures.

Yeasts: *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, *Z. bailii*

Molds: *Aspergillus niger*, *Byssoschlamys nivea*, *Penicillium roqueforti*



Preservation of Fanta Orange

AM-1 dissolved at **5 ppm** use level

Without AM-1, rapid growth of yeasts and molds is observed within 1 – 7 days (die Limo) or 8 – 14 days (Fanta) under test conditions.



Application Examples: Fruit drink with AM-1 versus sorbate/benzoate

Capri-Sonne Multivitamin (12% juice)

Declared ingredients with possible preserving properties: none

AM-1 dissolved at **10 ppm** use level

No microbial growth after 4 weeks despite inoculation with molds or yeasts.

Yeasts: *Saccharomyces cerevisiae*, *Zygosaccharomyces rouxii*, *Z. bailii*

Molds: *Aspergillus niger*, *Byssoschlamys nivea*, *Penicillium roqueforti*

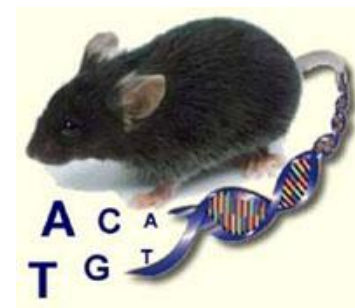


- Without AM-1, rapid spoilage is observed within 1 – 7 days under the test conditions
- Using benzoate or sorbate, 1000 µg/ml are needed to obtain identical results
→ **100-fold lower use level of AM-1 as compared to benchmarks!**

AM-1 is a very safe product

- Ames test: Not mutagenic
- Micronucleus test: Not clastogenic and/or aneugenic
- Topical safety tests negative: Skin sensitization (OECD406_Buehler test), Skin irritation (OECD 439) Phototoxicity (OECD 432) Eye irritation (HCE assay)
- Acute toxicity, rat (OECD 423): At 2,000mg/kg no signs of toxicity
- 90d sub-chronic tox (rat and dog): NOAEL > 1,200mg/kg/d
- Favorable ADME study with low bioavailability
- Favorable repro- and developmental tox study results

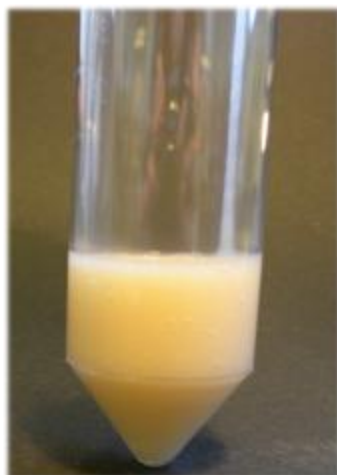
... very safe for chronic human penetration e.g. in foods and cosmetic preserving applications



AM-1: from mushroom to product



USP



Harvested culture broth



DSP



Product in DSP process



DSP



Final AM-1 product

Set up of manufacturing unit for large scale AM-1 production in progress



**AM-1 product at
INS: kg scale**

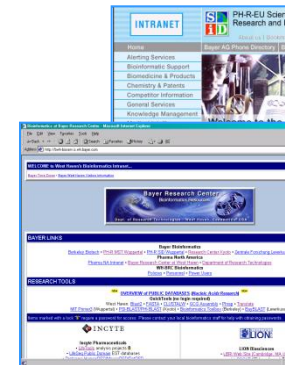


**AM-1 product in
production:
Multi-kg scale**

**Off-white micro-crystalline powder
>90% purity
Food/cosmetic compatible specification**

AM-1 Key Features

- Very safe in application (daily use)
- Highly efficacious against pathogens and spoiling germs
- No cross resistance to available solutions
- No tissue irritant or sensitizing
- Favorable PK parameters
- Soluble in water, taste- and colorless
- Surface active (as to attack biofilms)
- “All Natural” label
- Reasonable cost in use
- No regulatory hurdles
- Strong IP protection



... may be the most innovative product candidate in this field...

AM-1 market roll out



- R&D time lines: 6 (cosmetic) – 8 (food) years
- Target markets: Cosmetic, Food

Launch 2017-2019





AM-1

A promising “all natural” anti-microbial agent