ANTIMICROBIAL POLYMERS OF BACTERIAL ORIGIN

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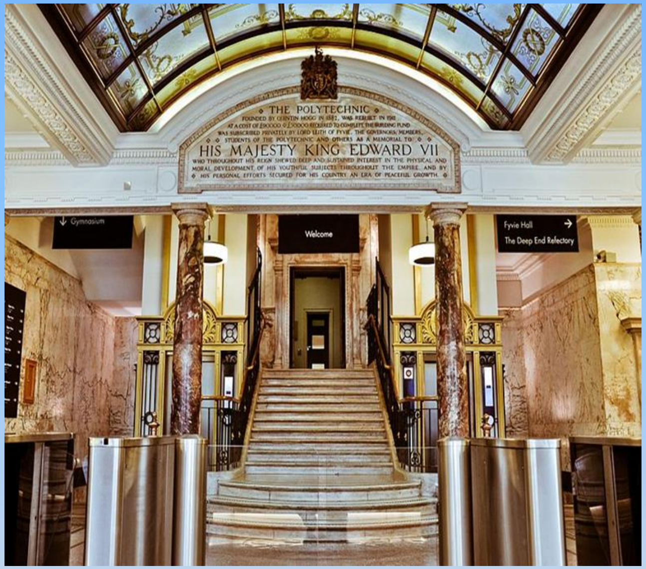

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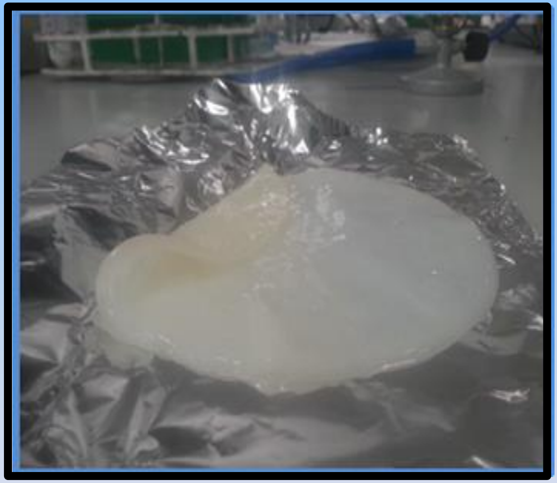
Polyhydroxyalkanoates



Bacterial Cellulose

Polyhydroxyalkanoates, the biodegradable and biocompatible polymers

Polyhydroxyalkanoates are water-insoluble storage polymers which are polyesters of 3-, 4-, 5- and 6-hydroxyalkanoic acids produced by a variety of bacterial species under nutrient-limiting conditions. They are biodegradable and biocompatible, exhibit thermoplastic properties and can be produced from renewable carbon sources.

Philip *et al*., 2007, JCTB, 82 (3):233-247

Akarayonye *et al*., 2010, JCTB, Volume 85 (6): 732-743

Keshavarz *et al*., 2010, Current Opinion in Microbiology 13 (3): pp. 321-326

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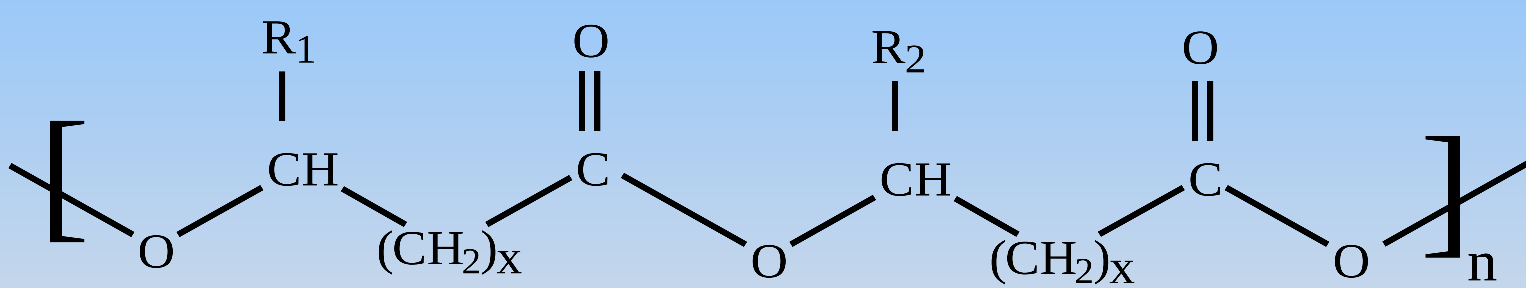
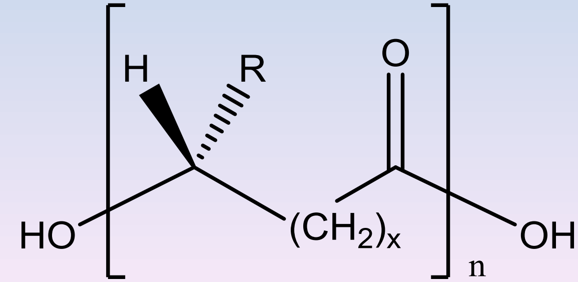
The general structure of Polyhydroxyalkanoates



R_1/ R_2 = alkyl groups (C_1-C_{13})
x = 1,2,3,4



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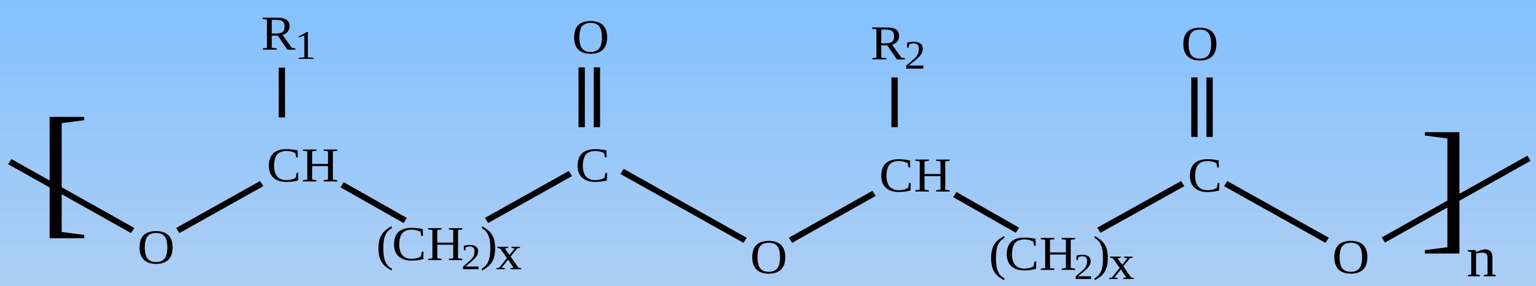
SCL and MCL Polyhydroxyalkanoates



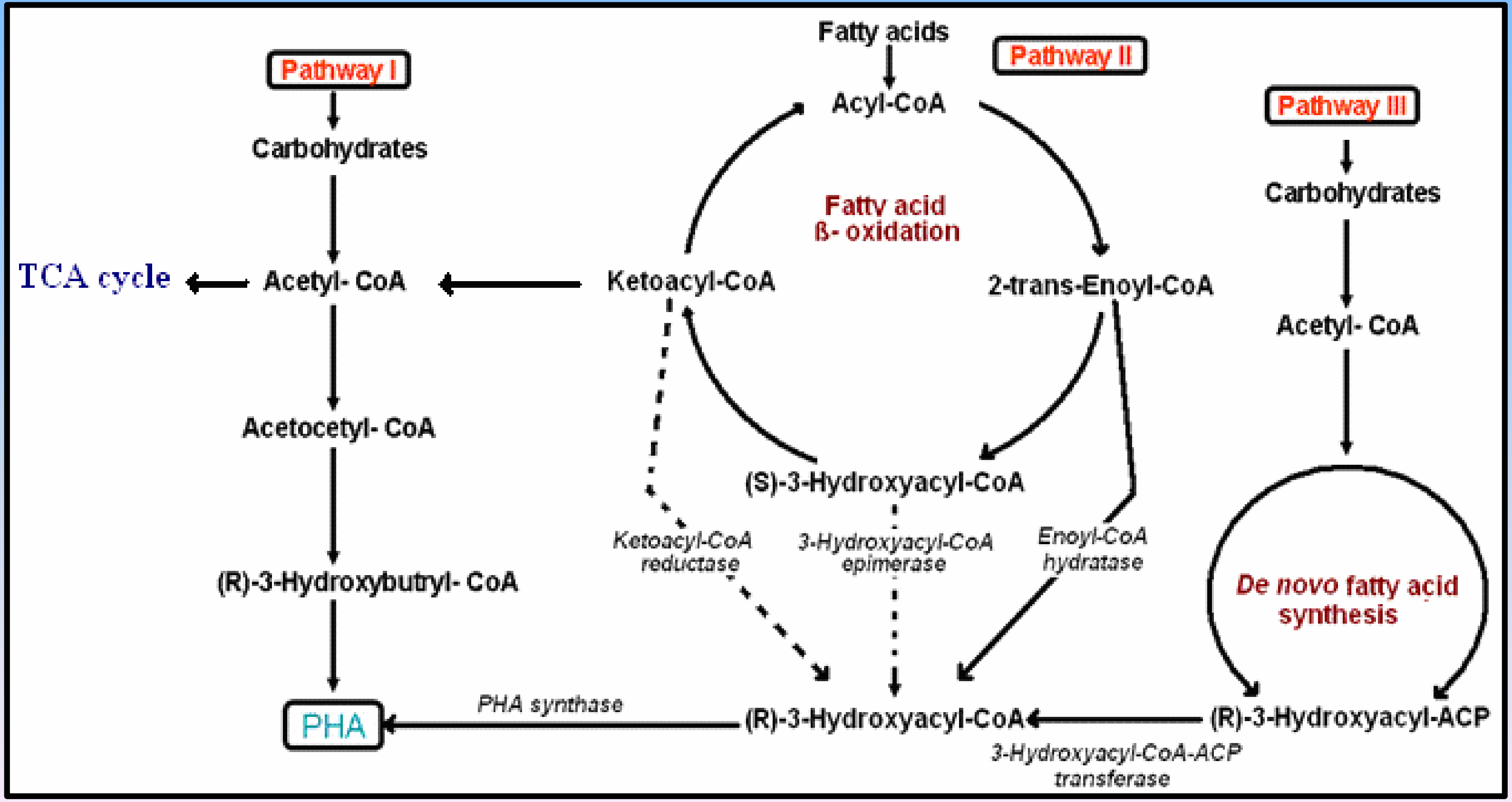
Total Carbon chain length in monomer = 4-5;**SCL PHAs**
Total Carbon chain length in monomer =6-14;**MCL PHAs**

SCL-PHAs- Thermoplastics
MCL-PHAs-Elastomerics

Properties of SCL and MCL Polyhydroxyalkanoates

Type of PHA	Melting Temp (°C)	Glass Transition Temp (°C)	Young's Modulus (GPa)	Elongation at break (%)	Tensile strength (MPa)
P(3HB)	171	2.7	3.5	1	40
P(3HB-co-20%3HV)	145	-1	1.2	3.84	32
P(4HB)	60	-50	0.149	1000	104
P(3HB-co-16%4HB)	152	-8	ND	444	26
P(3HO-co-18%3HHx)	61	-35	0.008	400	9
P(3HB-co-3HHx)	120	-2	0.5	850	21

Metabolic Pathways involved in PHA Biosynthesis



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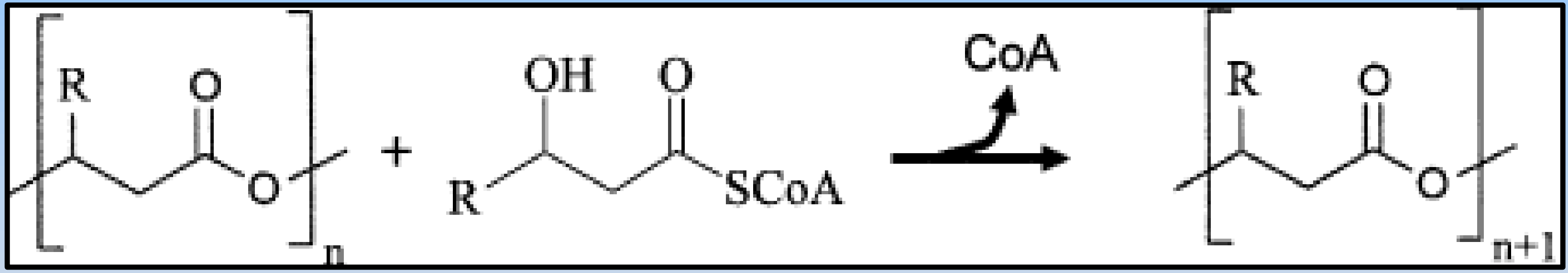
Polyhydroxyalkanoate Synthases, the enzymes involved in PHA Biosynthesis



PHA synthases catalyse the stereo-selective conversion of (*R*)-3-hydroxyacyl-CoA substrates to PHAs with the concomitant release of CoA

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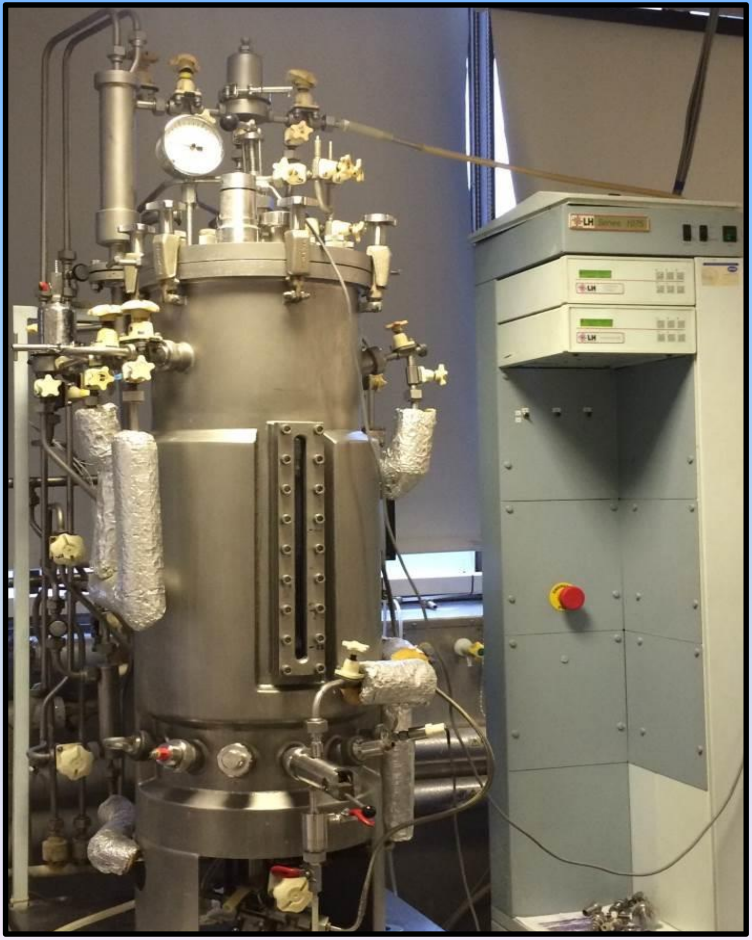
Production of Polyhydroxyalkanoates in Large Scale Fermenters



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Production of SCL-Polyhydroxyalkanoates using *Bacillus cereus* SPV, a Gram positive bacteria

Valappil *et al*., 2007, Journal of Biotechnology, Volume 127(3), 475-487

Valappil *et al*., 2008, Journal of Applied Microbiology Jun; 104(6):1624-35

Philip *et al*., 2009, Biomacromolecules 10(4): 691 – 699

Akarayonye *et al*., 2010, Biotechnology Journal 7(2) 293-303

Akarayonye *et al*., 2016, Polymer International,65 (7) 780–791

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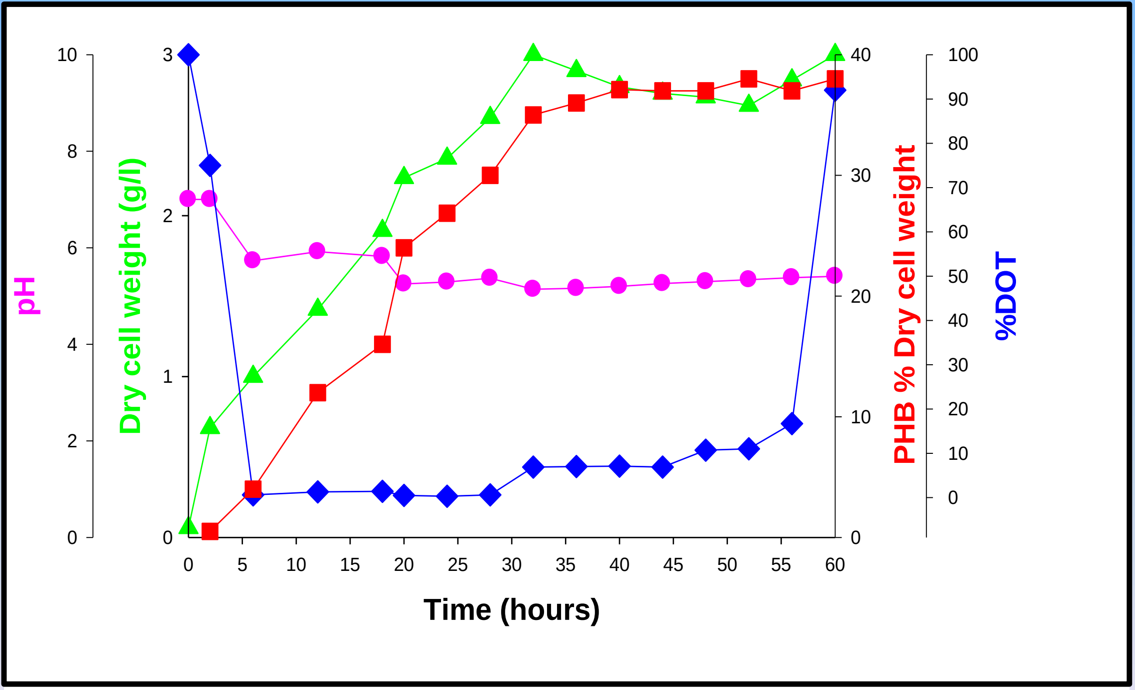
Large scale production of P(3HB) using fed batch fermentation in Kannan and Rehacek medium (Yield 38% dcw)



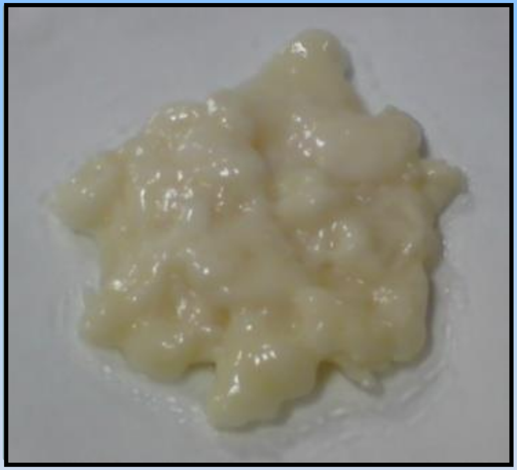
GLUCOSE as the main Carbon Source

Valappil *et al.*, 2007, Journal of Biotechnology, 132; 251-258

Material and Thermal Properties of the P(3HB) produced

Type of PHA	Melting Temp (^{o}C)	Glass Transition Temp (^{o}C)	Young's Modulus (GPa)	Elongation at break (%)	Tensile strength (MPa)
P(3HB)	169	1.9	1.7	3.8	25.7

Production of MCL-Polyhydroxyalkanoates using *Pseudomonas mendocina*, a Gram negative bacteria



Rai *et al*., 2011, Material Science Engineering (Reviews) 72(3) 29-47
Rai *et al*., 2011, Biomacromolecules, 12 (6), pp 2126–2136
Rai *et al*., 2011, Journal of Applied Polymer Science, 122, (6), 3606-3617
Lizzaraga *et al*., 2015, Engineering in Life Sciences 15(6) 612-621
Bagdadi *et al*., 2016, Journal of Tissue Engineering and Regenerative Medicine, doi: 10.1002/term.2318.

Material and Thermal Properties of the P(3HO) produced

Type of PHA	Melting Temp (°C)	Glass Transition Temp (°C)	Young's Modulus (MPa)	Elongation at break (%)	Tensile strength (MPa)
P(3HO)	42	-38	0.8	1200	8.6

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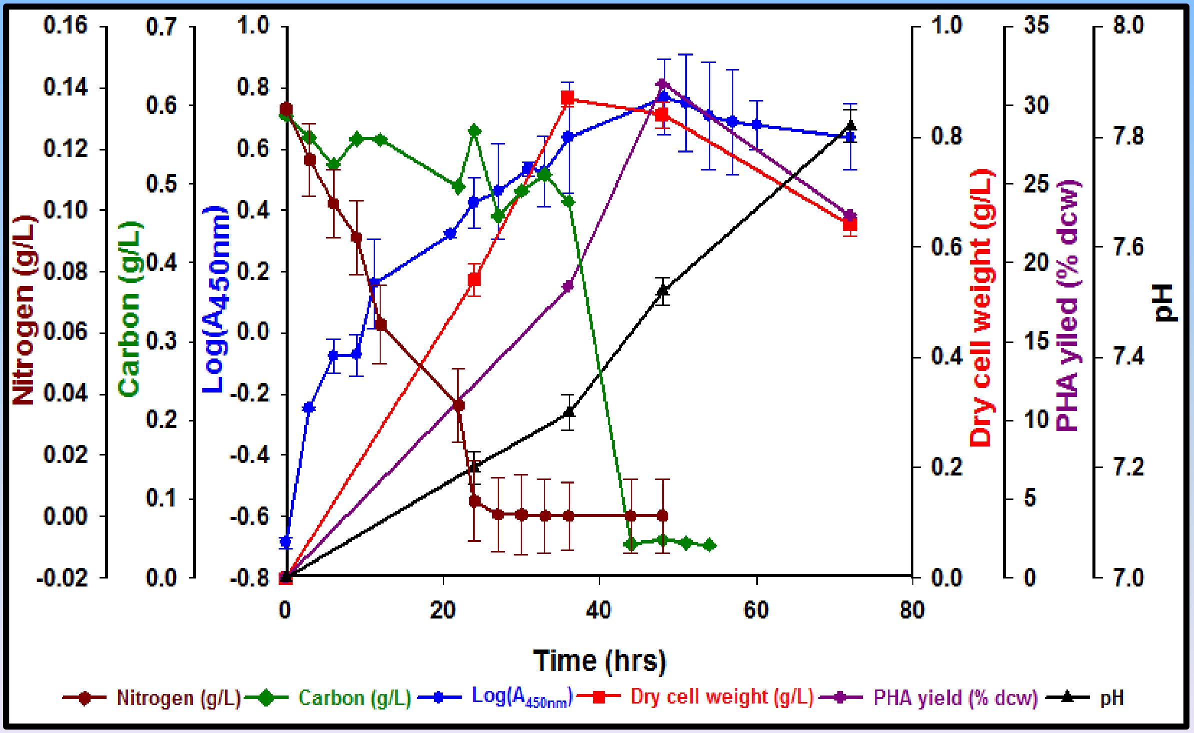
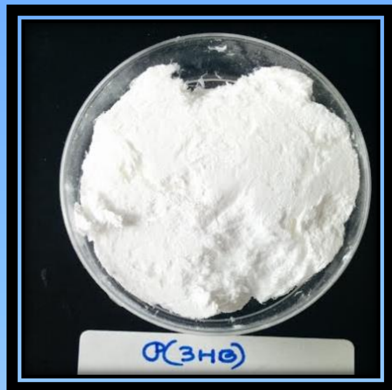
Large scale production of P(3HO) using batch fermentation in MSM media

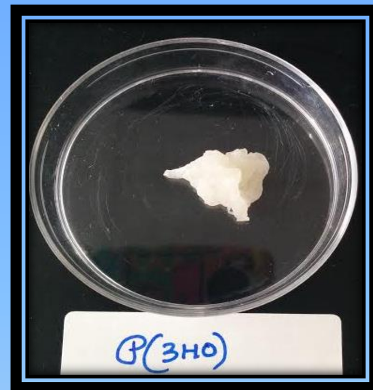
(Yield 31% dcw)

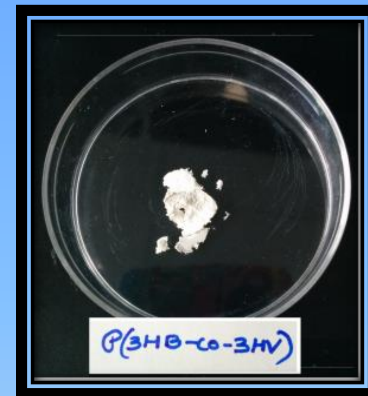


SODIUM OCTANOATE as the main Carbon Source

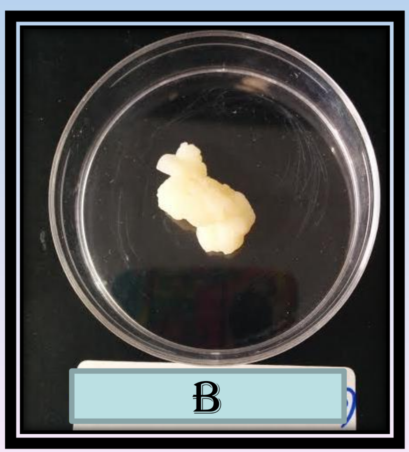
Production of a range of SCL-PHAs and MCL-PHAs

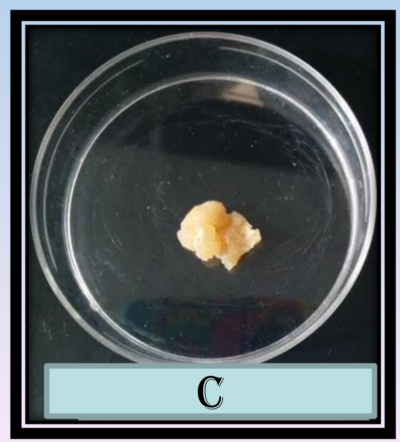


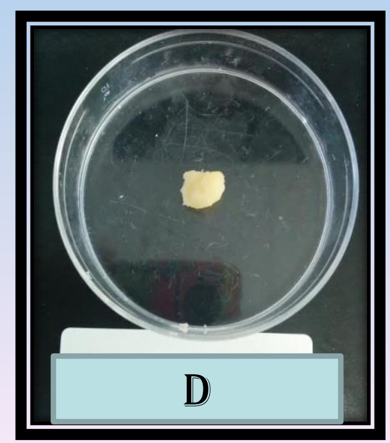




Polyhydroxyalkanoates produced using a range of different carbon sources



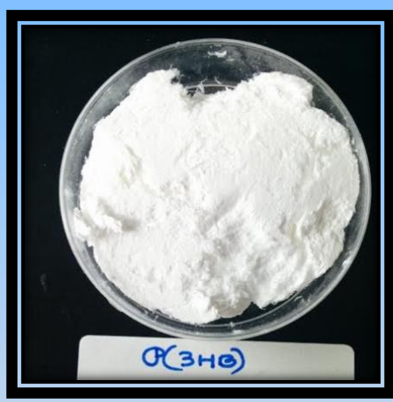




Production of Antimicrobial PHAs

Production of Antimicrobial PHAs by the addition of Antimicrobial agents of natural origin

Production of Antimicrobial PHAs



PHA

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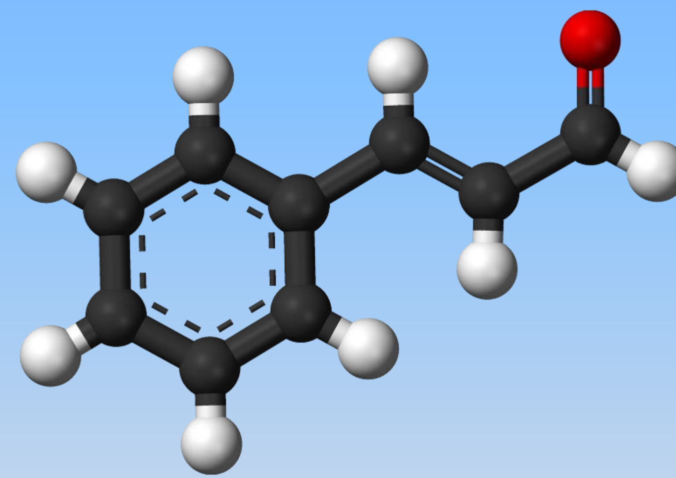
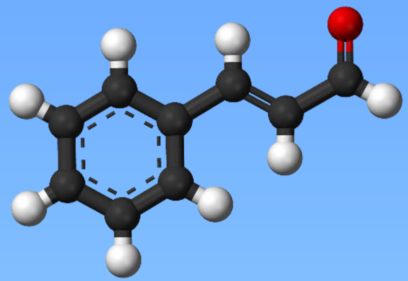
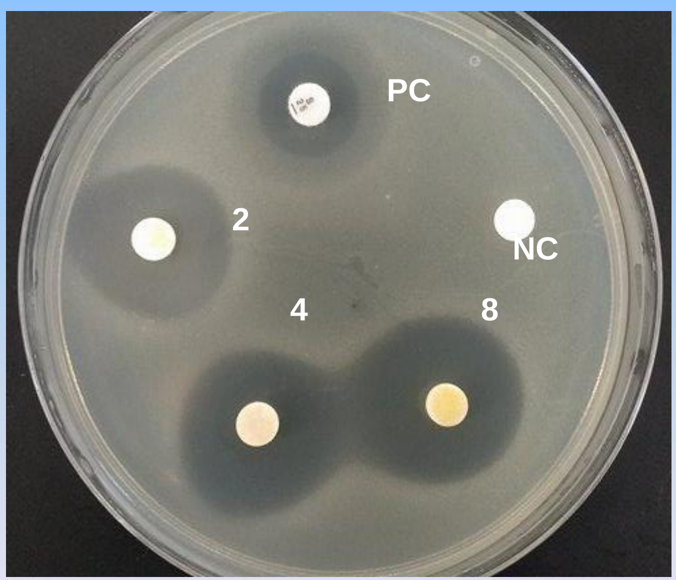


Trans-cinnamaldehyde

Production of Antimicrobial PHAs with Trans-cinnamaldehyde

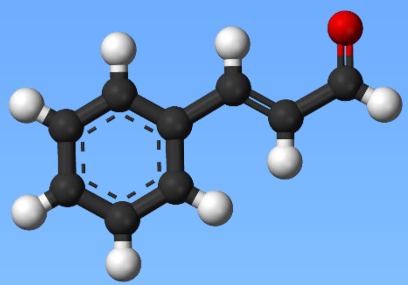


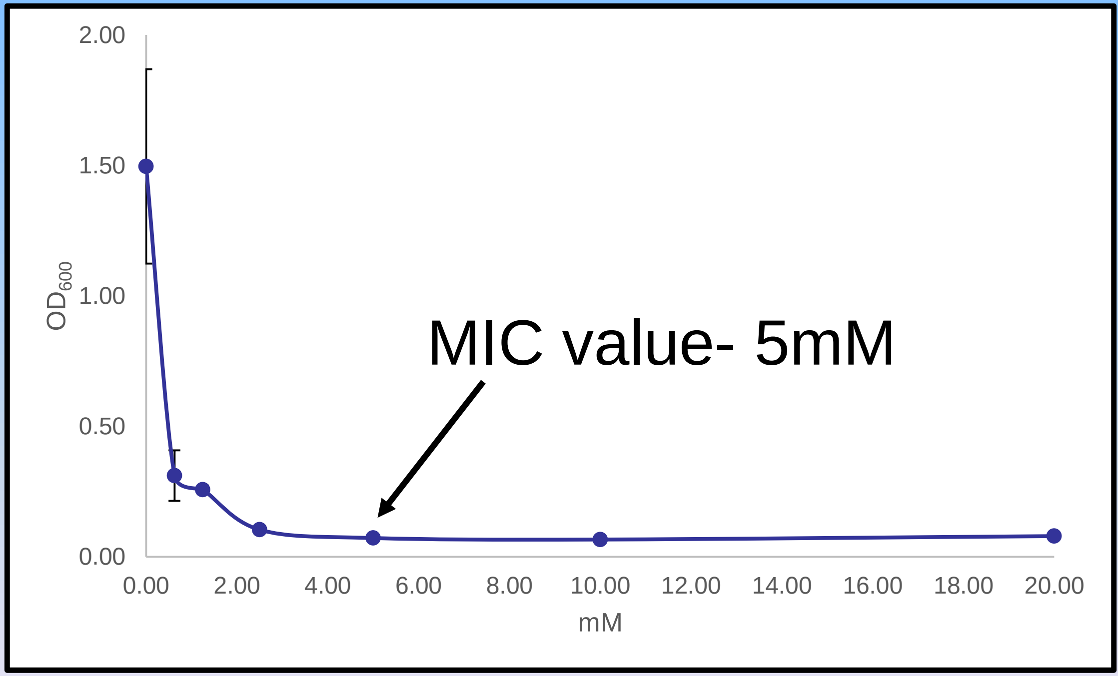


TC (µL)	Inhibition zone (cm)
PC	1.5; 2.5
2	2.5
4	2.5
8	2.5

Antimicrobial activity against *S. aureus* ATCC® 6538P™

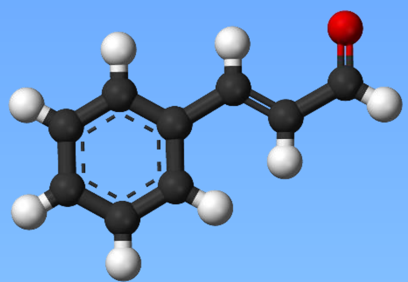
Production of Antimicrobial PHAs with Trans-cinnamaldehyde

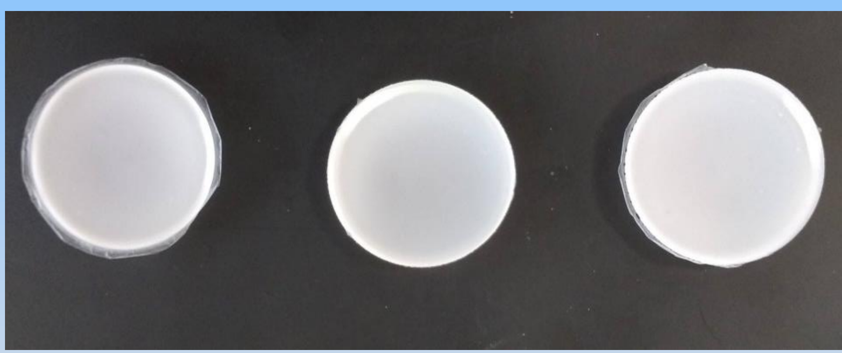




Antimicrobial activity against *S. aureus* ATCC® 6538P™

Production of Antimicrobial PHAs with Trans-cinnamaldehyde





P(3HB)/TC

	Tensile strength MPa	Young's Modulus MPa	Extension at break (%)
P(3HB)	21.5	1091.5	28.9
P(3HB), 8 wt%	16.8	802.3	53.5
P(3HB), 11 wt%	15.6	625.1	116.3
P(3HB), 13 wt%	10.7	444.8	109.1

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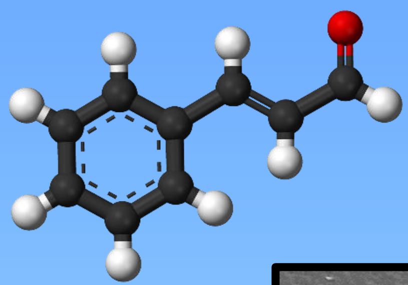
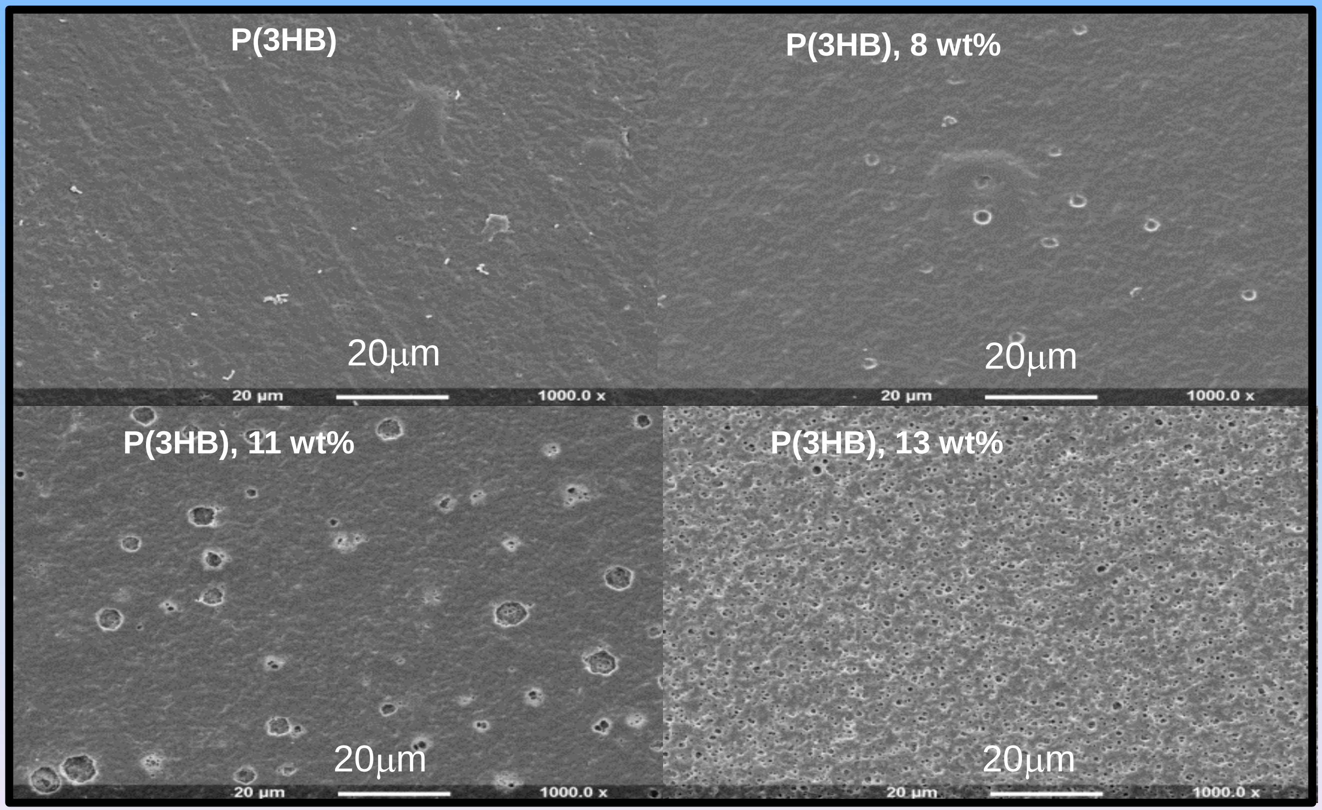
Production of Antimicrobial PHAs with Trans-cinnamaldehyde





P(3HB)/TC

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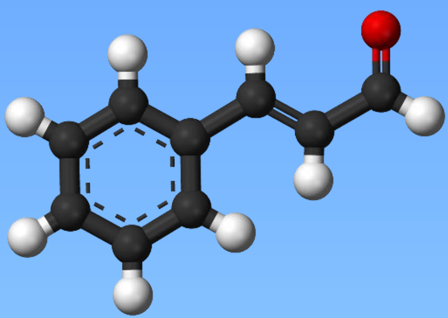
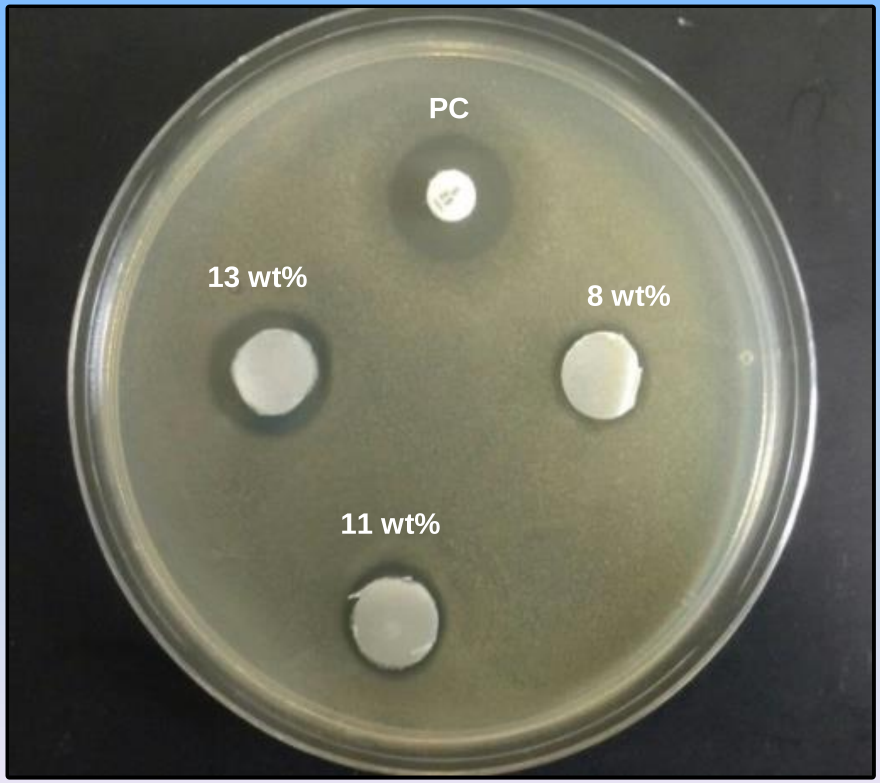
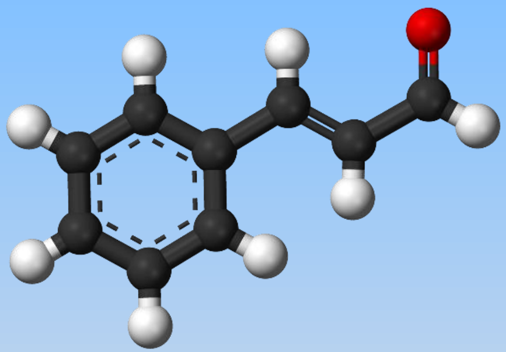
Production of Antimicrobial PHAs with Trans-cinnamaldehyde





P(3HB)/TC

S. aureus ATCC® 6538P™



Antimicrobial PHAs with Trans-cinnamaldehyde are effective against *S. aureus*

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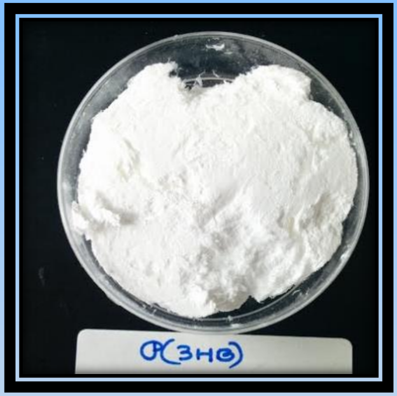
Production of Antimicrobial PHAs



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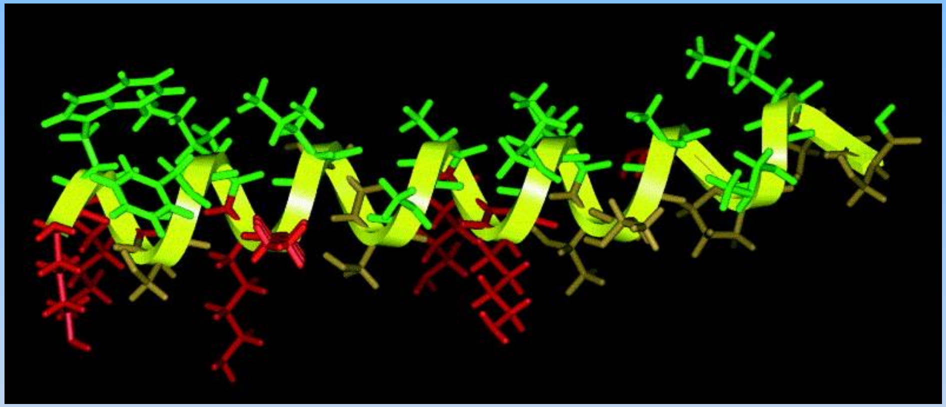

PHA

Antimicrobial peptides

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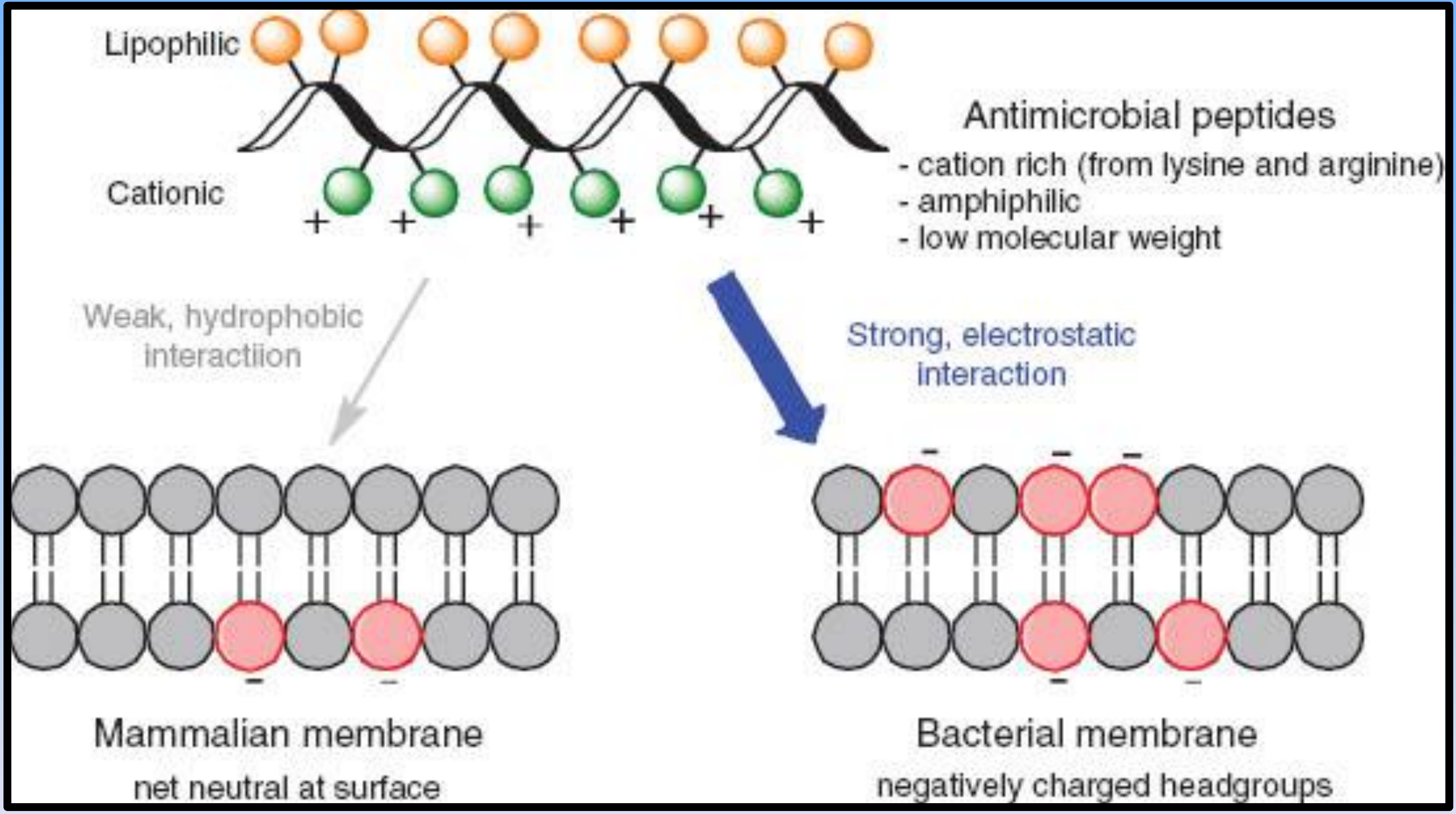
Production of Antimicrobial PHAs



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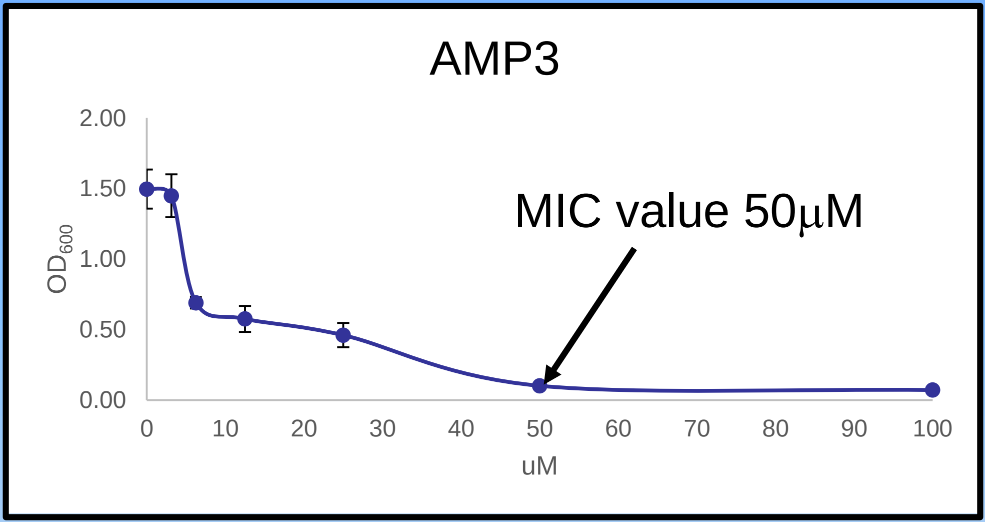
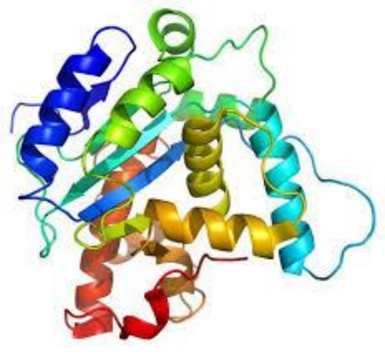
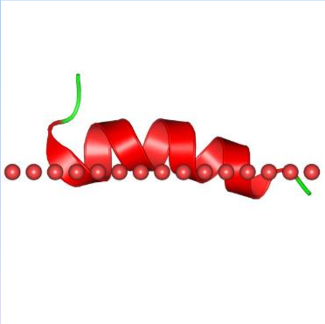
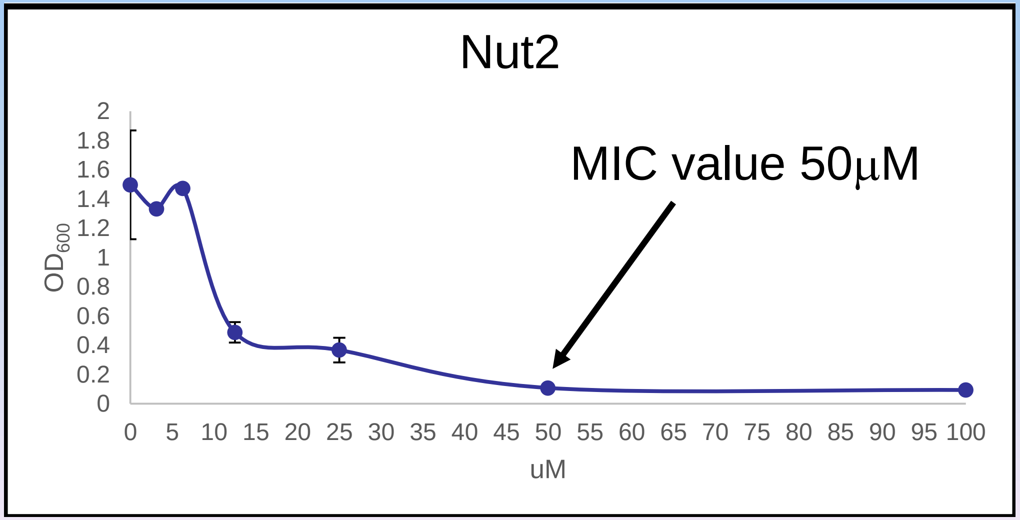
Production of Antimicrobial PHAs









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S. aureus ATCC® 6538P™

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Production of Antimicrobial PHAs

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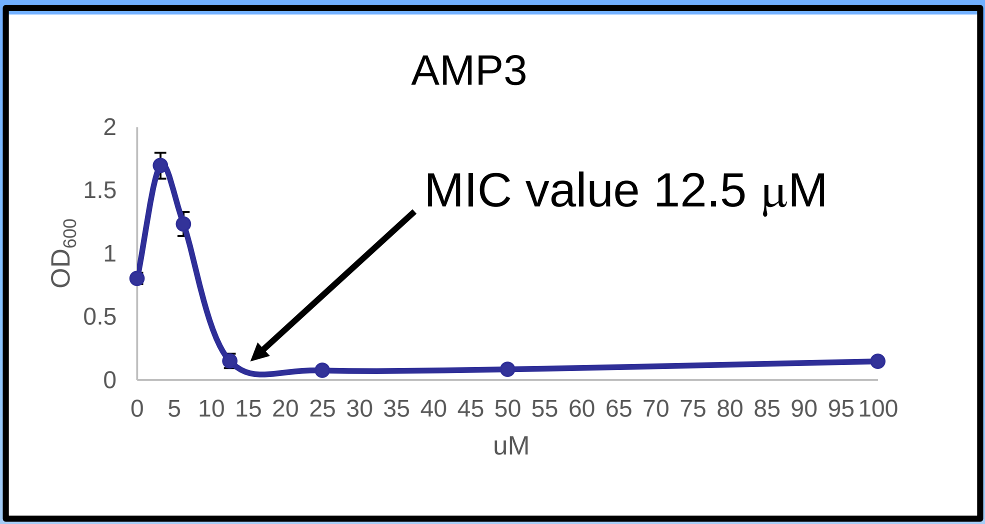

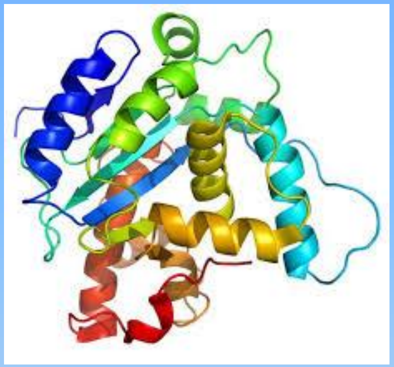

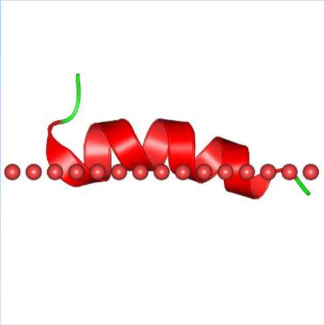

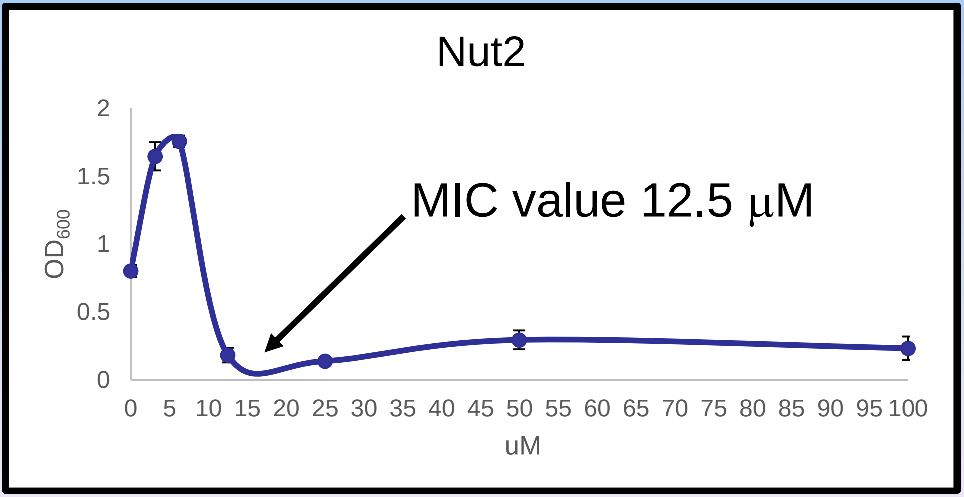

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E. coli ATCC 8739

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Antimicrobial PHAs with Antimicrobial peptides are effective against *S. aureus and E. coli*

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Production of Antimicrobial PHAs

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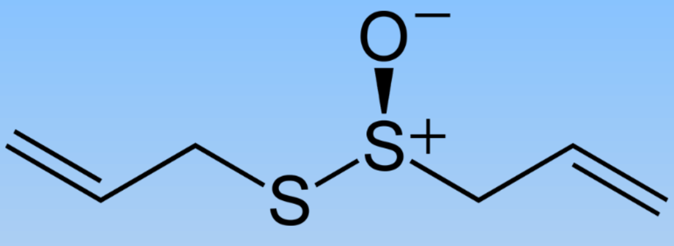

PHA

Garlic extract (allicin)

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Production of Antimicrobial PHAs

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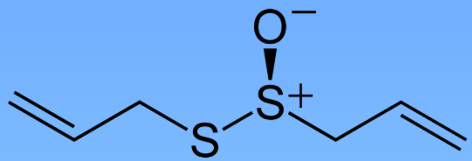

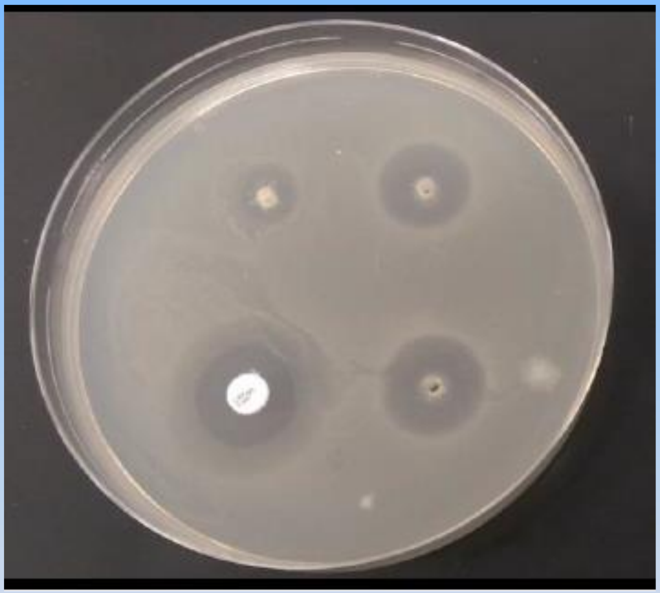

Concentration of dehydrated garlic	Dehydrated garlic/ Inhibition zone (cm)
3 mg/ml	1.1
5 mg/ml	1.5
7 mg/ml	1.7

Antibacterial assay-agar well diffusion against *S. aureus* ATCC® 6538P™

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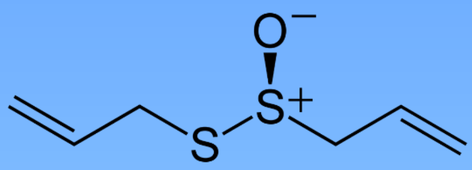
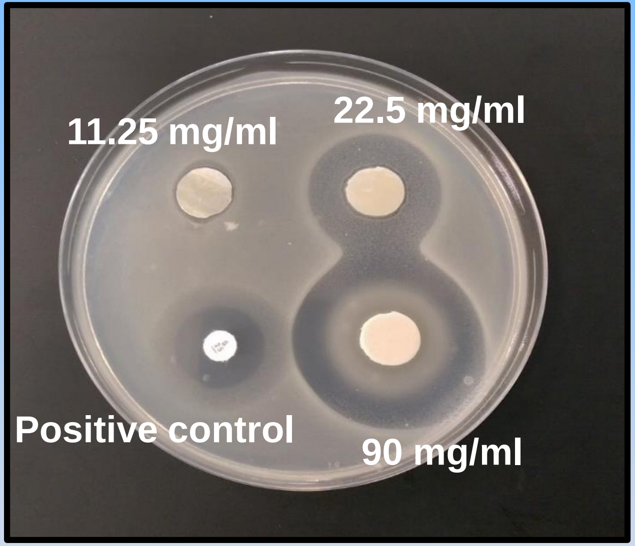
Production of Antimicrobial PHAs MCL-PHA

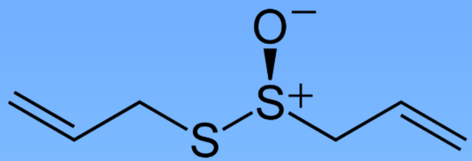


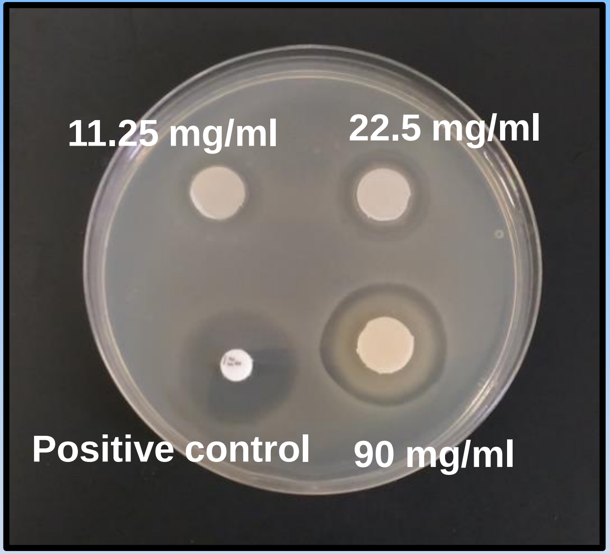


Concentration of agent	Dehydrated garlic/ Inhibition zone (cm)
11.25 mg/ml	1.5
22.5 mg/ml	2.7
90 mg/ml	3.8

Antibacterial activity of P(3HO-co-3HD) films against *S. aureus* ATCC® 6538P™

Production of Antimicrobial PHAs MCL-PHA





Concentration of agent	Dehydrated garlic/ Inhibition zone (cm)
11.25 mg/ml	1.7
22.5 mg/ml	2.2
90 mg/ml	2.7

Antibacterial activity of P(3HO-co-3HD) films against *E.coli* ATCC 8739

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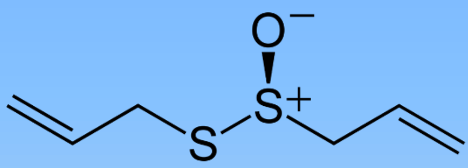

Antimicrobial PHAs with Dehydrated Garlic (Allicin) are effective against *S. aureus and E. coli*

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Inherently Antimicrobial PHAs
Thio-PHAs

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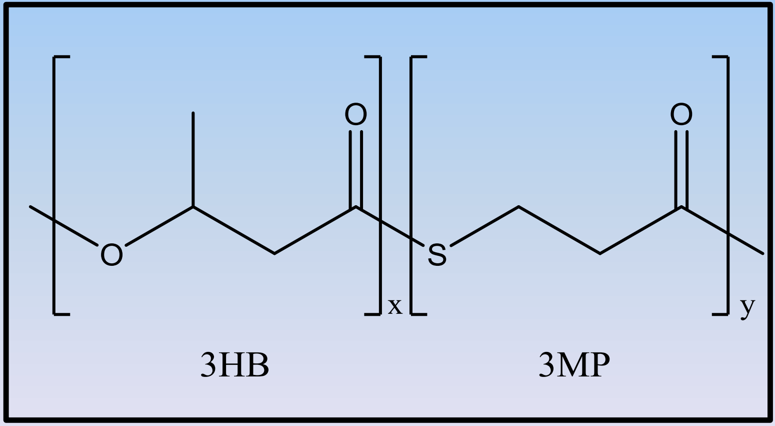
Thio-Polyhydroxyalkanoates

Sulphur containing PHAs

Sulphur in the backbone

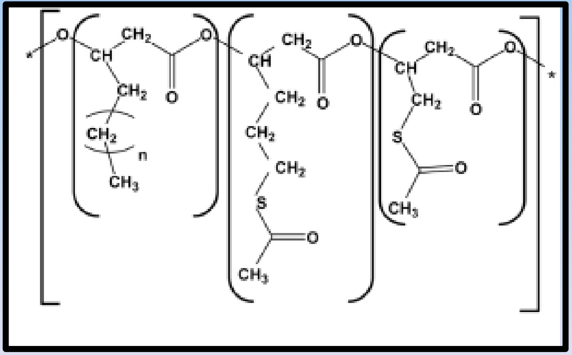
- 3-mercaptopropionate (3MP)
- 3-mercaptobutyrate (3MB)
- 3-mercaptovalerate (3MV)

copolymers with 3-hydroxybutyrate (3HB)

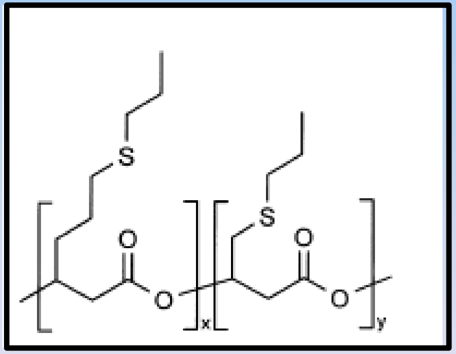


Sulphur in the side chains

Thioester groups



Thioether groups



Lütke-Eversloh, T. and Steinbüchel, A., Microbial Polythioesters. Macromol. Biosci., 4: 165–174, (2004).

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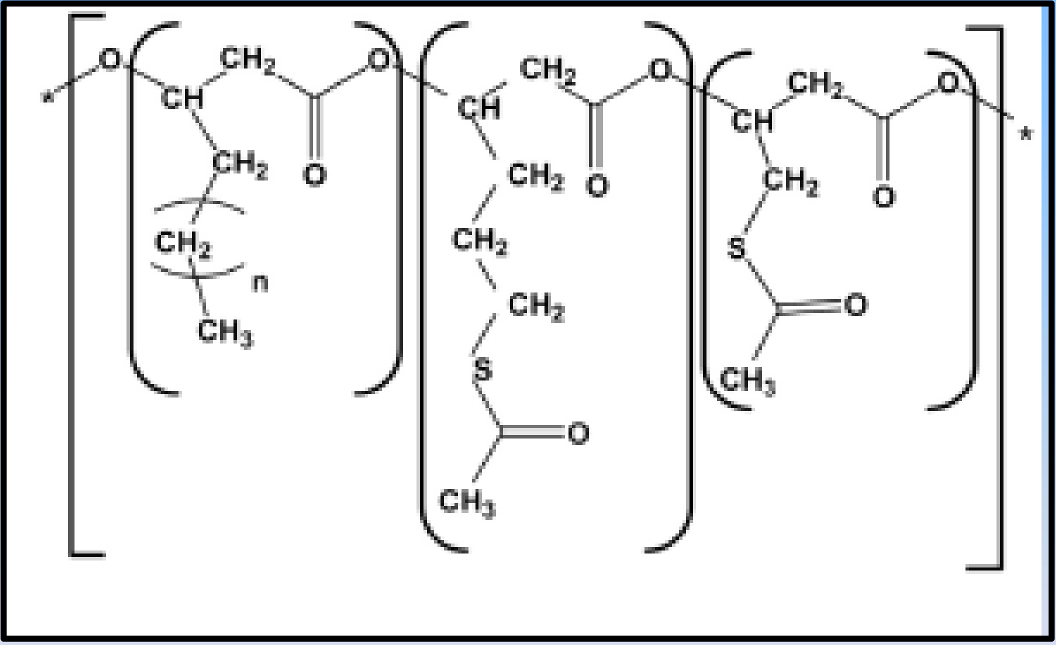
Thio-Polyhydroxyalkanoates

Sulphur containing PHAs

Sulphur in the side chains: ***Thioester groups***



<u>Proven intrinsic antimicrobial properties</u>

Against methicillin-resistant *Staphylococcus aureus* (MRSA) both *in vitro* and *in vivo*

HyMedPoly

Production of Thio-Polyhydroxyalkanoates

Co-feeding experiment:
- Decanoic acid
- 6 acetylthiohexanoic acid

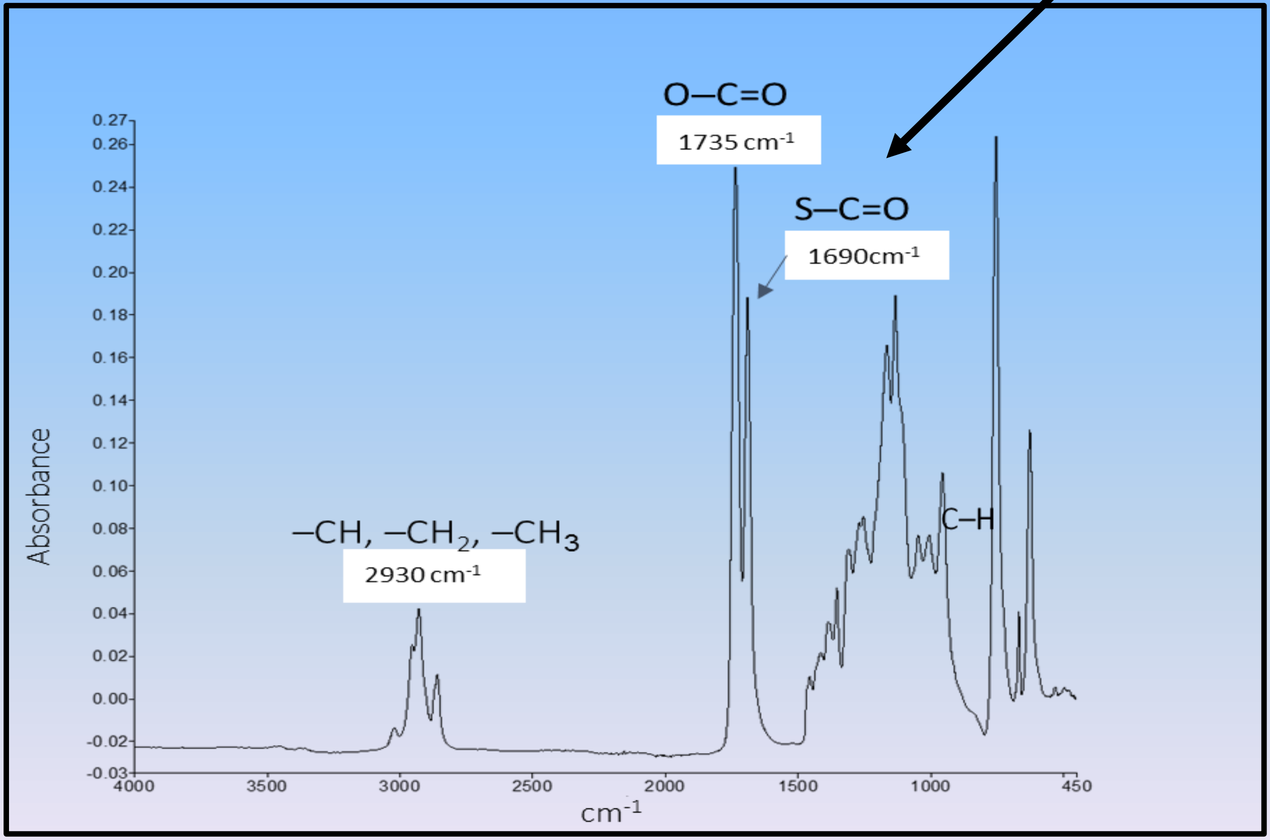
	Quantity
KH_2PO_4	13.6 g/L
$(NH_4)_2SO_4$	0.2 g/L
$FeSO_4 * 7H_2O$	0.5 mg/L
Trace elements	1 mL/L

Characterisation of the Thio-PHAs

Thio-PHAs

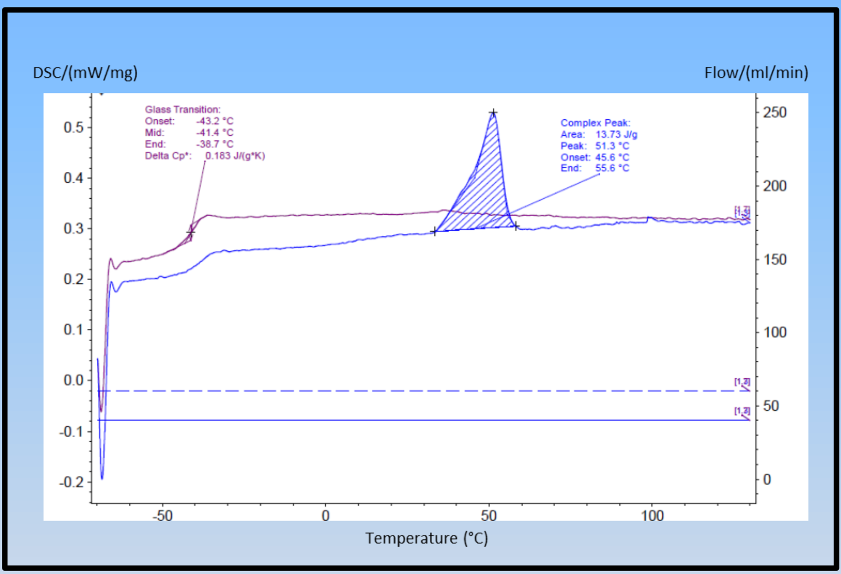
Chemical characterization: FT-IR

Thio-PHA

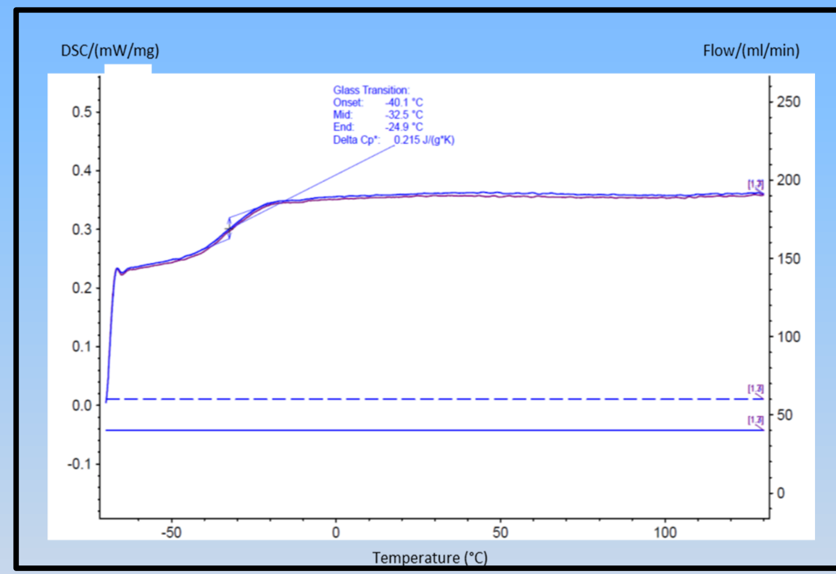


Thio-PHAs

Thermal characterization: DSC



P(3HHx-3HO-3HD)



P(3HHx-3HO-3HD-3H4ATB-3H6ATH)

Polymer	T_m (°C)	T_g (°C)
P(3HHx-3HO-3HD)	51.3	-41.4
P(3HHx-3HO-3HD-3H6ATH)	-	-32.5

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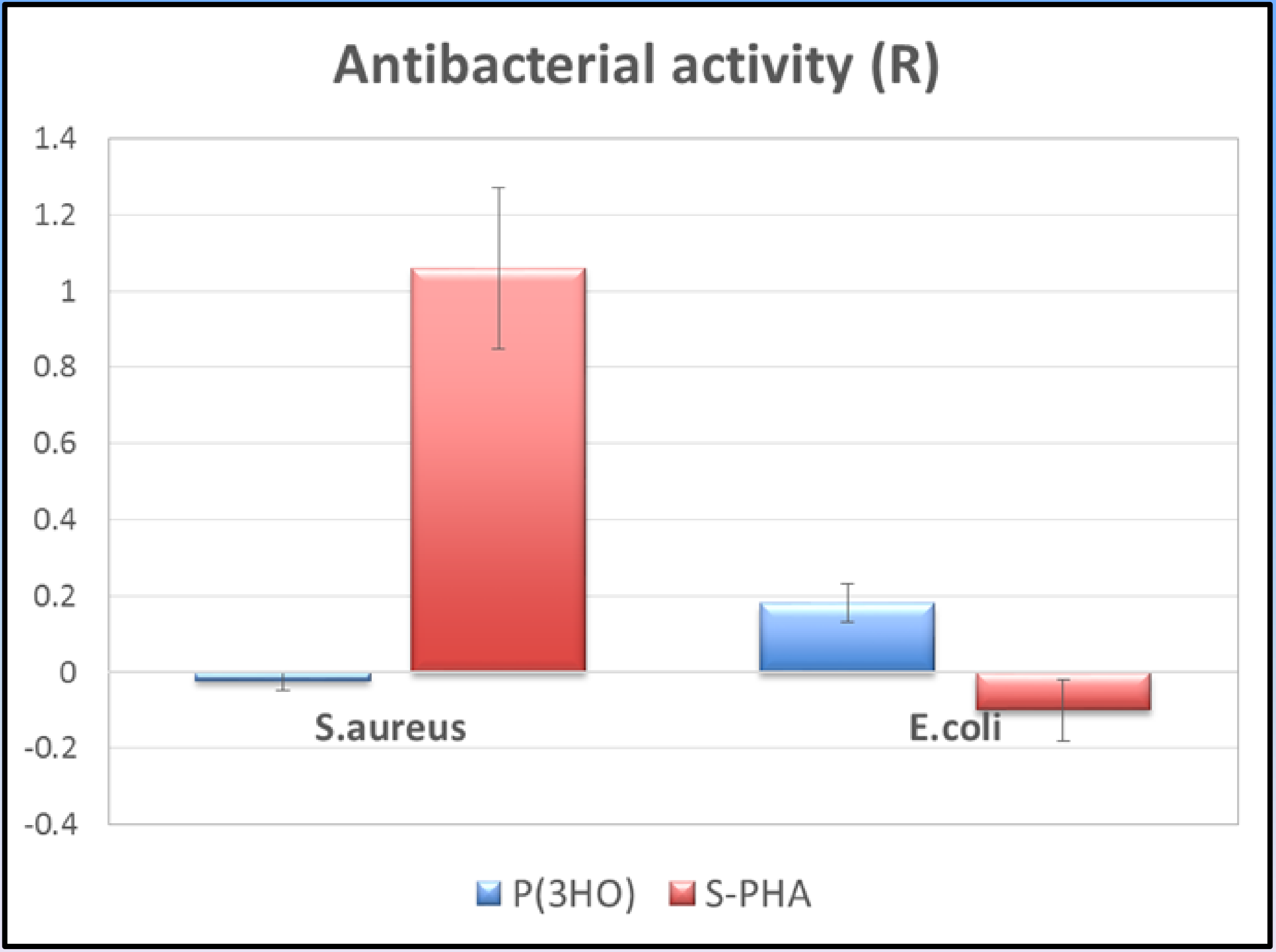
Thio-PHAs


Antibacterial activity (R)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
S.aureus
E.coli
P(3HO)
S-PHA

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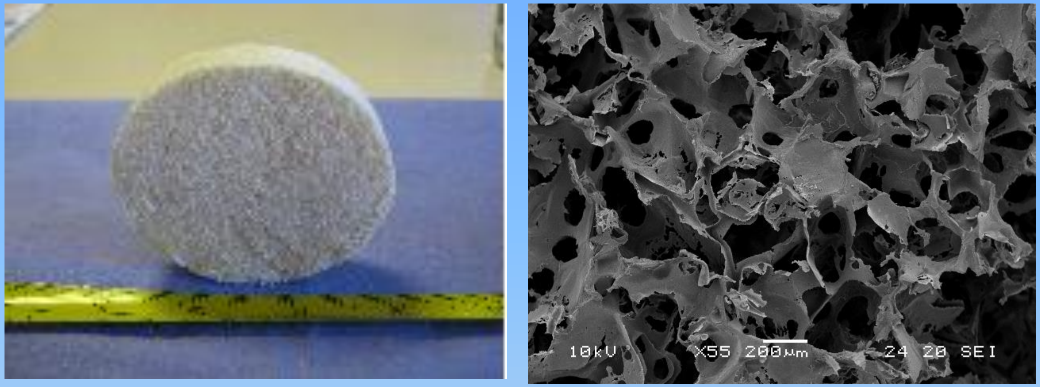
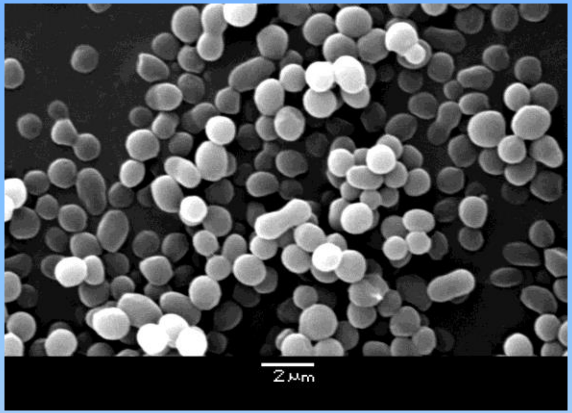
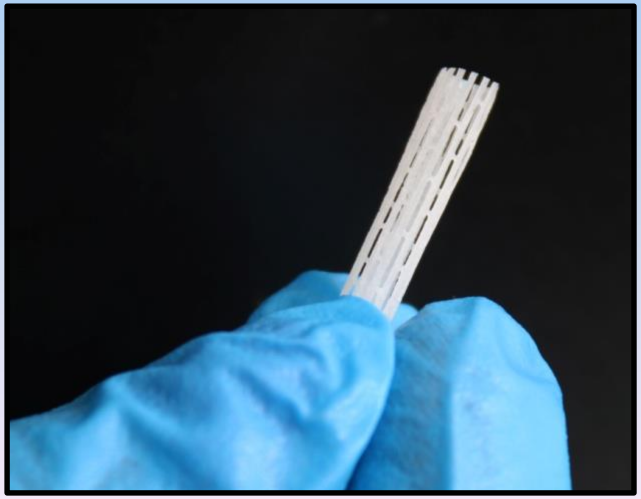
Scaffolds/devices/structures made using PHAs



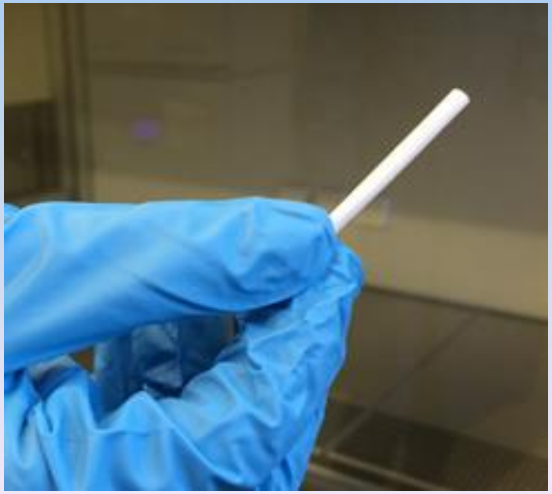
P(3HB) and P(3HB)/Bioglass® composites



Drug Delivery



Biodegradable Drug Eluting Stents



Biodegradable Nerve Conduits

PHAs
The new emerging medical materials!

Valappil *et al*., 2006; *Expert Review in Medical Devices* **3(6):** 853-868
Rai *et al*., 2010; Material Science Engineering (Reviews) **72(3)**:29-47
Dubey *et al*., 2014 Novel cardiac patch development using biopolymers and biocomposites; ISBN13: 9780841229907

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Regulatory Body Approval of Polyhydroxyalkanoates for Medical Applications

❖ **Apr 2, 2007** Tepha, Inc. Receives **FDA Clearance** for TephaFLEX® Absorbable Suture product for marketing in the U.S. TephaFLEX® is the first medical device derived from PHAs developed by Tepha and the MIT.

❖ **May 1, 2009** Tepha, Inc. announced that its corporate partner, Aesculap AG, has received a **CE Mark** and is launching its MonoMax monofilament absorbable suture for general surgical indications in Europe. The product is made with TephaFLEX® fibre.

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Medical applications of PHAs being explored in my Group

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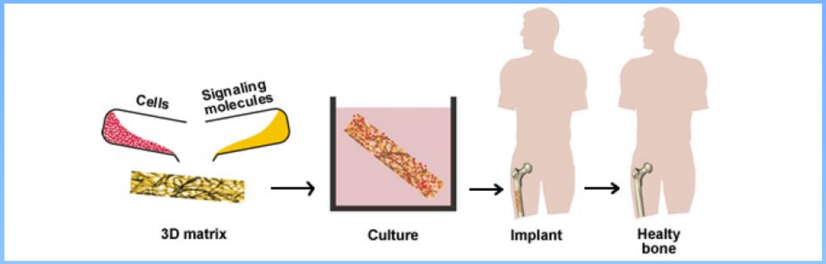
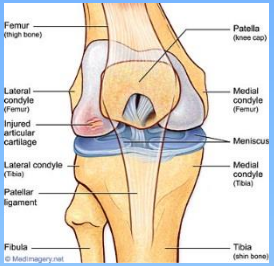
Bone tissue engineering



P(3HB) and P(3HB)/Bioglass® composites

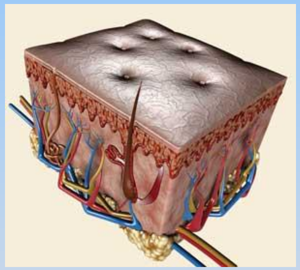
Cartilage Tissue Engineering



P(3HB)/MFC composites

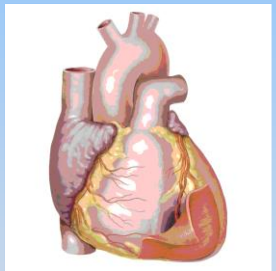
Semiartificial Pancreas

P(3HO)/P(3HB) Blends



Skin Tissue Engineering/ Wound Healing

P(3HO)/NanoBioglass Composites



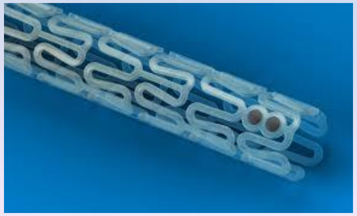
Cardiac Tissue Engineering

P(3HO) and P(3HN-co-3HP)

Drug Delivery

P(3HB)/P(3HB-co3HV)

Medical Device Development:



Biodegradable Drug Eluting Stents

SCL/MCL PHAs

Biodegradable Nerve Conduits

SCL/MCL PHAs

Bacterial cellulose based antimicrobial materials

Bacterial cellulose

Bacterial cellulose (BC) produced by bacteria from different genera (for example *Gluconacetobacter*). Bacterial cellulose shows a peculiar tridimensional structure. It is produced as nanosized fibrils with high degree of purity and crystallinity, giving it unique physical and mechanical properties like strength and water retention. Moreover, it is much purer than plant cellulose which is normally in the form of lignocellulose and is known to be highly biocompatible, so it is very well suited for applications in the biomedical field.

Helenius, *et al*., 2006, Journal of Biomedical Materials Part A 76 (2) 431-438.

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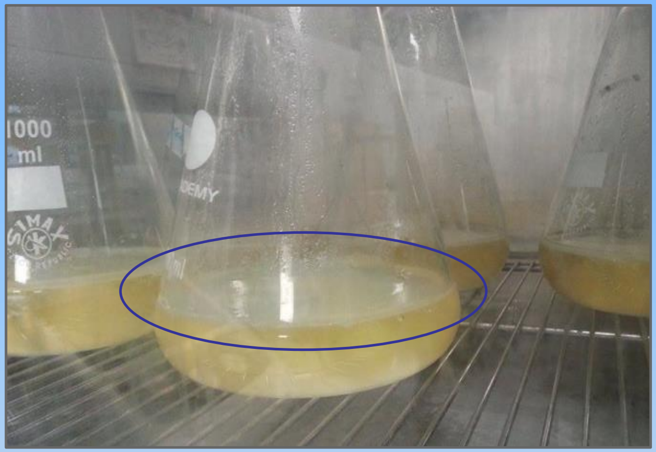
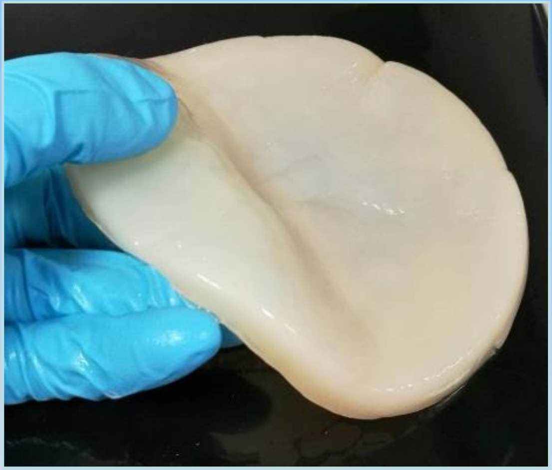
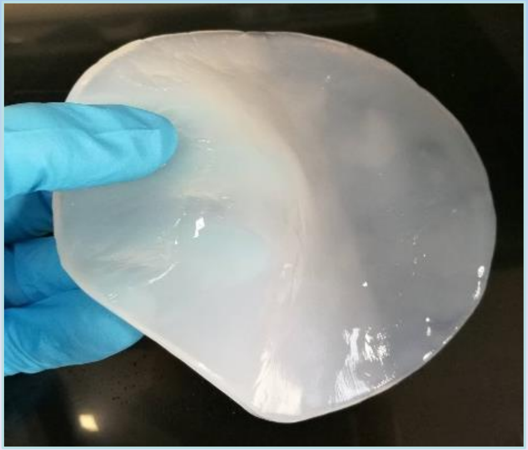
Production of Bacterial cellulose



Gluconacetobacter xylinus
5-7 days at 30 °C

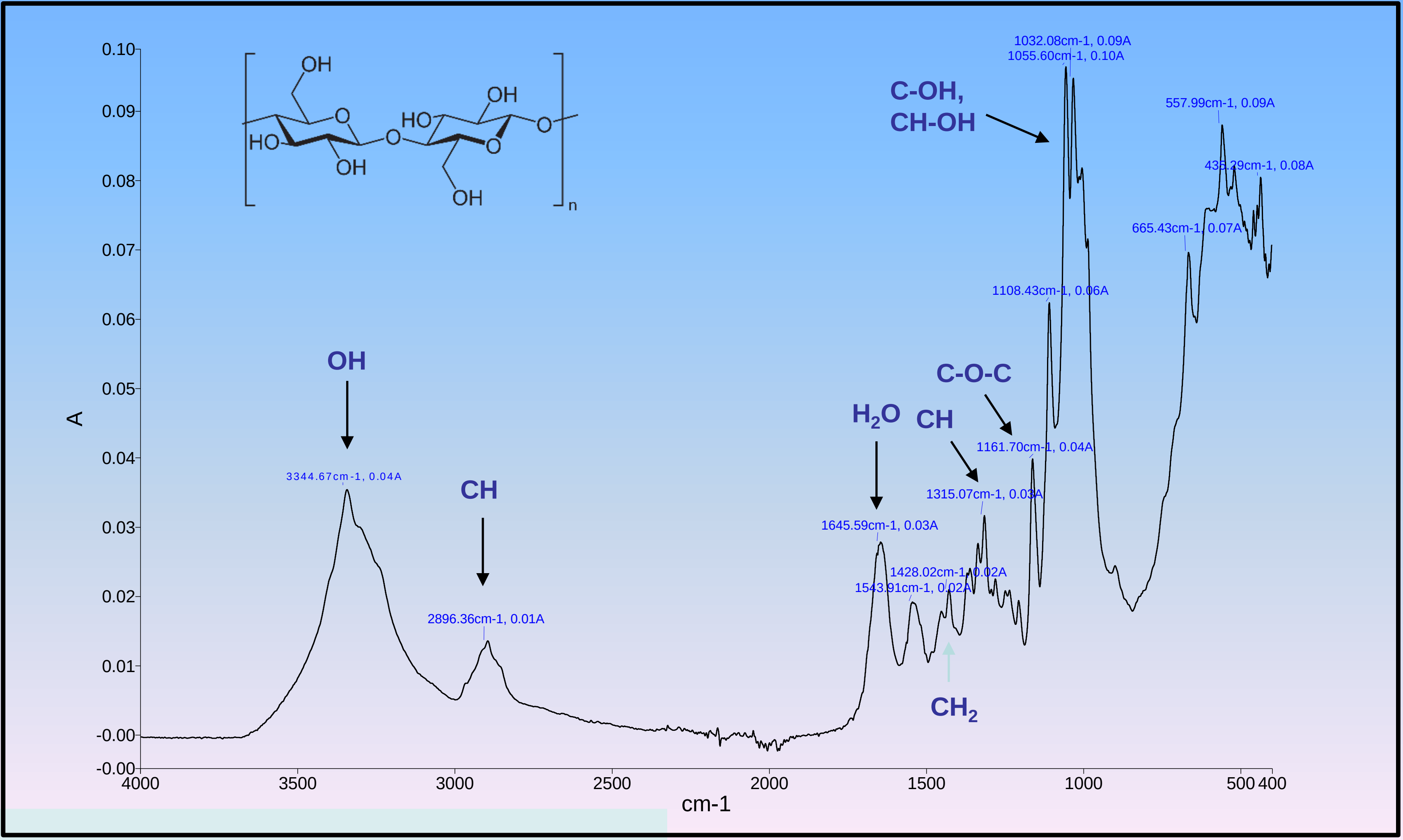


Bacterial cellulose pellicle



Bacterial cellulose pellicle
after washing

Characterisation of Bacterial cellulose



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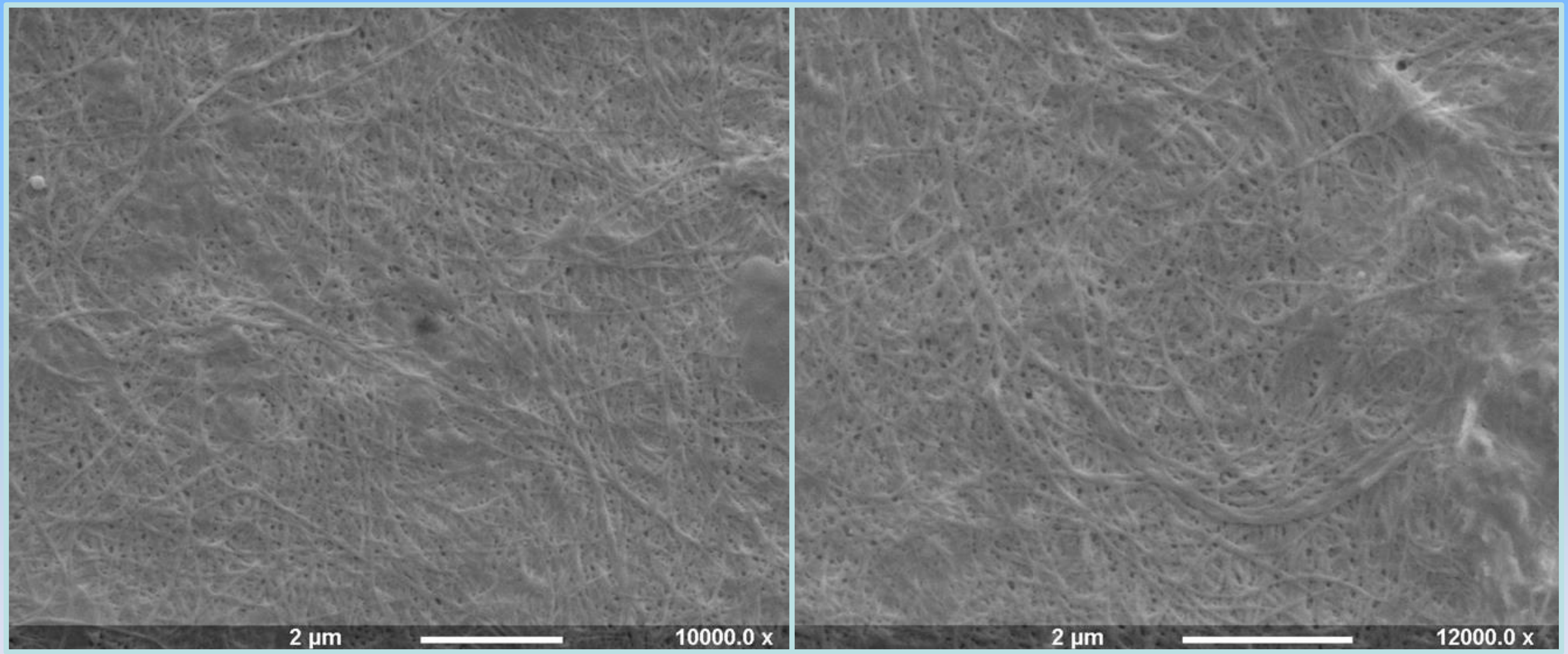
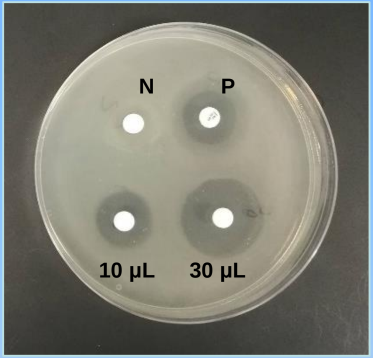
Characterisation of Bacterial cellulose

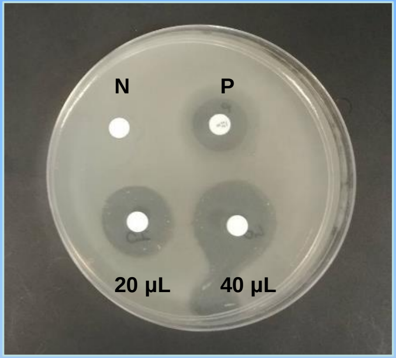


SEM

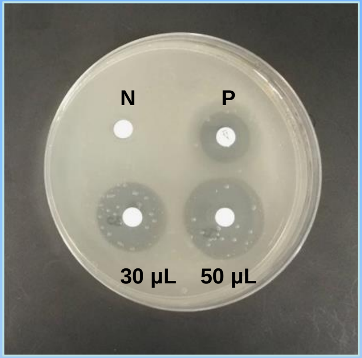
Antibacterial activity of additive ‘a’



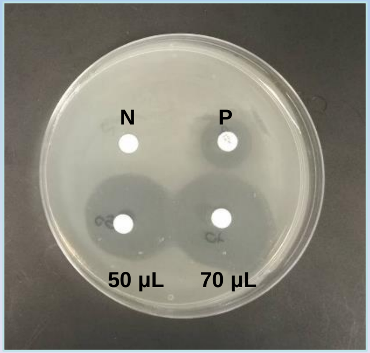
10 µL, ZOI = 1.7 cm
30 µ, ZOI = 2.3 cm



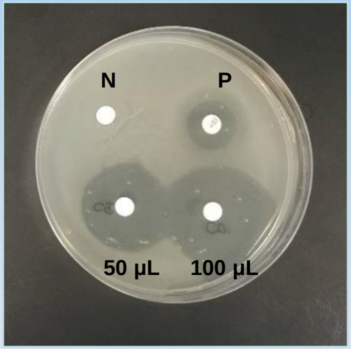
20 µL, ZOI = 2.1 cm
40 µL, ZOI = 2.5 cm



30 µL, ZOI = 2.3 cm
50 µL, ZOI = 2.8 cm



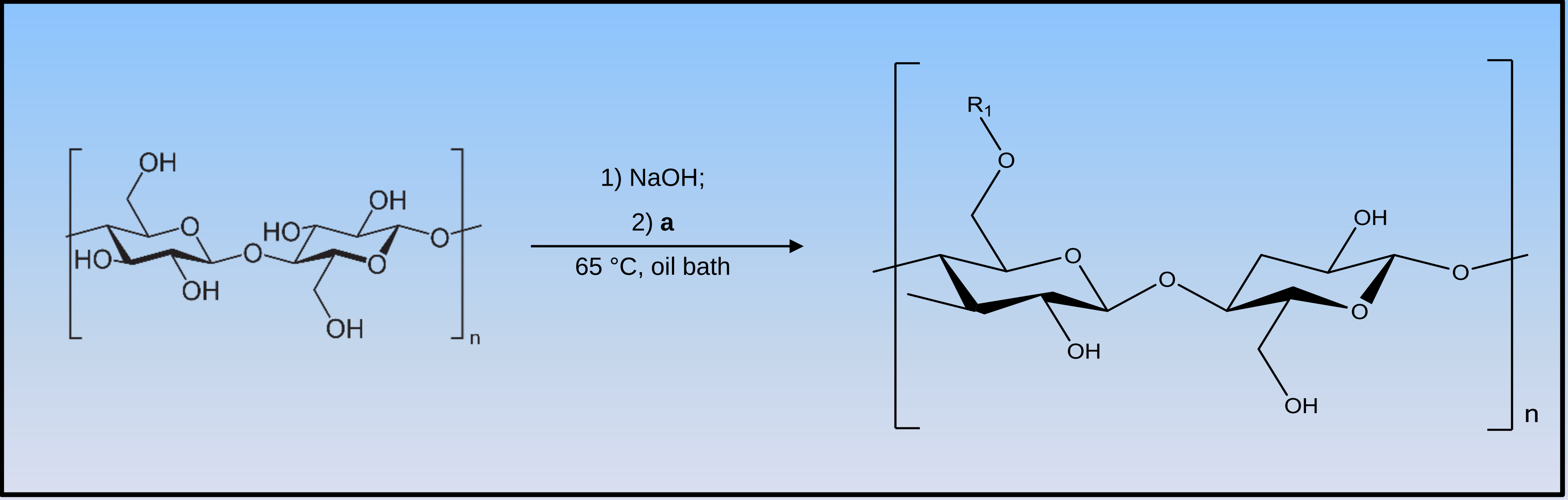
50 µL, ZOI = 2.8 cm
70 µL, ZOI = 3.1 cm



50 µL, ZOI = 2.8 cm
100 µL, ZOI = 3.3 cm

S. aureus ATCC® 6538P™

Surface modification of Bacterial cellulose



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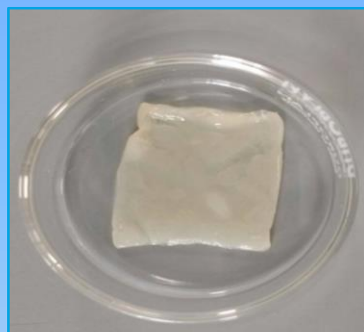
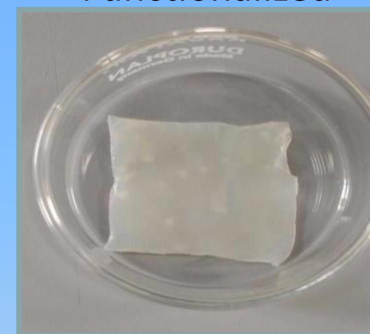
Bacterial Cellulose

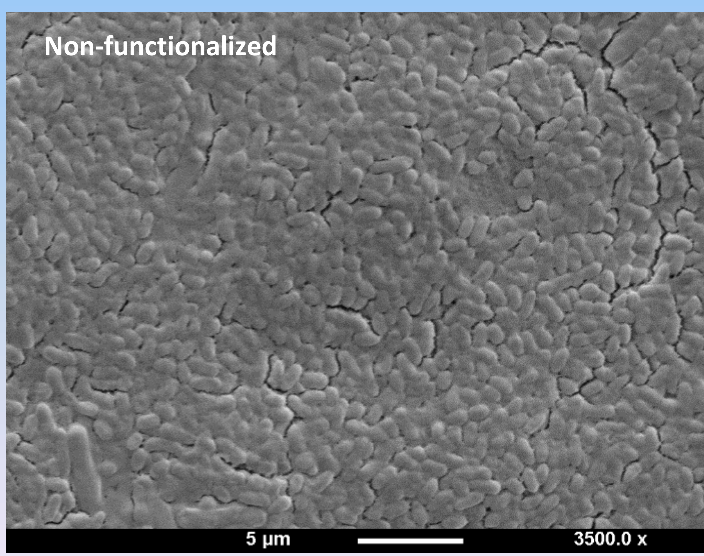
Surface Antibacterial testing

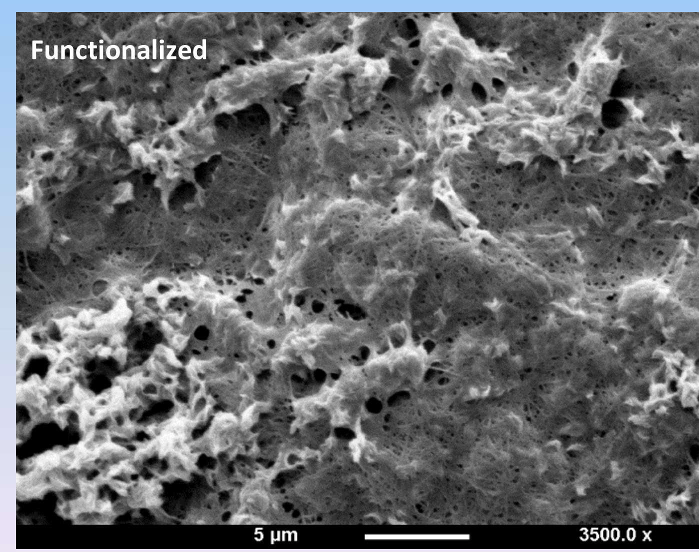
Non-functionalized



Functionalized







S. aureus ATCC® 6538P™

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Conclusions

- Polyhydroxyalkanoates (PHAs) are an emerging class of biodegradable and biocompatible polymers of natural origin with huge potential in biomedical applications.
- *Bacillus* sp. and *Psuedomonas* sp. have been used in the Roy lab to produce SCL-PHAs and MCL-PHAs respectively.
- The PHAs produced have been used successfully in development of antimicrobial polymers using additives-TC, AMP, Allicin.
- Thio-PHAs are another emerging class of antibacterial polymers
- Bacterial Cellulose is another natural polymer with potential in biomedical applications including wound healing.

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Key Scientists

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Sheila Piarali
(TC, AMP and PHA)

Isabel Orlando
(Bacterial Cellulose)

Dr Pooja Basnett
(All aspects)

Elena Marcello
(Thio-PHA)

Alexandra Paxinou
(Allicin and PHA)

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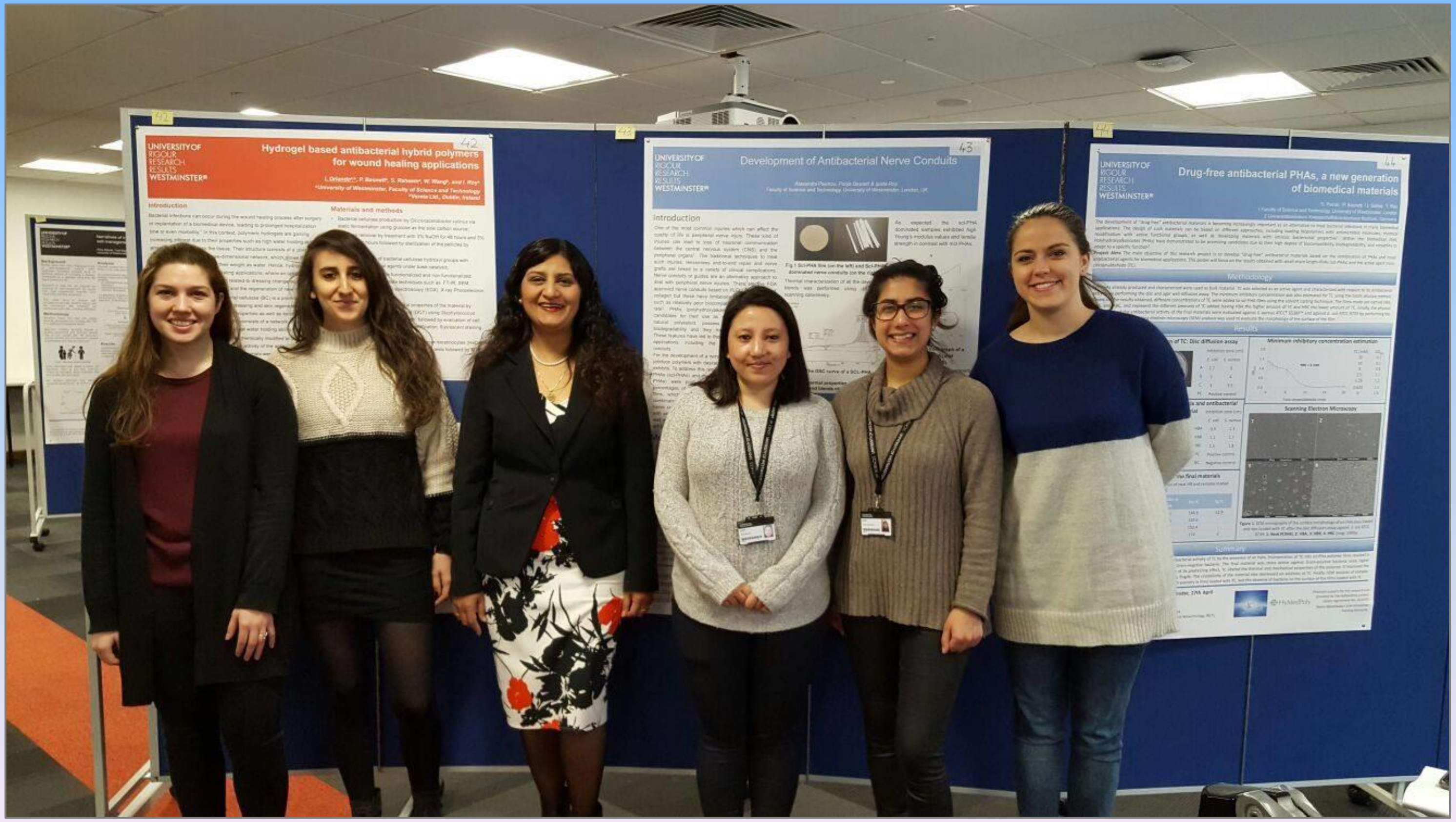
The HyMedPoly Group from UoW



Funding for this work was provided by the European Commission's "Horizon 2020 Programme" under Grant agreement No. 643050 (HyMedPoly).

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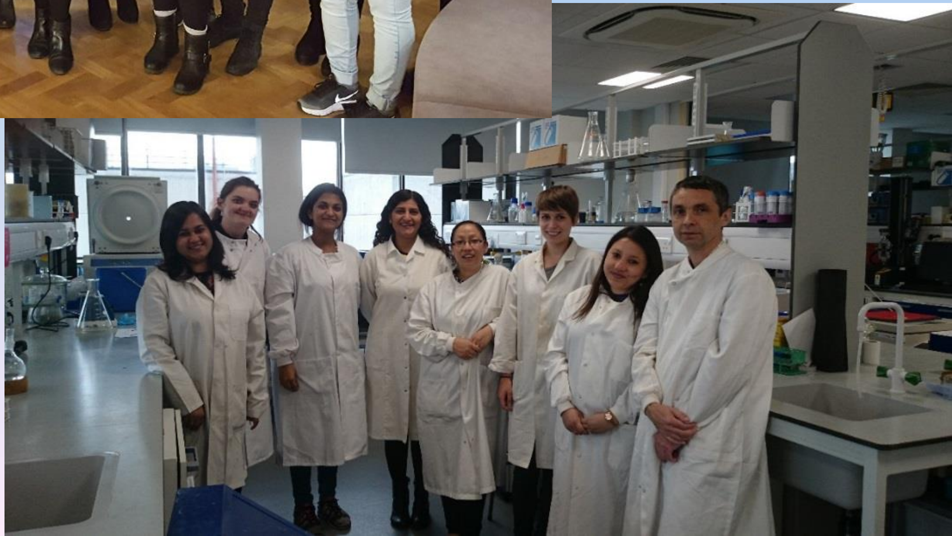

My Group

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Thanks for your attention!