Fusarium spp. in Tropical Asia: Their Taxonomy, Pathogenic, Genetic and Molecular Aspects

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ABSTRACT

Fusarium is one of the most interesting genera of pathogenic and toxigenic fungi widely distributed all over the world, including Southeast Asia. Several *Fusarium* species are able to produce mycotoxins; some of which have been implicated in various animal disorders and human diseases. Furthermore, the mycotoxins have also received significant attention as potential non-conventional warfare agents in Asia. A number of species could cause opportunistic human infections. A considerable numbers, however, are destructive plant pathogens. In this presentation, four major topics of the fungi will be discussed i.e. taxonomy, pathogenic, genetic, and molecular aspects of particularly phytopathogenic fusaria obtained from this region.

As far as plant diseases are concerned, *Fusarium* spp. in this region are becoming more significant since the introduction of intensive and high yielding production systems and genetically uniform cultivars. Our continuous study in nearly thirty years showed that *Fusarium* spp. have been associated with a wide range of host plants and agricultural products as well as naturally infested soils (93%), feeds and food (5%) and to some extend (<1%) with immuno-deficient animals and human in this region. Here, most exported and/or imported plants and feeds are also harboured by this fungus. To date, more than 5,500 *Fusarium* accessions, mostly associated with plant diseases in the fields, have been isolated and classified into more than 35 species. Some were sent by researchers or institutions in the tropical Asia for identification and preservation. All life isolates are preserved at the *Fusarium* Culture Collection Unit, School of Biological Sciences, USM. The role and function of the Unit, being the biggest in Asia, are also discussed.

A. K. Sudesh Kumar. Presented at the International Symposium on Biological Polyesters, 22-28 Aug. 2004, Beijing China.

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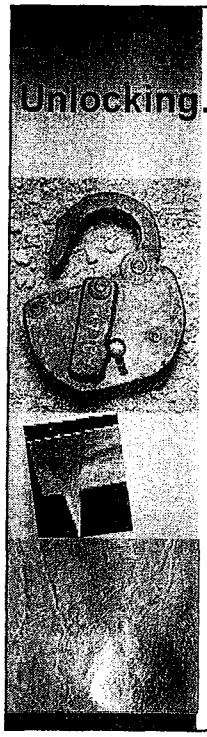

The Structure of Native Polyhydroxyalkanoate Granules Based on Atomic Force Microscopy

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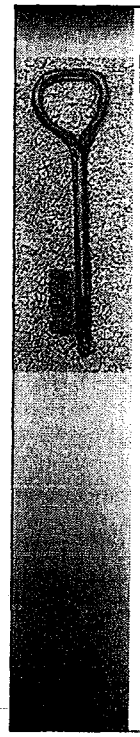
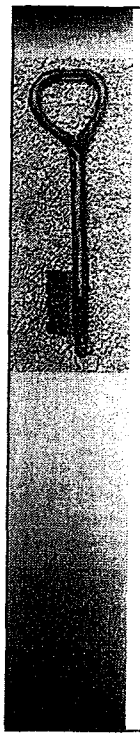


Polyhydroxyalkanoate (PHA)

- Carbon and energy **storage** material
- Exists in the form of **amorphous**, water insoluble cytoplasmic granules
- Rapidly synthesized and mobilized
- Most PHAs are highly **crystalline** but crystallization *in vivo* is effectively prevented

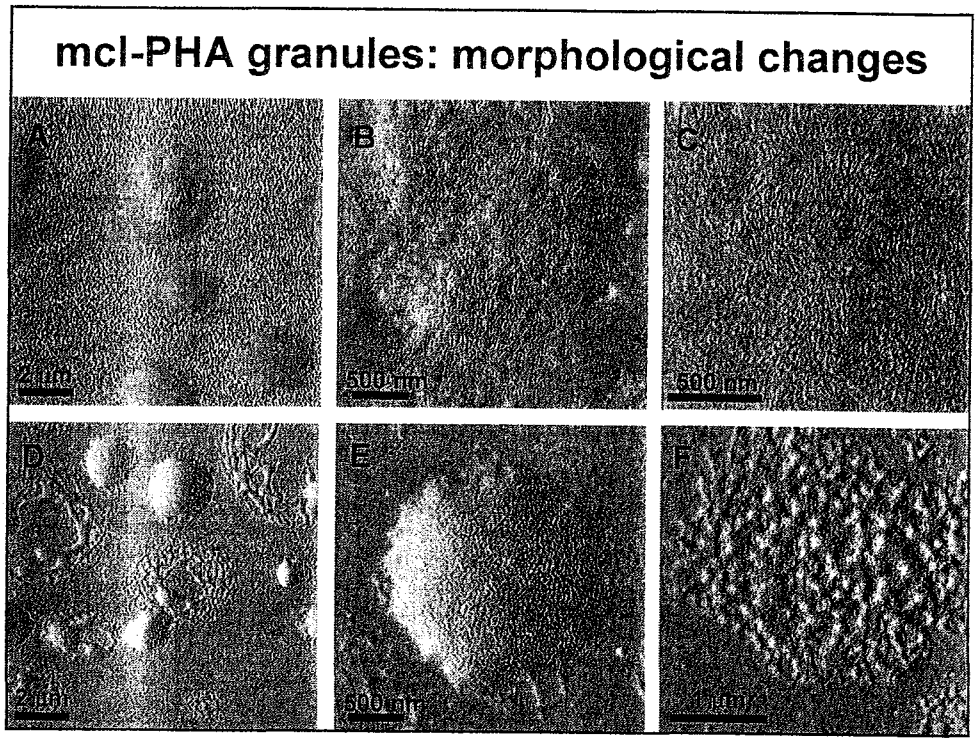


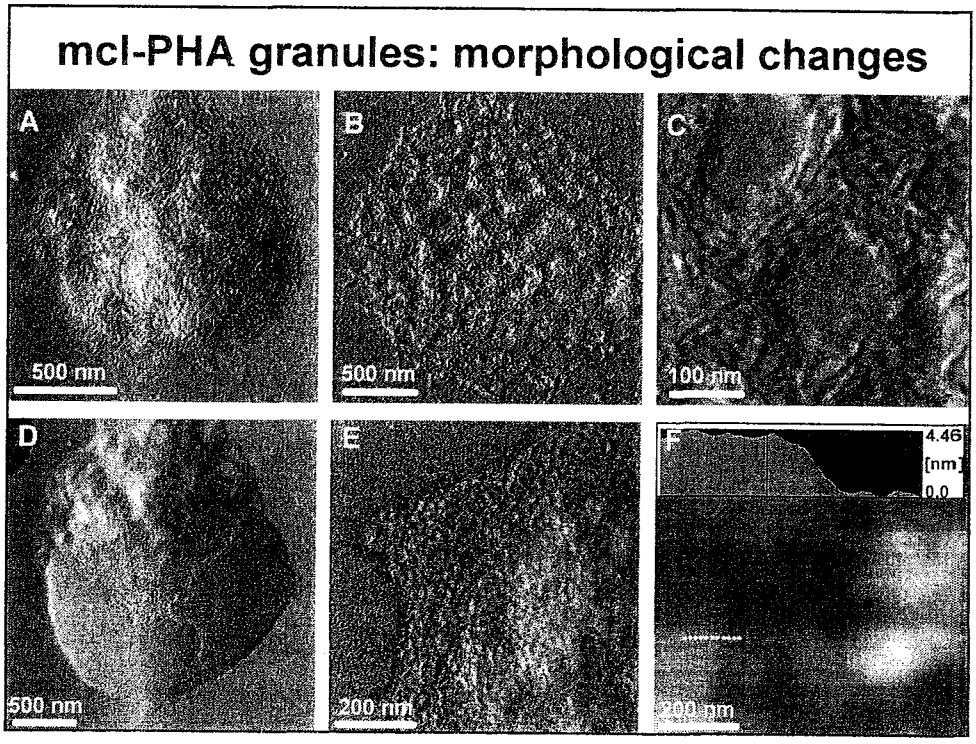
Granule crystallization

- Preventing crystallization is very important as it can affect both synthesis and mobilization of PHA
- How bacteria accomplish this?
- Do bacteria have a mechanism to prevent crystallization during synthesis, storage and especially during mobilization?

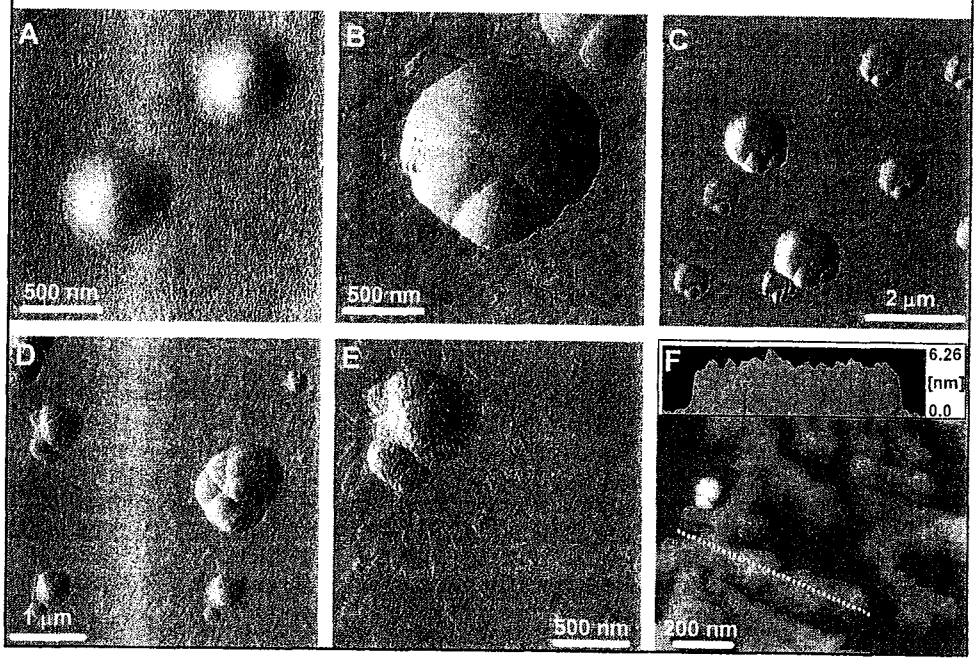
Rationale for using AFM

Granule characteristics	AFM characteristics
♦ Amorphous, extremely delicate	♦ No pretreatment
♦ Tendency to crystallize	♦ Rapid
♦ Enzymes/proteins on granule surface	♦ Gentle
♦ Presence of a lipid monolayer (?)	♦ Quantitative & qualitative
	♦ Ability to follow changes

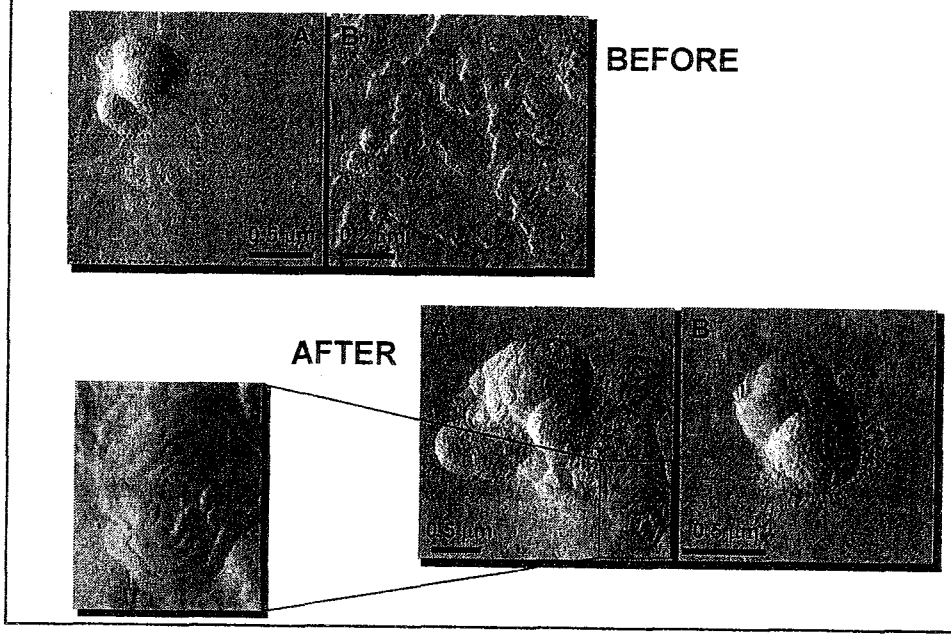

mcl-PHA granules: morphological changes
A
B
C
500 nm
D
E
F
500 nm


mcl-PHA granules: morphological changes
A
500 nm
B
500 nm
C
100 nm
D
500 nm
E
200 nm
F
4.46
[nm]
0.0
200 nm

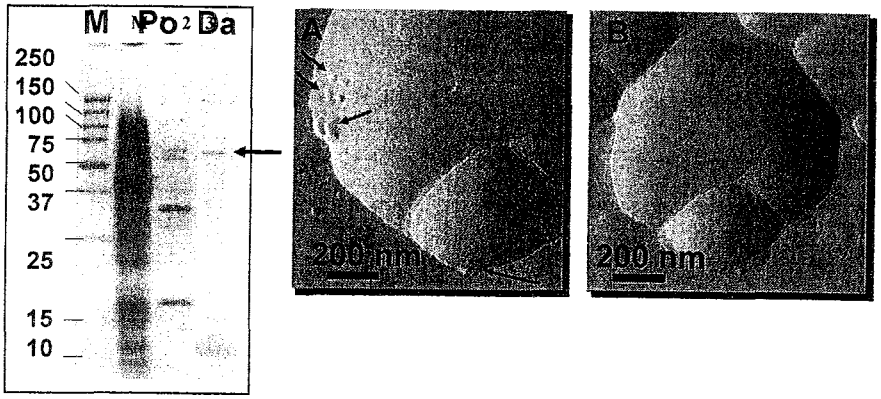
P(3HB-*co*-3HV) granules: morphological changes



Extracellular depolymerase treatment

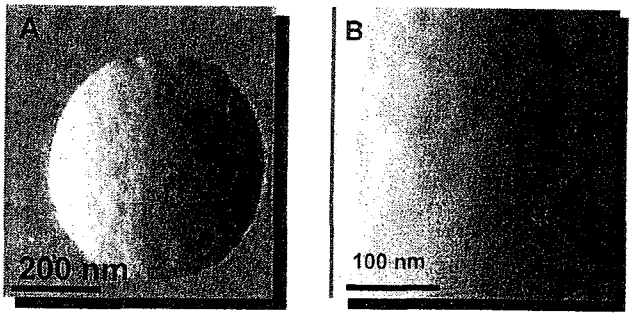


Granule associated proteins (GAPs)



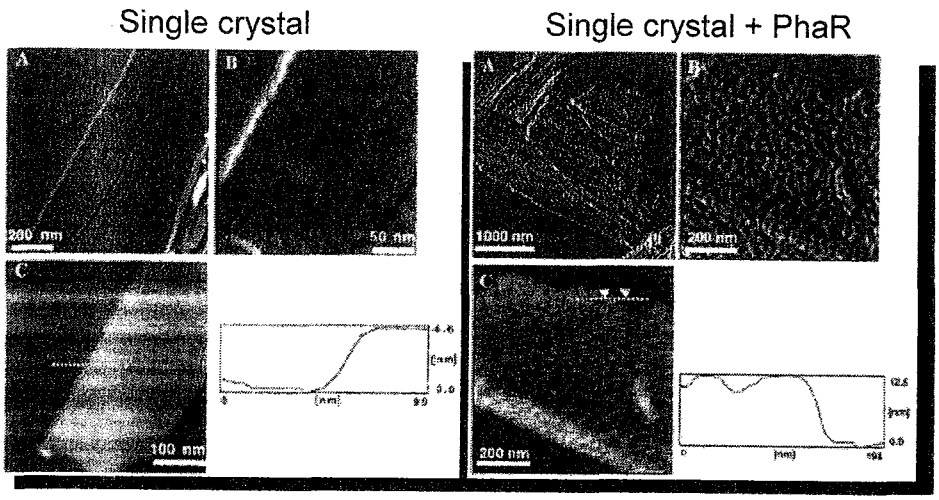
Globular structures on granule surface

Globular structures on granule surface

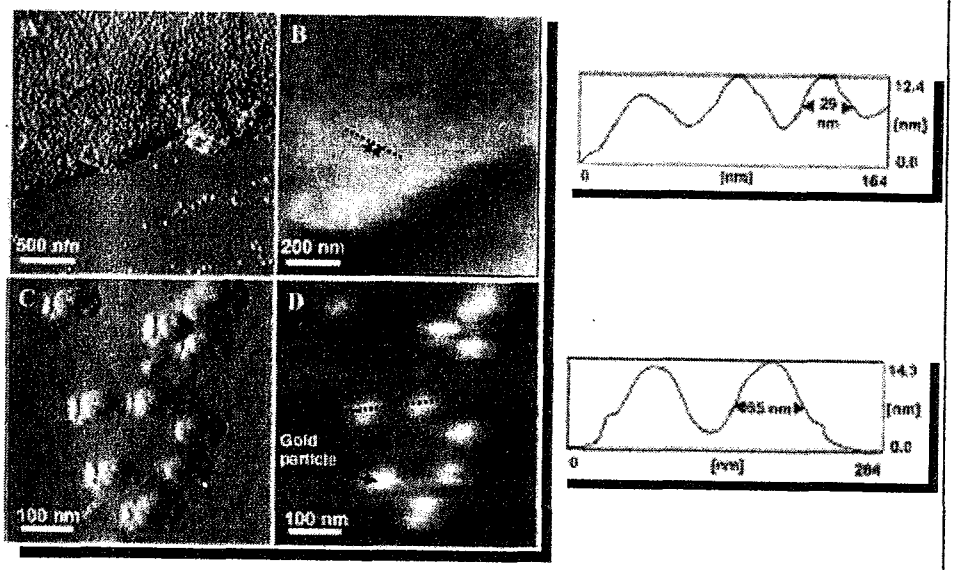


After 6 months of exposure to ambient condition: no signs of crystallization

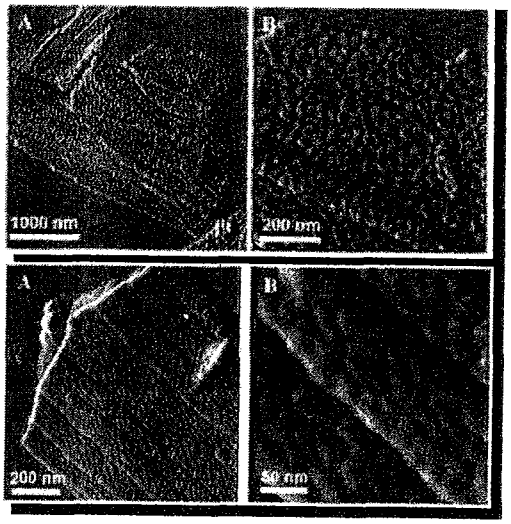
PhaR on PHB single crystal



Immunogold labeling



PhaP on PHB single crystal



PhaR: 22 kDa

Both proteins bind homogenously to the PHB single crystal to form a monolayer

PhaP: 16 kDa

GAPs bind directly to the granule surface

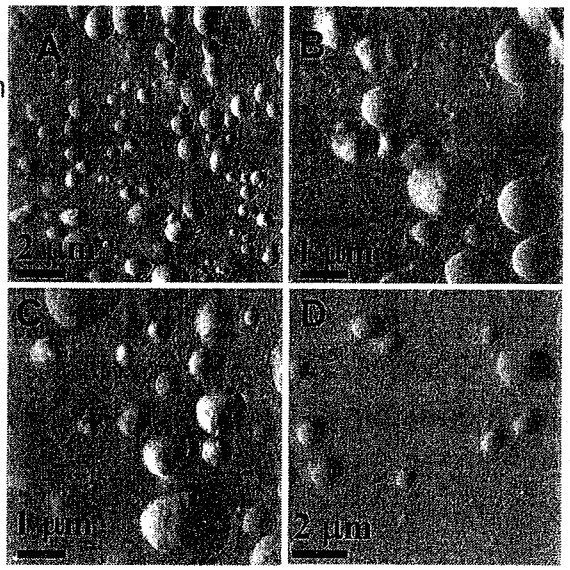
GAPs are easily removed from granule surface: weak interactions

GAPs can form a monolayer on the granule surface

GAPs may have a role in preventing granule crystallization

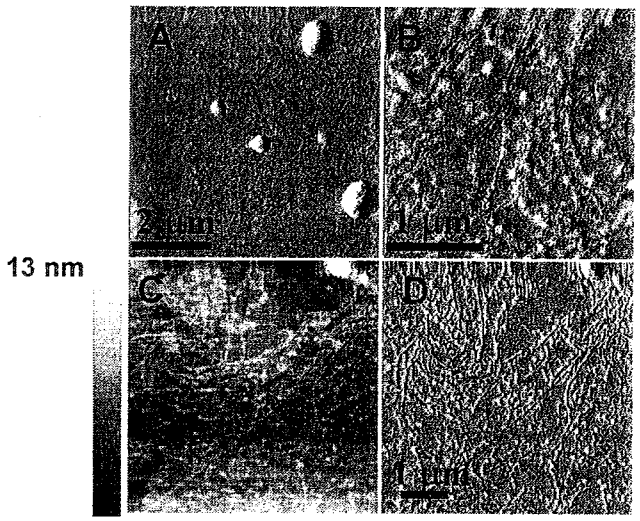
Worm-like fibrillar structures

Granules from *R. eutropha* (recombinant)

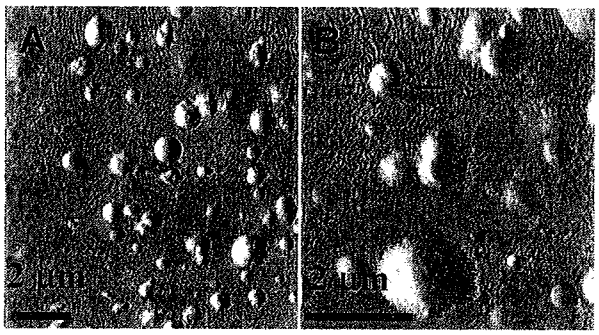


Granules from *R. eutropha* (wild-type)

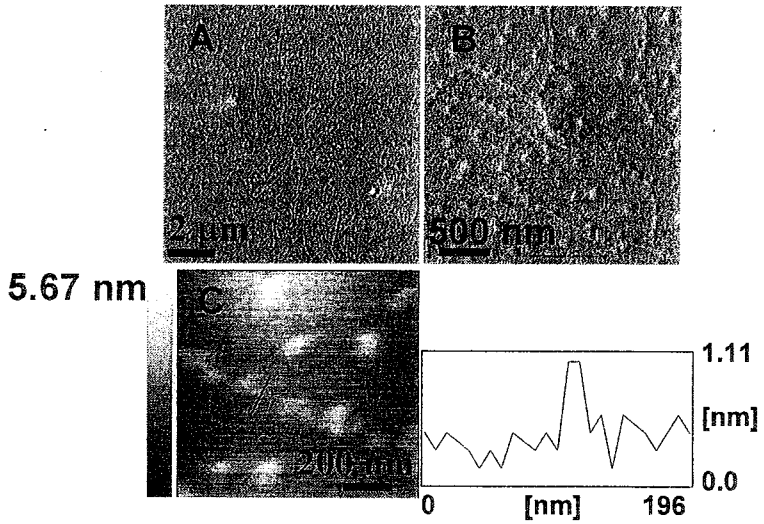
Long, winding, regularly sized fibrillar structures



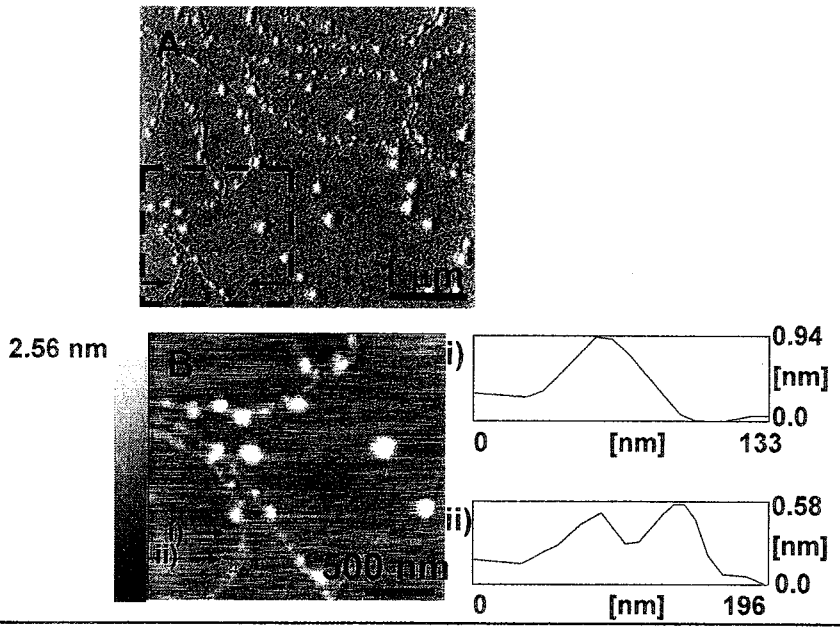
Fibrils released from the granule



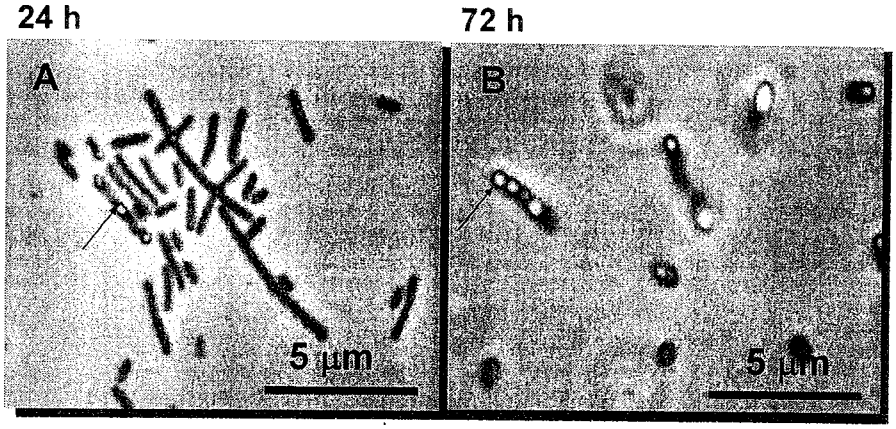
Aqueous acetone treatment



Finer protofibrils

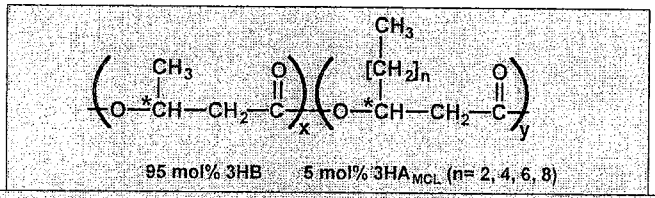


Phase contrast light microscopy



Phase contrast light microscopy of recombinant *R. eutropha* cells grown in 2% (wt/vol) fructose as the sole carbon source

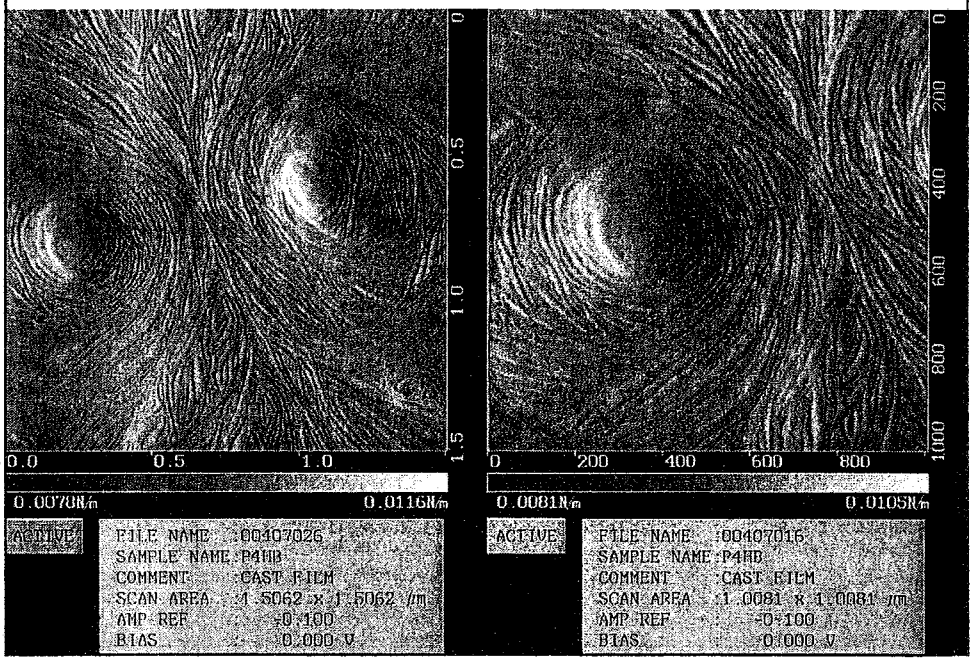
Characteristics of the PHA



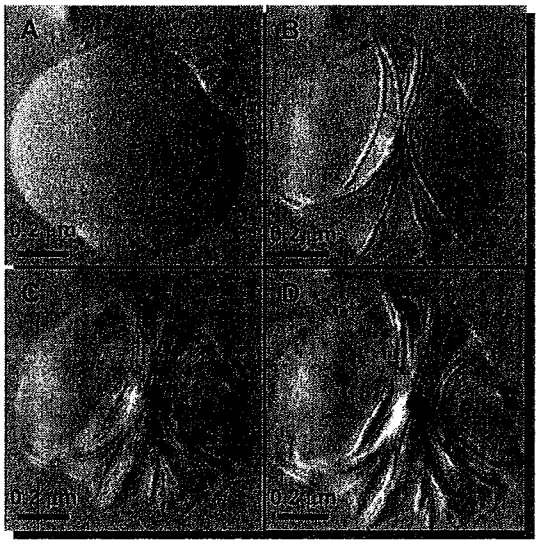
	PHA composition (mol%)[a]					Molecular weight		Thermal properties		
	C4	C6	C8	C10	C12	$Mn \times 10^{-3}$	Mw/Mn	Tg (°C)	Tm (°C)	ΔHm (J/g)
24 h	95.2	0.7	1.2	1.6	1.3	nd	nd	nd	nd	nd
72 h	96.7	0.5	0.9	1.2	0.7	188	2.7	-2	142, 155	77

The contents of PHA at 24 h and 72 h were 13 and 26 wt% respectively, of the dry cell weight as determined by gas chromatography
[a]Composition determined by NMR

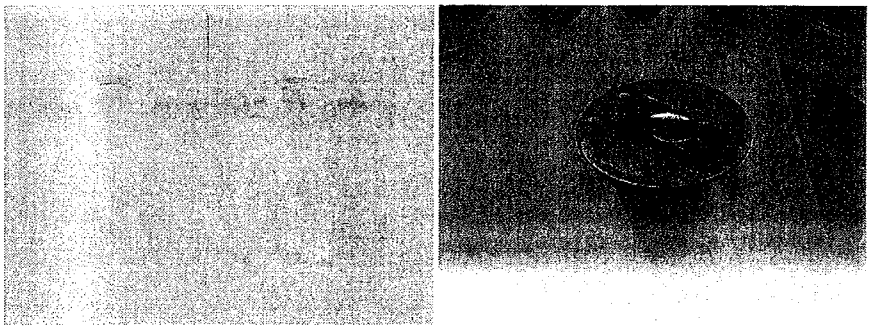
Edge-on crystals of P(4HB) ultrathin film



Crystalization of a single P(4HB) granule



In vitro polymerization reaction

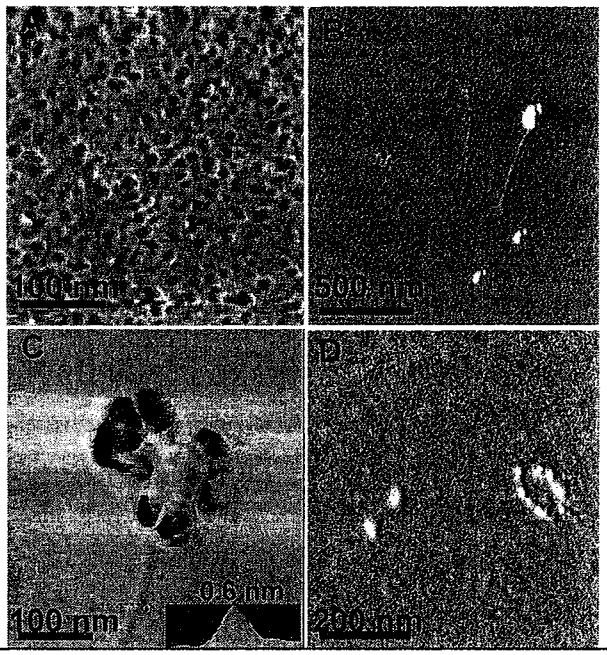


In eppendorf tube

Directly on HOPG

In vitro synthesis of PHB

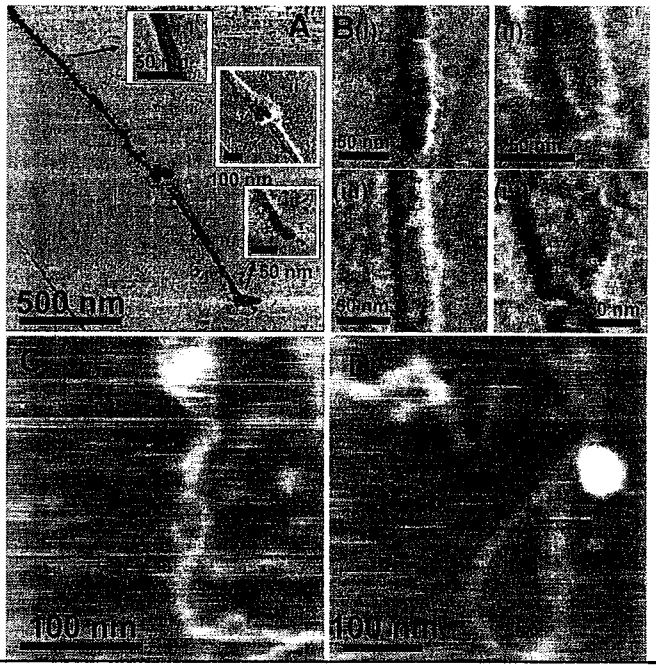
Synthase before the addition of 3HB-CoA →



Organization of polymer chains

On hydrophobic surface (HOPG)

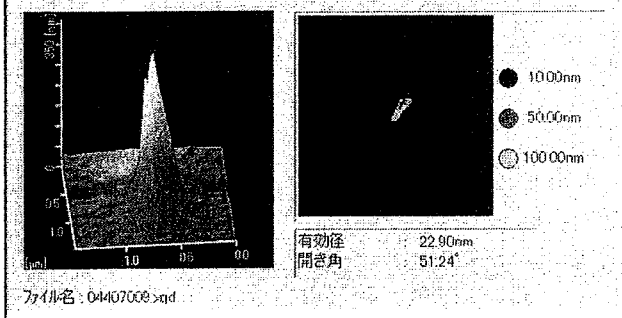
On hydrophilic surface (mica)

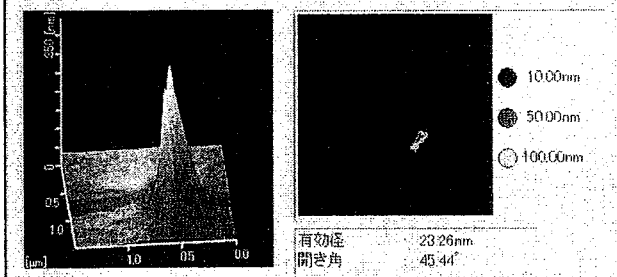


Tapping-Mode AFM

- Works by vibrating a tip which is at the end of a cantilever and bringing the tip into intermittent contact with a sample surface.

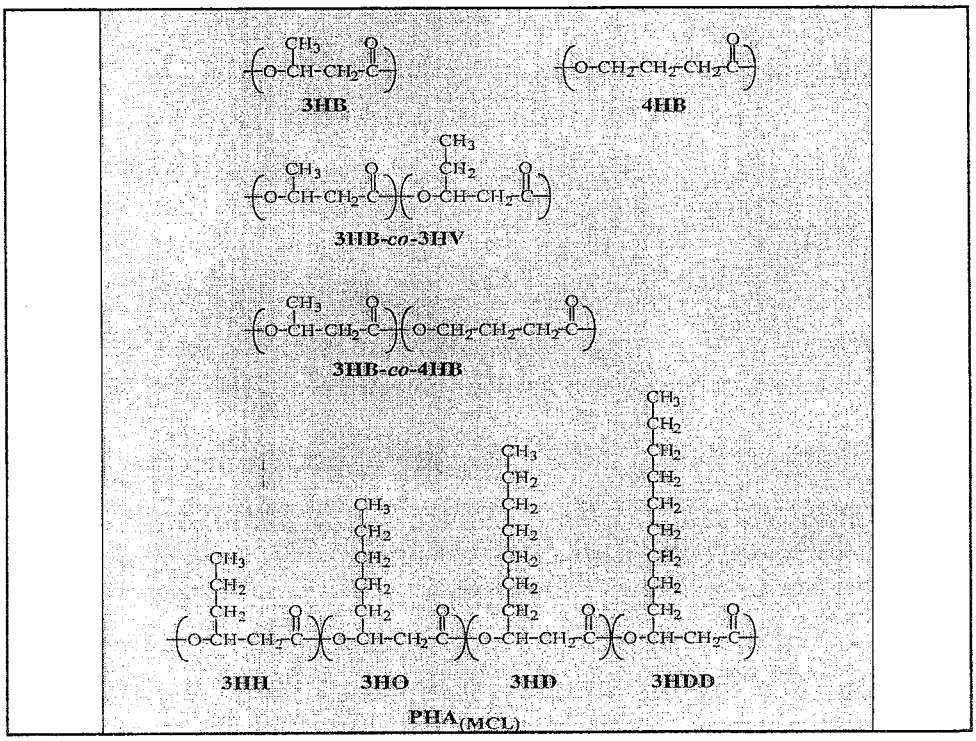
- TM-AFM has the ability to image soft samples without damaging them, and for this reason TM-AFM is very useful for imaging biological and polymer samples.
- It also has the advantage of being fairly easy to use and requires little sample preparation.





Average tip radius = 10.4±1.3 nm

Images kindly provided by Y. Kikkawa



Conclusions

- GAPs interact directly with PHA
- GAPs are capable of forming a continuous monolayer on granule surface
- GAPs help prevent granule crystallization
- Polymer chains in granule are organized in the form of fibrillar aggregates
- Both ends of a growing polymer chain are attached to the enzyme complex
- PHA granules remain amorphous not simply because of slow crystallization kinetics

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Thank you!