

Table 1. Percentage wilt of black pepper cuttings in the nursery

Treatment	Percentage wilt of black pepper cuttings ^{a,b}	
	60 DAP	90 DAP
PN-026	40.60 (39.44)	45.00 (41.92)
Pathogen control	95.83 (79.78)	97.92 (83.99)
Healthy control	6.25 (14.00)	6.25 (14.00)
0.2% COC drenching	27.08 (30.83)	27.08 (30.83)
CD ($P = 0.05$)	6.15	6.02

^aMean of three replications having fifteen cuttings each; ^bFigures in parenthesis are arc-sine transformed values.

cuttings. The isolate PN-026, which showed maximum suppression of lesion development in the screening (8.22 mm compared to 59.22 mm in the pathogen control after 96 h of incubation), reduced the incidence of the disease significantly compared to other bacterial antagonists (Table 1). In general, isolates giving greater suppression of lesion development in the shoot assay, irrespective of their antagonism on the dual culture plate assay, showed higher degree of biocontrol efficiency. Our study suggests that the screening procedure described in the present investigation can be used as a rapid and more accurate technique for selecting bacterial antagonists against soil-

borne infection by *P. capsici* in black pepper nursery, as it gives a direct relationship between the lesion suppression and *in vivo* biological control activity.

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K. N. ANITH^{†,*,}
N. V. RADHAKRISHNAN[‡]
T. P. MANOMOHANDAS[‡]

[†]*Department of Plant Pathology,
College of Agriculture,
Kerala Agricultural University,
Vellayani,*

Thiruvananthapuram 695 522, India

[‡]*Regional Agricultural Research Station,
Kerala Agricultural University,
Ambalavayal,
Wayanad 673 593, India*

[#]*Present address:*

*North Florida Research and
Education Center,*

*Institute of Food and Agricultural Sciences,
University of Florida,*

*155 Research Road,
Quincy, FL 32351, USA*

**For correspondence*

e-mail: knanith@yahoo.com

Characterization of polyhydroxy alkanates – Biodegradable plastics from marine bacteria

A wide variety of bacteria accumulate polyhydroxy alkanates (PHAs) as intracellular storage material^{1–4}. Because of their physical and structural properties and amenability to biodegradation, PHAs are considered potential substitutes for petrochemical plastics. PHAs vary in their mechanical properties depending on the composition of the monomeric units⁵. The medium chain-length PHAs are semi-crystalline elastomers with a low melting point (T_m), low tensile strength and high elongation to break^{6,7}, and can be used as biodegradable rubber. Polyhydroxy butyrate (PHB), smallest known PHA displays a similar degree of crystallinity and T_m as polystyrene⁸, and is stiffer and more brittle than polypropylene^{8,9}; but its copolymerization with hydroxy valerate (HV) monomer units reduces its stiffness and increases its toughness, giving a pro-

duct with desirable properties for commercial applications¹. Polymers may fail in specific applications, simply because they do not possess the necessary strength to carry the designed load or occasional overload¹⁰. Hence, it is important to study the mechanical and physical properties of such commercially important polymers before their use in the industry.

Tropical mangrove and marine ecosystems from the mid-west coast of India were screened for promising bacteria, with capability of accumulating high amounts of PHA¹¹. The isolates designated as 61/4, 64/4, 87/4, 182/5, 12/BL, 85/6 and 86/6, which accumulated more than one gram PHA per litre culture broth were studied for physico-chemical factors influencing quantitative yield of PHA (unpublished results). The physical and mechanical properties of the PHA

produced by these organisms are presented here.

The selected isolates were grown routinely in 50 ml of E2 mineral medium¹² consisting of $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$, 3.5 g; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 7.5 g; KH_2PO_4 , 3.7 g per litre; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.17 g, and microelements stock solution, 1 ml containing $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.78 mg; $\text{MnCl}_4 \cdot 4\text{H}_2\text{O}$, 1.98 mg; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 2.81 mg; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.47 mg; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.17 mg, and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.29 mg supplemented with yeast extract 0.04% (w/v), glucose 2% (w/v), for 48 h on an Orbitek shaker at 28°C and 150 rpm. The cells were washed with saline by centrifugation. The PHA extracted from the cell pellet by the hypochlorite method¹³, was washed with methanol and acetone consecutively and centrifuged at 8000 rpm for 20 min. The polymers were then dissolved in hot

chloroform (60°C) and the solution poured onto glass trays. The chloroform was allowed to evaporate slowly by placing the trays in the cold room at 4°C. The film of PHA so obtained was used for further analysis.

PHA from the seven cultures was esterified by acidified methanolysis¹⁴. Thin layer chromatogram (TLC) on silica gel of the methyl esters, run in benzene and ethyl acetate (1 : 1), visualized with iodine vapours, exhibited two different patterns. Esters of PHA extracted from five isolates, viz. 61/4, 64/4, 87/4, 182/5 and 12/BL exhibited two distinct spots with R_f 0.75 and 0.6. Methyl esters of homopolymers of PHB are known to exhibit two spots, one being that of the ester of 3-hydroxy butyrate, and the other with higher R_f that of the dimeric hydroxy butyrate which is more non-polar and separates first¹⁵. The methyl ester of PHA of R_f 0.6 was eluted in benzene with 50% of ethyl acetate on the silica gel column. The IR (Shimadzu FT IR 8101 A) (Figure 1 a and b) and the ¹H NMR (Bruker WT 300 MHz FT NMR) spectra (Figure 1 c and d) of the PHA films and the purified methyl ester recorded in duplicates, confirmed the polymer to be homopolymers of 3-hydroxy butyrate.

The TLC profiles of the methyl esters of PHA extracted from isolates 85/6 and 86/6 exhibited four distinct spots with R_f 0.75, 0.6, 0.44 and 0.3. These PHAs were therefore initially thought to be heteromers, with 3-hydroxy butyrate as one of the components. Surprisingly, the ¹H NMR spectrum of the methyl esters of the monomer with R_f 0.44 also resembled that recorded for the methyl ester of 3-hydroxy butyrate, except for the signal at 3.5 ppm, of the methyl ester moiety. Re-esterification of the fraction by acidified methanolysis showed a single spot of R_f 0.6, confirming it to be methyl ester of 3-hydroxy butyrate. Hence, the fraction with 0.44 was also confirmed as 3-hydroxy butyric acid, a fraction of the PHA which got hydrolysed but not esterified, possibly because of the high number average molecular weight of the PHA (Table 1). The spot at 0.3 was confirmed to be that of the oligomers of PHA, on *co*-TLC with the PHA polymer. The ¹³C NMR spectrum of PHA (Figure 1 e), recorded four distinct peaks at 20, 40, 67 and 170 ppm, confirming the polymer to be homopolymer of 3-hydroxy butyrate.

Although all the seven polymers were confirmed to be homopolymers of PHB,

their physical properties such as molecular weight, crystallinity, enthalpy of fusion and melting temperature were found to vary (Table 1). The number average molecular weight of the polymers dissolved in chloroform, was determined viscometrically on a Brookfield Rheometer DV III, using spindle number 42 at 150 rpm and Mark-Houwink equation¹⁶. The melting temperature of the polymers was recorded on a Perkin Elmer Differential Scanning Calorimeter (DSC) Model 7. The pans containing the polymer film, 5–10 mg, were uniformly heated at 10°C/min up to 190°C to check the thermal stability of the polymers. Wide-angle X-ray diffraction measurement of the polymer films of dimension 20 mm × 1.5 mm × 1 mm was made on Rigaku RAD II C system, using CuK α radiation at 25°C in the range $2\theta = 0 - 40^\circ$ with a scan speed of 2°/min. The percentage crystallinity was calculated from the diffraction intensity data, according to Vonk's method¹⁷. The XRD pattern

obtained for PHA from isolates 87/4 and 85/6 is given in Figure 2.

All the seven polymers exhibited molecular weights much higher than the reported values of $0.1-0.75 \times 10^6$, for PHB from *R. eutropha* grown on glucose or on long-chain hydrocarbons^{8,15,18}. The molecular weight of the polymers should be directly proportional to their crystallinity and melting temperature^{19,20}, and in turn, the heat of fusion (ΔH_m). Thus the PHA from isolates 85/6 and 86/6, with high number average molecular weights, are found to be much more crystalline in nature (59%) with higher ΔH_m than those from cultures 61/4, 64/4, 87/4 and 182/5, having lower moderate molecular weights ($0.2-1.8 \times 10^6$), (45–41%) crystallinity and with lower ΔH_m . This phenomenon, however, is not uniformly observed as in the case of polymers from 87/4, 182/5 and 12/BL, possibly due to determinations in the form of number average molecular weight rather than average molecular weight.

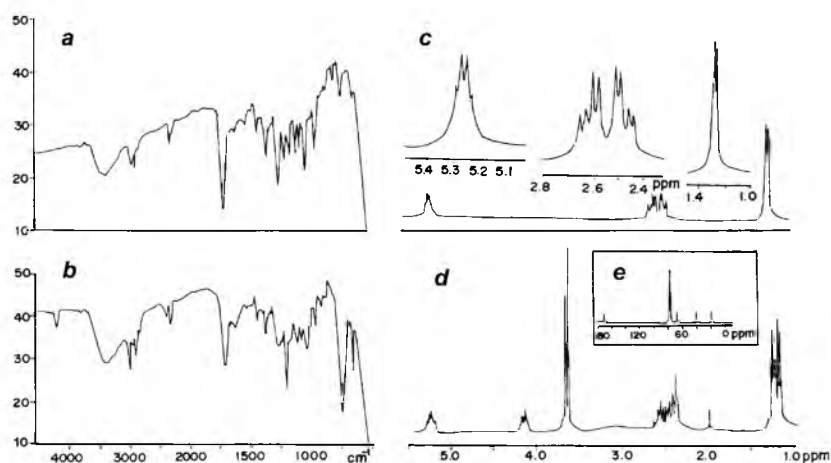


Figure 1. Infra-red spectra of *a*, PHA film from isolate 61/4 and *b*, methyl ester of the PHA monomer with 0.6. ¹H NMR (300 MHz) spectra of *c*, PHA film from isolate 61/4 and *d*, methyl ester of PHA monomer with 0.6; *e*, ¹³C NMR spectrum (300 MHz) of PHA film from isolate 61/4.

Table 1. Molecular weight, degree of crystallinity and temperature of melting of PHA samples from seven marine isolates

Isolate	Number average molecular weight/ 10^6	Crystallinity (%)	Enthalpy of fusion (ΔH_m) (J/g)	Melting temperature T_m (°C)
61/4	0.199	45.63	46.55	166.71
64/4	0.189	45.52	46.72	166.75
87/4	0.870	41.29	32.54	162.97
182/5	0.829	41.44	32.46	162.36
12/BL	0.785	50.56	71.69	174.95
85/6	1.870	59.83	65.40	176.95
86/6	1.423	59.25	65.82	177.02

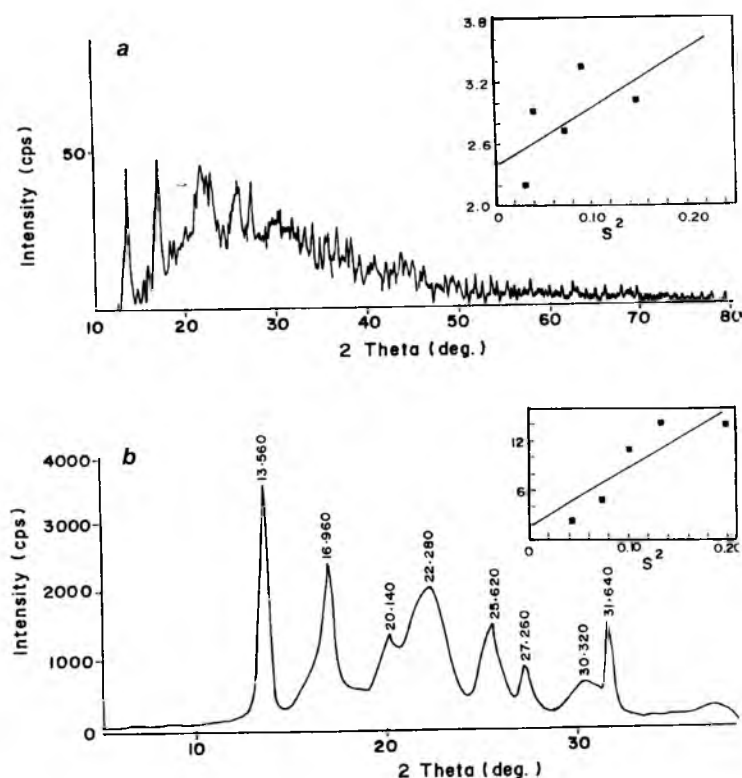


Figure 2. X-ray diffraction pattern of PHA from isolates (a) 87/4 and (b) 85/6. (Inset) Plot of I/I_c against S^2 obtained for the respective isolates.

Table 2. Mechanical properties of PHB from isolates 61/4 and 85/6

Polymer	Stress at break (MPa)	Young's modulus (MPa)	Toughness (MPa)	Strain at break (%)
61/4	35.51	1785.00	33.44	11.40
85/6	30.71	1474.00	25.81	15.41

Crystallinity and molecular weight have marked effect on the mechanical properties of polymers. On the basis of their molecular weight, the isolates 61/4 and 85/6 were selected for the study. The mechanical measurements were done using PHA bars of 2 cm × 7 cm, with 1.0–1.5 mm thickness on an Instron Corporation series IX Automated Materials Testing System 1.04, with sample rate 10 point/s and cross head speed 20 mm/min, at 27°C with 50% humidity. The mechanical tensile data were calculated as an average of three specimens. The toughness of the polymer is measured in terms of its impact strength, while the flexibility is related to the capacity for elongation to break^{8,15,18}. The elongation to break (%) for PHB from isolate 61/4 (Table 2) was found to be much lower than that reported for polyethylene, but higher than that reported for PHB^{8,15,18}. Placed in the increasing order in terms of

toughness and Young's modulus, these are polyethylene < 85/6 < 61/4 < polypropylene (Table 2). Since the PHB from 61/4 and 85/6 is tough, it can find applications in load-bearing.

Overall, the PHA extracted from the bacterial isolates in the tropical marine ecosystem possessed varied physical and mechanical properties, thus reflecting the diversity of the ecosystem to harbour such bacteria.

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T. RAWTE
S. MAVINKURVE*

Department of Microbiology,
Goa University, Taleigao Plateau,
Goa 403 206, India
*e-mail: suneela@unigoa.ernet.in