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In vitro degradation of poly[(r)-3-hydroxybutyrate] and BIOPOL™ by marine-derived fungi

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Abstract. As part of efforts to conserve global resources and reduce pollution, the search for new bio-synthetic/biodegradable materials remains an urgent task. Poly[(R)-3-hydroxyalkanoates] (PHAs) are synthesized by bacteria and display thermoplastic properties. The most common bacterial bio-synthetic PHAs are poly[(R)-3-hydroxybutyrate] (PHB), poly[(R)-3-hydroxyvalerate] (PHV) and their copolymers. PHB and PHV are already commercially produced and used as BIOPOL™ (ICI) for packaging purposes. Oceans and estuaries often serve as major landfills and fungi are an important component of their biodegradation microbiota. In order to characterize the role of fungi in marine biodegradation processes, a simple degradation test suitable for fungi in partially simulated marine conditions had to be developed. Thirty-two strains of yeasts and 102 strains of mycelial fungi isolated from marine habitats and belonging to different systematic and ecological groups were tested for their ability to degrade PHAs. Only approximately 4.5% of the strains were able to degrade BIOPOL™ and about 6.7% depolymerized pure PHB homopolymers. This is in sharp contrast to the results of our previous experiments with 143 strains of terrestrial fungi which showed that more than 55% were able to degrade BIOPOL™. Among ascomicetous fungi, one strain of *Asteromyces cruciatus,* one strain of *Candida guilliermondii,* and two strains of *Debaryomyces hansenii* were able to degrade PHB or both PHB and BIOPOL™. Active basidiomicetous fungi were represented by one strain of *Nia vibrissa* and one strain of *Rhodosporidium sphaerocarpum* that were able to depolymerize PHB, but not BIOPOL™.

Key words: biodegradation, BIOPOL, marine fungal isolates, PHA, PHB, Polyhydroxyalkanoates, screening.

INTRODUCTION

The current global decrease in our natural fossil resources has stimulated increased interest in the search for renewable, bio-synthetic raw materials. Furthermore, current concerns about global environmental pollution associated with petrochemical-based plastics has inspired investigations into possible options for the replacement of such non-degradable synthetic polymers with acceptable bio-degradable substitutes (Hartley 1987; Lafferty et al. 1988; Anderson and Dawes 1990; Brandl et al. 1990; Abe et al. 1995). In some bacteria PHAs constitute a major carbon and energy storage material (Holmes 1988; Fuller 1990). PHAs have generated increased research interest in recent years because of their biodegradability, biocompatibility, and excellent mechanical properties compared with traditional nondegradable thermoplastics, such as polypropylene and polyethylene. Poly[(R)-3-hydroxybutyrate] (PHB) is the most extensively studied type in the PHA family and has been used successfully in the medical and packing fields as a renewable and biodegradable plastic (Guo et al. 2016). Recently, PHB and PHV, often co-polymerized as PHB-co-HV, were demonstrated to possess interesting thermoplastic properties. Thus, these biopolymers have attracted great industrial interest. BIOPOL™, a new biodegradable plastic material, is one such copolymer and is already being produced on a large scale for use as packaging material (ICI, Imperial Chemical Industries, England, 1990).

For the environment, one of the most important properties of PHAs is their ability to undergo complete microbial bio-degradation to CO_2, water and energy (Byrom 1990; ICI 1990). In addition, due to a decline in production costs, PHAs are today used more and more for the production of

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biodegradable containers, packages, and disposable dishware and household goods. Therefore, PHAs are ecologically important alternative materials that can replace synthetic plastics (Thị Vân Anh 2010; Angelini et al. 2014). In the natural environment, PHAs are decomposed by degrading microorganisms to carbon dioxide and water under aerobic conditions, or methane and water under anaerobic conditions. (Matavulj et al. 1995, 2006; Boyandin et al. 2012a, 2012b). Enzymolysis by PHB-depolymerase has been considered to be the root cause of PHB degradation (Guo et al. 2016).

PHAs have been shown to be completely bio-degradable by bacteria both in culture or under environmental (terrestrial, freshwater, anthropogenic) conditions (Macrae and Wilkinson 1958; Merrick and Doudoroff 1964; Delafield et al. 1965; Lusti and Doudoroff 1966; Hippe und Schlegel 1967; Fedulova et al. 1980; Tanio et al. 1982; Nakayama et al. 1985; Fukui et al. 1988; Janssen and Harfoot 1990; Manna and Paul 2000; González García et al. 2013). However, few reports have addressed PHA bio-degradation in marine environments (Akmal et al. 2003; Rutkowska et al. 2008; Rydz 2015; Volova et al. 2015).

In early reports on microbial PHAs and PHA-based plastic decomposers, fungi received little attention and (with a few exceptions) were mostly neglected (Delafield et al. 1965; Lepidi et al. 1972). However, in recent decades molds, yeasts and even mushrooms have attracted greater attention with respect to plastic biodegradation (Holmes 1985; McLellan and Halling 1988; Dave et al. 1990a, 1990b; Matavulj and Molitoris 1990, 1991a, 1991b, 1991c; 1992; Matavulj 1991; Matavulj et al. 1992; Neumeier et al. 1994a, 1994b; Kim and Rhee 2003). Fungi are an important part of the biodegradation microbiota, playing a very active role in mineralization of organic matter and in element cycling. Because they are equipped with extracellular multienzyme complexes, fungi are often highly efficient "bio-degradation machines", especially for the breakdown of natural polymeric compounds. In addition, due to their fast-growing hyphal systems they are also able to colonize substrates rapidly, and to transport and redistribute nutrients within their mycelium (Brucato and Wong 1991; Cooney et al. 1993; Andersen et al. 2011).

Mycorrhizal fungi are also an important component of soil life and soil chemistry. Fungi possess important degradative capabilities that have implications for the recycling of recalcitrant polymers, and for the elimination of hazardous wastes from the environment (Joutey et al. 2013; Katanić et al. 2013, 2015; Pilipović et al. 2014). Previously we reported that, unlike bacteria which degrade only the surface of organic substrates, through the use of their hyphae mycelial fungi are able to penetrate deeply into organic materials. Moreover, being equipped with a broader spectrum of enzymes, they are able to efficiently degrade and decompose all natural polymers (Matavulj et al. 1992, 1995, 1997, 2000; Rohrmann and Molitoris 1992; Gassner and Matavulj 1997; Molitoris et al. 1997; Matavulj and Molitoris 1999; Karaman et al. 2012). The available data on fungal bio-degradation of PHAs or PHA-based plastics are summarized chronologically in Table 1.

The ultimate fate of many organic materials is mineralization by microorganisms. Ever-increasing demand for materials leads to a concomitant increase in waste production. The fact that marine environments (sea- and estuarine waters, sea-bed sediments, coastal sand/rock/soil areas), often serve today as major landfills (O'Brine and Thompson 2010) means that new generations of bio-synthetic plastics likely must be degraded in marine environments (Pruter 1987; Doi et al. 1992; Mukai and Doi 1995; Tsui and Suzuyoshi 2002). Marine fungi would thus be expected to participate significantly in waste bio-plastic materials decomposition.

Obligate marine fungi are those that grow and sporulate exclusively in a marine or estuarine habitat; facultative marine fungi are those from freshwater and terrestrial milieus that are able to grow and possibly also sporulate in a marine environment (Kohlmeyer and Kohlmeyer 1979). Marine fungi grow on a wide variety of substrata: ranging from wood, sediments, mud, soil, sand, algae, coral, decaying leaves (e.g. mangroves), intertidal grasses and living animals, to the guts of crustaceans or other organisms. The need for a concerted effort to investigate the biodiversity and role of marine fungi globally and on as many substrata as possible has already been emphasized (Hyde 1989; Matavulj and Molitoris 1992; Hyde et al. 1998).

Fungi possess important bio-degradative capabilities that have implications for recycling recalcitrant polymers (e.g. lignin) and for the elimination of hazardous wastes from the environment (Mohapatra 2006). In addition to aromatic and aliphatic hydrocarbon compounds, microfungi may transform numerous other aromatic pollutants cometabolically: including polycyclic aromatic hydrocarbons, various pesticides, and plasticizers (Joutey et al. 2013). Some marine-derived fungi were even reported as potential catalysts for bio-remediation of oil spills and other potential pollutants (Cooney et al. 1992; Matavulj and Molitoris 2009; Volova et al. 2010).

It is currently well-accepted that fungi play significant roles in microbial bio-degradation processes. However, their role in the bio-transformation of allochthonous organic matter – particularly in marine habitats – remains largely unknown. In order to address this gap in our current knowledge, a simple screening method for fungal bio-degradation of BIOPOL™ and its components (PHB homopolymer and triacetin) must be developed. The method should provide at least semi-quantitative data and enable screening large numbers of fungal marine isolates under partially simulated marine conditions (seawater mineral composition, level of salinity, pH). Screening of bio-degradation of PHAs by fungi isolated from marine environments should elucidate the capacity of marine fungal population to participate in biodegradation of novel PHA-based pollutants reaching ma-

Table 1. Chronological review of fungal species degrading poly[(R)-3-hydroxyalkanoates].

Species	Taxon	Strain	Substrate	Reference
Penicillium simplicissimum	A		PHB	McLellan and Halling 1988a,b
Eupenicillium sp.	A	IMI 300465	PHB	McLellan and Halling 1988a
Aspergillus sp.	A		PHB	Püchner 1988
Penicillium funiculosum Syn. *Talaromyces funiculosus*	A		PHB	Dave et al. 1990a,b
Penicillium funiculosum Syn. *Talaromyces funiculosus*	A		PHB	Brucato and Wong 1991
Penicillium simplicissimum	A		BIOPOL	Matavulj and Molitoris 1991a
Penicillium atrovenetum	A		BIOPOL	Matavulj and Molitoris 1991a
Trichoderma polysporum	A		BIOPOL	Matavulj and Molitoris 1991a
Collybia peronata	B		BIOPOL	Matavulj and Molitoris 1991a
Lentinus edodes	B		BIOPOL	Matavulj and Molitoris 1991a
Pleurotus ostreatus	B		BIOPOL	Matavulj and Molitoris 1991a
Serpula lacrymans	B		BIOPOL	Matavulj and Molitoris 1991a
Nectria episphaeria	A		BIOPOL	Matavulj and Molitoris 1991a
Mucor hiemalis	Z		BIOPOL	Matavulj and Molitoris 1991a
Mucor sp.	Z		BIOPOL	Matavulj and Molitoris 1991a
Phlyctochytrium africanum	C		BIOPOL	Matavulj and Molitoris 1991a
Phlyctochytrium palustre	C		BIOPOL	Matavulj and Molitoris 1991a
Dictyostelium discoideum	My-Am		BIOPOL	Matavulj and Molitoris 1991a
Physarum polycephalum	My-Am		BIOPOL	Matavulj and Molitoris 1991a
Polyporus circinatus	B		BIOPOL	Matavulj and Molitoris 1992
Mucor sp.	Z		BIOPOL	Matavulj and Molitoris 1992
Penicillium simplicissimum	A	IMI 300465	BIOPOL	Matavulj and Molitoris 1992
Cephalosporium sp.	A		BIOPOL	Matavulj et al. 1992b; 2000
Aspergillus sp.	A		BIOPOL	Matavulj et al. 1992b; 2000
Verticillium sp.	A		BIOPOL	Matavulj et al. 1992b; 2000
Cladosporium sp.	A		BIOPOL	Matavulj et al. 1992b; 2000
Penicillium sp.	A		BIOPOL	Matavulj et al. 1992b; 2000
Penicillium restricum	A		PHB	Mergaert et al. 1992
Penicillium orchrochloron	A		PHB	Mergaert et al. 1992
Penicillium daleae	A		PHB	Mergaert et al. 1992
Aspergillus penicilloides	A		PHB	Mergaert et al. 1992
Paecilomyces marquandii	A		PHB	Mergaert et al. 1992
Penicillium adametzii	A		PHB	Mergaert et al. 1992
Acremonium sp.	A		PHB; 3-PHB/3-PHV	Mergaert et al. 1993
Aspergillus fumigatus	A		PHB	Mergaert et al. 1993, 1994
Verticillium leptobactrum	A		PHB	Mergaert et al. 1994
Penicillium chermisinum	A		PHB	Mergaert et al. 1995
Penicillium janthinellum	A		PHB	Mergaert et al. 1995
Penicillium simplicissimum	A		PHB	Mergaert et al. 1995
Penicillium funiculosum	A		PHB	Oda et al. 1995
Aspergillus fumigatus	A		PHB	Hocking et al. 1996
Aspergillus fumigatus	A		PHB	Scherer 1996
Paecilomyces lilacinus	A	D218	PHB	Oda et al. 1997
Penicillium pinophilum	A	ATCC 9644	PHB	Han et al. 1998
Rhizopus delamar	Z		(R)-3-HB-co-HH	Abe et al. 1995
Penicillium crysosporium	A		3-PHB/3-PHV	Renstad et al. 1999
Penicillium simplicissimum	A		3-PHB/3-PHV	Renstad et al. 1999
Phanerochaete chrysosporium	A		3-PHB/3-PHV	Renstad et al. 1999
Aspergillus fumigatus	A	M2A	PHB	Scherer et al. 1999
Aspergillus fumigatus	A		PHB-co-PHV	Eldsäter 1999
Aspergillus sp.	A		PHB	Sanchez et al. 2000
Thermoascus aurantiacus	A	IFO 31910	PHB	Sanchez et al. 2000
Aspergilus ustus	A	T-221	PHB	Gonda et al. 2000
Paecilomyces sp.	A		PHB	Savenkova et al. 2000
Cephalosporium sp.	A		PHB	Savenkova et al. 2000
Trichoderma sp.	A		PHB	Savenkova et al. 2000
Penicillium sp	A		PHB	Savenkova et al. 2000

Table 1. Chronological review of fungal species degrading poly[(R)-3-hydroxyalkanoates]. (continued)

Species	Taxon	Strain	Substrate	Reference
Aspergillus fumigatus	A		PHB	Scherer et al. 2000
Aspergillus fumigatus	A	Pdf1	PHB	Iyer et al. 2000
Fusarium solani	A	LAR 11	PHB	Kim et al. 2000
Penicillium minioluteum	A	LAR 14	PHB	Kim et al. 2000
Penicillium pinophilium	A	LAR 15	PHB	Kim et al. 2000
Penicillium pinophilium	A	LAR 13	PHB	Kim et al. 2000
Penicillium simplicissimum	A	IFO 6345	PHB	Miyazaki et al. 2000
Trichoderma reesei	A		PHA polymers	Cutright, 2002
Paecilomyces lilacinus	A	F4-5	PHB-co-PHV	Sang et al. 2002
Paecilomyces farinosus	A	F4-7	PHB-co-PHV	Sang et al. 2002
Fusarium oxisporum	A	F1-3	PHB-co-PHV	Sang et al. 2002
Penicillium simplicissimum	A	LAR13	PHB	Han and Kim 2002
Emericellopsis minima	A	W2	PHB; 3-PHB/3-PHV	Kim et al. 2002
Penicillium funiculosum *Syn. Talaromyces funiculosus*	A	ATCC 11797	PHB-co-HV/PHEMA	Gracida et al. 2004
Penicillium sp.	A	DS9701-09a	PHB	Liu et al. 2006
Penicillium sp.	A	DS9713a-01	PHB	Ci et al. 2006
Penicillium funiculosum *Syn. Talaromyces funiculosus*	A		PHB	Hisano et al. 2006
Paecilomyces lilacinus *Syn. Purpureocillium lilacinum*	A	F4-5	PHB PHB-co-PHV	Sang et al. 2006
Penicillium funiculosum *Syn. Talaromyces funiculosus*	A		PHB	Numata et al. 2007
Penicillium simplicissimum	A	T-154	BIOPOL	Matavuly and Molitoris 2009
Penicillium sp.	A	DS9701-D2	PHB	Zhou et al. 2009
Aspergillus fumigatus	A	202	PHB	Bhatt et al. 2010
Malbranchea sp.	A		PHB; 3-PHB/3-PHV	Volova et al. 2010
Aspergillus sp.	A		PHB; 3-PHB/3-PHV	Volova et al. 2010
Verticillium sp.	A		PHB; 3-PHB/3-PHV	Volova et al. 2010
Penicillium sp.	A		PHB; 3-PHB/3-PHV	Volova et al. 2010
Trichoderma sp.	A		PHB; 3-PHB/3-PHV	Volova et al. 2010
Mucor sp.	Z		PHB; 3-PHB/3-PHV	Volova et al. 2010
Aspergillus fumigatus	A		PHB	Lodhi et al. 2011
Gongronella sp.	Z		PHB; PHB-co-PHV	Boyandin et al. 2012a
Paecilomyces sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012a,b
Penicillium sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012a,b
Trichoderma sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012a
Acremonium sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012a
Verticillium sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012ab
Zygosporium sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012a
Aureobasidium sp.	A		PHB; PHB-co-PHV	Boyandin et al. 2012b
Aspergillus sp.	A	NA25	PHB-co-PHV	Nadhman et al. 2012a
Penicillium expansum	A		PHB	Shivakumar 2012
Fusarium solani	A		PHB	Shivakumar 2013
Penicillium pinophilum	A	ATCC 9644	PHB	Panagiotidou et al. 2014
Penicillium expansum	A		PHB	Gowda and Shivakumar 2015
Acremonium butyri	A		P(3HB)	Volova et al. 2015
Penicillium sp.	A	BP-1	P(3HB)	Volova et al. 2015
Penicillium sp.	A	BP-2	P(3HB)	Volova et al. 2015
Purpureocillium lilacinum	A		P(3HB)	Volova et al. 2015
Zygosporium masonii	A		P(3HB)	Volova et al. 2015
Verticillium lateritium	A		P(3HB)	Volova et al. 2015
Acremonium recifei	A		P(3HB)	Volova et al. 2015
Gongronella butleri	Z		P(3HB)	Volova et al. 2015
Trichoderma pseudokoningii	A		P(3HB)	Volova et al. 2015
Penicillium oxalicum	A		P(3HB)	Volova et al. 2015

A = Ascomycota, B = Basidiomycota, Z = Zygomycota, C = Chytridiomycota, My-Am = Myxomycota – Amoebozoa.

rine environments recently.

The scarce available data on marine fungi or fungal strains isolated from marine environments that are capable of biodegradation PHAs or PHA-based plastics are summarized in Table 2.

MATERIAL AND METHODS

Media

In developing media for fungal growth and detection of PHA biodegradation, three media with a low content of non-PHA organic nutrients were tested comparatively:

1) Glucose-peptone-yeast extract agar (GPY), containing 0.1% glucose, 0.05% peptone, and 0.01% yeast extract (Molitoris and Schaumann 1986).

2) Reduced glucose-peptone-yeast extract agar (rGPY): 0.01% glucose, 0.01% peptone, and 0.01% yeast extract.

3) Basal mineral medium with peptone and yeast extract (MPY): Mineral salts: 0.14% $NaNO_3$, 0.10% $NH_4H_2PO_4$, 0.10% KH_2PO_4, 0.06% K_2HPO_4 x $3H_2O$, 0.04% $MgSO_4$ x $7H_2O$, 0.02% $CaCl_2$ x $2H_2O$. Organic substances: 0.01% peptone, 0.01% yeast extract. Microelement solution A (1 ml/l medium): 0.40% $CuSO_4$ x $5H_2O$, 0.40% $ZnSO_4$ x $7H_2O$, 0.40% Na_2MoO_4, 0.20% $MnSO_4$ x H_2O, 0.20% $CoCl_2$ x $6H_2O$, 0.20% H_3BO_3, 0.10% KI, 0.10% Na_2SeO_3 x $5H_2O$, 0.10% Na_2Wo_4 x $2H_2O$, 0.10% $KAl(SO_4)_2$ x $12H_2O$. Microelement solution B (1 ml/l medium): 0.05% $FeS0_4$ x $7H_2O$.

In all cases 1.60% agar and artificial seawater were used for the preparation of media. The pH was adjusted to 7.0 before autoclaving at 121 °C for 20 min. The composition of artificial seawater was as described earlier (Lorenz and Molitoris 1992) resulting in the following concentrations (weight/volume of distilled water): NaCl - 2.270%, KH_2PO_4 - 0.788%, $MgSO_4$ x $7H_2O$ - 0.700%, $MgCl_2$ x $6H_2O$ - 0.550%, KCl - 0.065%, NaBr - 0.010%, H_3BO_3 - 0.003%, $SrCl_2$ x $6H_2O$ - 0.0015%, $C_6H_8O_7$ x H_2O - 0.001%. $CaCl_2$ was added as a separate solution to make a final concentration of 0.15%.

A modified solution of microelements as proposed by Balch and Wolfe (1976) and Balch et al. (1979), was added to give final concentrations of: $C_6H_9NO_6$ (titriplex I) - 0.0015%, $MnSO_4$ x H_2O - 0.0005%, $Ni(NH_4)_2(SO_4)_2$ - 0.0002%, $FeSO_4$ x $7H_2O$ - 0.0001%, $CoCl_2$ x $6H_2O$ - 0.0001%, $ZnSO_4$ x $7H_2O$ -0.0001%, $CuSO_4$ x $5H_2O$ - 0.0001%, $KAl(SO_4)_2$ x $12H_2O$ - 0.00001%, Na_2MoO_4 x $2H_2O$ - 0.00001%, Na_2WO_4 x $2H_2O$ - 0.00001%, Na_2SeO_3 x $5H_2O$ - 0.00001%, KI - 0.000005%.

According to the visually recorded the most abundant growth of all tested fungi, the GPY medium was chosen for further experimental work.

Preparation of assay medium

Powdered commercial BIOPOL™ ("Bottle formulation") (1.00 g) or powdered PHB homopolymer (1.00 g) was suspended in 100 ml of seawater, sonicated for 10 min (Bandelin Sonorex RK 106 S) and autoclaved separately. After sonication for another 10 min, the opaque suspension was added aseptically to 900 ml of hot sterile GPY medium, which was

Table 2. Fungal isolates from marine habitats - degrading poly[(R)-3-hydroxyalkanoates].

Species	Taxon	Strain	Substrate	Reference
Doratomyces sp.	A		PHB, BIOPOL	Matavulj and Molitoris 1991c
Gliomastix sp.	A		PHB, BIOPOL	Matavulj and Molitoris 1991c
Debaryomyces hansenii	Ay		PHB, BIOPOL	Matavulj and Molitoris 1991c
Candida guilliermondii	Ay		PHB, BIOPOL	Neumeier 1994
Debaryomyces hansenii	Ay		PHB, BIOPOL	Neumeier 1994
Rhodosporidium sphaerocarpum	By		PHB, BIOPOL	Neumeier 1994
Aspergillus ustus	A		PHB	Neumeier 1997
Aspergillus ustus	A	M-224	PHB	Gonda et al. 2000
Candida guilliermondii	Ay		PHB	Gonda et al. 2000
Debaryomyces hansenii	Ay		PHB	Gonda et al. 2000
Rhodosporidium sphaerocarpum	B		PHB	Gonda et al. 2000
Gliomastix sp.	A	M-51	BIOPOL	Matavuly and Molitoris 2009
Fusarium merismoides	A	M-46	BIOPOL	Matavuly and Molitoris 2009
Doratomyces sp.	A	M-50	BIOPOL	Matavuly and Molitoris 2009
Asteromyces cruciatus	A	M-1	BIOPOL, PHB	Matavuly and Molitoris 2009
Debaryomyces hansenii	Ay	M-111	BIOPOL, PHB	Matavuly and Molitoris 2009
Debaryomyces hansenii	Ay	M-113	BIOPOL, PHB	Matavuly and Molitoris 2009
Candida guilliermondii	Ay	M-122	BIOPOL	Matavuly and Molitoris 2009
Nia vibrissa	B	M-167	BHB	Matavuly and Molitoris 2009
Talaromyces verruculosus Syn. *Penicillium verruculosum*	A		PHB	Devi et al. 2014

A = Ascomycota, B = Basidiomycota, Ay = ascomycetous yeast, By = basisiomycetous yeast.

then sonicated for another 10 min, agitated, cooled to 50 °C and poured into sterile Petri plates or test tubes, which resulted in a fine and evenly distributed turbidity caused by the PHA. During autoclaving of the media, precipitates of mineral salts occurred, which interfered with the readability of the clearing reaction in plates, however no interference occurred in test tubes where the larger particles of the precipitate were allowed to settle before use. To prevent the sedimentation of fine PHA particles during solidification of the media, test tubes were cooled quickly in a cold water bath. Powdered commercial BIOPOL™ was kindly supplied by ICI, England.

Biological materials

One hundred thirty-four pure strains of marine fungi or marine-derived fungi (Culture collection of the Botanical Institute, University of Regensburg), belonging to different systematic and ecological groups (Table 1) were used for testing the three basic media. The halotolerant strain *Penicillium simplicissimum* (IMI 300465) was used as reference strain because of its known high PHB-degrading activity (McLellan and Halling 1988a; Matavulj and Molitoris 1992).

Cultivation

Fungal strains were cultured on GPY agar at 22 °C. Approximately 3 × 3 mm squares from the actively growing area of the agar plates were used as an inoculum. All cultures in test tubes were incubated at 22 °C and approximately 65% relative humidity with a diurnal periodicity of 12 hours light and 12 hours darkness.

Evaluation of degradative activity

Fungal ability to degrade commercial BIOPOL™ and to depolymerize pure PHB homopolymer and use triacetin, was determined by recording the depth of clearing in mm of the opaque media in test tubes at daily (for the first week), or weekly intervals for a period up to 9 weeks.

RESULTS AND DISCUSSION

In our work on PHA biodegradation in terrestrial fungi (Matavulj and Molitoris 1992) we recommended using test tubes instead of Petri plates for the PHA clearing test because the clearing reaction on Petri plates may be concealed by the growing mycelium, in particular if working with fastgrowing strains. The same applies here to our work with marine fungi. Since used commercial BIOPOL™ contains several components including PHB and PHV as a heteropolymer (79% PHB, 8% PHV, 9% triacetin, 3% titanium dioxide, 1% boron nitride), it was important to know whether there are differences between media containing PHB-V heteropolymer and media containing pure PHB homopolymer for the purpose of specifying depolymerase activity.

Test results for 134 marine-derived fungi for the biodegradation of commercial BIOPOL™, PHB homopolymer and triacetin are summarized in Table 3.

A total of 134 strains were tested, representing 85 species and (conditionally) 55 genera of different systematic and ecological groups. Interestingly, only 6 strains (4.48%) were found to be able to degrade commercial BIOPOL™, 9 strains (6.72%) depolymerized PHB homopolymer, and 124 strains (92.53%) were able to use triacetin as the sole carbon source. This is in sharp contrast with our results using terrestrial fungi, where approximately 55% were able to degrade commercial BIOPOL™, and nearly 68% depolymerised PHB homopolymer (Matavulj and Molitoris 1992). Triacetin was also degraded by more than 90% of the terrestrial fungi tested. The easy degradability of triacetin could be an important initial step in the destruction of this plastic material in nature, especially under oligotrophic marine conditions.

Mergaert and Swings (1996), using polyhydroxyalkanoates as another pollutant, were unable to isolate any marine fungus able to degrade these substances *in situ.* Using pure fungal cultures, Matavulj and Molitoris (1991c), Neumeier (1994a), and Molitoris et al. (1995) also found considerably less marine isolates of filamentous fungi and yeasts (vs. terrestrial) capable of PHB biodegradation. One possible explanation for this disparity lies in the fact that almost all producers of PHAs are isolated from freshwater or terrestrial environments, while producers of PHAs from marine environments are almost unknown.

The results of a study by Neumeier (1997) showed less degradation of PHB by marine and terrestrial isolates of *Aspergillus ustus* (T-221, M-224) in artificial seawater vs. the same terrestrial isolate of *A. ustus* (T-221) grown in freshwater medium under otherwise identical conditions (GPY medium with PHB). Even more convincing is the failure of marine yeast isolates studied to degrade PHB in artificial seawater medium since on solid freshwater medium they all degraded PHB, at least in the clearing test (Neumeier 1994). The negative effects of salinity on the biodegradation of different substrates by fungi have been reported earlier. Rohrmann (1993) noticed a decrease in the degradation of wood with increasing media salinity. Decreased growth, substrate degradation and enzyme activity with increasing salinity was also found by Kurchenko et al. (1998), and Molitoris et al. (1998).

A study of bacterial biodegradation of PHAs by Jendrossek et al. (1993) revealed that only approximately 10% of aerobic PHB-utilizing bacteria were also able to degrade pure PHV-homopolymers. Furthermore, Doi et al. (1990) reported that the rate of polyester degradation by extracellular PHA depolymerase purified from the bacterium *Alcaligenes eutrophus*, was strongly dependent on the composition of the polyester. They observed that the presence of 3-hy-

droxyvalerate units significantly retarded the degradation of the polyester as compared with the rate of pure PHB degradation. Also, attenuation of the biodegradability of PHB by a lignin-rich filler used for production of bio-based blends was reported recently (Angelini et al. 2014).

Another important question is whether PHA-degrading activity correlates with the systematic or ecologic groups of fungi investigated. Because of the low number of active strains in marine fungal isolates, it is difficult to obtain statistically significant correlations. However, from Table 3 it can be seen that biodegradation activities are not evenly distributed among the different groups of tested marine-derived fungal species. Table 3 seems to indicate that PHA-degrading activity in marine fungal isolates is rather low among the Ascomycotina (BIOPOL™ – 5.71%, PHB – 6.67%) as well as in the group of Basidiomycotina (BIOPOL™ – 0.00%, PHB – 6.89%).

Considering the distribution of PHA-degrading activity among several members of a given genus or a given species, one can see from Table 3 that out of five strains of *Asteromyces cruciatus* tested, only the strain M-1 was able to degrade both commercial BIOPOL™ and pure PHB. Out of 9 strains of the genus *Candida*, only one strain of *Candida guilliermondii* (M-122) depolymerised PHB, but not commercial BIOPOL™. The yeast *Debaryomyces hansenii* was represented in our screen by 6 strains, of which only two showed the ability to degrade both PHB and BIOPOL™.

Investigation of 29 isolates belonging to Basidiomycotina, representing 4 genera (*Cryptococcus*, *Nia*, *Rhodosporidium* and *Sporobolomyces*) and 15 species, showed that only one strain of *Nia vibrissa* (M-167) (Agaricales) and one strain of *Rhodosporidium sphaerocarpum* species (M-185) (Sporidiobolales) were able to depolymerize PHB, but not commercial BIOPOL™. Earlier we reported that among 65 investigated terrestrial basidiomycetous mushroom strains, *Collybia peronata, Lentinus edodes, Pleurotus ostreatus*, *Serpula lacrymans, and Polyporus circinatus* were able to degrade BIOPOL (Matavulj and Molitoris, 1991a, 1992).

CONCLUSION

According to the data presented, it turned out that the biodegradation capacity of marine-derived fungal population against tested PHB and BIOPOL™ was pretty poor (4.5% degraded BIOPOL™ and 6.7% depolymerized pure PHB). This is in contrast to the results obtained from investigation of terrestrial fungi (more than 55% active). Moreover, our data indicate that in marine-derived fungi this characteristic is not related to taxonomic character of a particular species or genera, nor is it a feature of certain ecological groups. In contrast, PHA biodegradation activity seems rather to be a physiological characteristic of individual "physiological strains" of the studied marine-derived fungal species.

AGKNOWLEDGEMENTS

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Table 3. Test results for fungi isolated from marine environments on the degradation of BIOPOL™, PHB homopolymer and triacetin.

Species	Authority	Taxon	Origin	Code	Rg Code	Marine	Substrate	BP	PHB	Ta
								degradation		
Acremonium furcatum	Moreau & R. Moreau ex Gams, 1969	A	KMPB	H 750	M-039	MI	sand/beach	-	-	+
Acremonium potronii	Vuillemin, 1910	A	KMPB	H 707	M-037	MI	sand/beach	-	-	+
Acremonium sp.	-	A	KMPB	H 2046	M-038	MI	sediment	-	-	+
Amylocarpus encephaloides	Currey, 1859	A	PP	# 1002	M-141	MI	wood	-	-	-
Ascomycet indeterminatus	-	A	JK	R 13/2	M-066	-	Ni	-	-	+
Ascomycet indeterminatus	-	A	KMPB	H 344	M-011	-	Ni	-	-	+
Asteromyces cruciatus	Moreau & Moreau ex Hennebert, 1962	A	KMPB	H 54a	M-001	MI	Ni	+	+	+
Asteromyces cruciatus	Moreau & Moreau ex Hennebert, 1962	A	KMPB	H 76	M-084	MI	foam	-	-	+
Asteromyces cruciatus	Moreau & Moreau ex Hennebert, 1962	A	URB	SR 2	M-149	MI	foam	-	-	+
Asteromyces cruciatus	Moreau & Moreau ex Hennebert, 1962	A	JK	3678	M-153	MI	sand/beach	-	-	+
Asteromyces cruciatus	Moreau & Moreau ex Hennebert, 1962	A	URB	H 55	M-181	MI	wood/dune	-	-	+
Aureobasidium pullulans	(de Bary) G. Arnaud (1918)	Ay	PP	698	M-156	FM	Ni	-	-	+
Biconiosporella corniculata	Schaumann, 1972	A	KMPB	H 691	M-012	MI	Ni	-	-	+
Camarosporium roumeguerii	Saccardo, 1880	A	JK	3905	M-076	MI	*Salicornia* sp. leaves	-	-	-
Pichia guilliermondii Anamorph: *Candida guilliermondii*	Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-148	M-118	FM	seawater	-	-	+
Pichia guilliermondii Anamorph: *Candida guilliermondii*	Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-153	M-119	FM	sea gull liver	-	-	+
Pichia guilliermondii Anamorph: *Candida guilliermondii*	Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-155	M-120	FM	sea gull liver	-	-	+
Pichia guilliermondii Anamorph: *Candida guilliermondii*	Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-266	M-121	FM	seawater	-	-	+
Pichia guilliermondii Anamorph: *Candida guilliermondii*	Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-283	M-122	FM	seawater	-	+	+
Pichia guilliermondii Anamorph: *Candida guilliermondii*	Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-299	M-123	FM	seawater	-	-	+
Pichia guilliermondii Anamorph: *Candida guilliermondii*	(Wickerham, 1966 (Castellani) Langeron & Guerra, 1938	Ay	SC	NS-76-167	M-148	FM	seawater	-	-	+
Candida sorbophila	(Nakase) Meyer & Yarrow, 1978	Ay	URM	Cy4/1b	M-186	MI	Vulkanic rock	-	-	+
Candida tropicalis	(Castellani) Berkhout, 1923	Ay	SC	NS-76-162	M-124	FM	Sea gull gut content	-	-	+
Carbosphaerella leptosphaerioides	I. Schmidt, 1969	A	PP	4810	M-189	MI	Driftwood	-	-	+
Carbosphaerella leptosphaerioides	I. Schmidt, 1969	A	PP	4804	M-190	MI	Driftwood	-	-	+
Acremonium sclerotigenum An.: *Cephalosporium sclerotigenum*	(M.&R.Moreau exValenta) W.Gams,1972 M. & R. Moreau ex Valenta, 1948	A	KMPB	H 724	M-040	MI	Sand beach	-	-	+
Ceriosporopsis circumvestita	(Kohlm.) Kohlm., 1972	A	PP	0724	M-207	MI	Ni	-	-	+
Ceriosporopsis halima	Linder in Barghoorn & Linder, 1944	A	URB	M-178	M-178	MI	Wood	-	-	+
Ceriosporopsis halima	Linder in Barghoorn & Linder, 1944	A	URB	M-179	M-179	MI	Wood	-	-	+
Chaetomium ramipilosum	Schaumann, 1973	A	KMPB	H 890/2	M-013	MI	Ni	-	-	+
Chaetomium sp.	-	A	KMPB	H 2076	M-55	MI	Sediment	-	-	+
Cirrenalia macrocephala	(Kohlm.) Meyers & RT Moore, 1960	A	KMPB	H 210	M-002	MI	Ni	-	-	+
Cirrenalia macrocephala	(Kohlm.) Meyer, & RT Moore, 1960	A	JK	6073	M-159	MI	Wood	-	-	+
Cirrenalia pygmea	Kohlmeyer, 1966	A	PP	# 1036	M-138	MI	Wood	-	-	+
Cirrenalia tropicalis	Kohlmeyer, 1968	A	PP	# 1031	M-139	MI	Wood	-	-	+
Cladosporium cladosporioides	(Fresen.) de Vries, 1952	A	URM	1/1	M-201	MI	Ni	-	-	+
Cladosporium cladosporioides	(Fresen.) de Vries, 1952	A	URM	1/3	M-203	MI	Ni	-	-	+
Cladosporium cladosporioides	(Fresen.) de Vries, 1952	A	URM	2/1	M-204	MI	Ni	-	-	+

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Species	Authority	Taxon	Origin	Code	Rg Code	Marine	Substrate	BP	PHB	Ta
								degradation		
Cladosporiuum herbarum	(Pers.) Link ex S. F. Gray, 1816	A	URM	1/2	M-202	MI	Ni	-	-	+
Cladosporiuum herbarum	(Pers.) Link ex S. F. Gray, 1816	A	URM	2/2	M-205	MI	Ni	-	-	+
Corollospora colossa	Nakagiri & Tokura, 1988	A	PP	4794	M-191	MI	Driftwood	-	-	+
Corollospora lacera	(Linder in Bargh & Lind) Kohlm., 1962	A	JK	3897	M-058	MI	Wood	-	-	+
Corollospora maritima	Werdermann, 1922	A	KMPB	H671	M-015	MI	Ni	-	-	+
Corollospora maritima	Werdermann, 1922	A	JK	4414	M-059	MI	Ni	-	-	+
Corollospora maritima	Werdermann, 1922	A	URB	SR 8	M-150	MI	Sand	-	-	+
Corollospora maritima	Werdermann, 1922	A	URB	H 12/1	M--182	MI	Wood/Sandy beach	-	-	+
Corollospora quinqueseptata	Nakagiri ex Tokura, 1988	A	URB	M 206	M-206	MI	Ni	-	-	-
Corollospora trufurcata	(Höhnk) Kohlm., 1962	A	JK	4423/1	M-087	MI	Detritus	-	-	+
Cryptococcus albidus	(Saito) Skinner, 1950	By	JF	ML 2604	M-103	FM	Seawater	-	-	+
Curvularia sp.		A	PP	520	M-155	MI	Ni	-	-	+
Cytospora rhizophorae	Kohlmeyer & Kohlmeyer, 1971	A	JK	5241	M-176	MI	Ni	-	-	-
Debaryomyces hansenii	(Zopf) Lodder & Kreger-van Rij (1984)	Ay	SC	NS-75-9	M-111	FM	Seawater	+	+	+
Debaryomyces hansenii	(Zopf) Lodder & Kreger-van Rij (1984)	Ay	SC	NS-75-10	M-112	FM	Seawater	-	-	+
Debaryomyces hansenii	(Zopf) Lodder & Kreger-van Rij (1984)	Ay	SC	NS-75-11	M-113	FM	Seawater	+	+	+
Debaryomyces hansenii	(Zopf) Lodder & Kreger-van Rij (1984)	Ay	SC	NS-75-21	M-114	FM	Seawater	-	-	+
Debaryomyces hansenii	(Zopf) Lodder & Kreger-van Rij (1984)	Ay	SC	NS-75-35	M-115	FM	Seawater	-	-	+
Debaryomyces hansenii	(Zopf) Lodder & Kreger-van Rij (1984)	Ay	SC	NS-75-60	M-116	FM	Seawater	-	-	+
Dendryphiella salina	(Sutherland) Pugh & Nicot., 1964	A	kmpb	H/800/1	M-003	MI	Ni	-	-	+
Dendryphiella salina	(Sutherland) Pugh & Nicot., 1964	A	jk	2	M-007	MI	Ni	-	-	+
Dendryphiella salina	(Sutherland) Pugh & Nicot., 1964	A	urb	SR 22	M-151	MI	Seawater	-	-	+
Dendryphiella salina	(Sutherland) Pugh & Nicot., 1964	A	urb	H 3	M-183	MI	Wood/Sandy beach	-	-	+
Dictyosporium pelagicum	(Linder) Hugh. ex EBG Jones, 1963	A	KMPB	H652	M-004	MI	Ni	-	-	-
Doratomyces sp.		A	KMPB	H 2086	M-050	MI	Sediment	+	+	+
Drechslera halodes	(Drechsler) Subram. & BL Jain, 1966	A	JK	4094	M-062	MI	Detritus	-	-	+
Fusarium merismoides	Corda, 1838	A	KMPB	H 635	M-046	MI	Sand/beach	+	+	+
Fusarium sambucinum Teleomorph: *Gibberella pulicaris*	Fuckel, 1870 (Fr.) Sacc. (1877)	A	KMPB	H 796	M-045	MI	Sand/beach	-	-	+
Gliomastix sp.		A	KMPB	H 2001	M-051	MI	Sediment	+	+	+
Halosarpheia ratnagiriensis	Patil & Borse, 1982	A	PP	1907	M-193	MI	Wood	-	-	+
Halosarpheia trullifera	(Kohlm.) Jones, Moss & Cuomo, 1983	A	PP	4912	M-194	MI	Driftwood	-	-	+
Halosarpheia trullifera	(Kohlm.) Jones, Moss & Cuomo, 1983	A	PP	4913	M-195	MI	Driftwood	-	-	+
Halosphaeriopsis mediosetigera	(Cribb & Cribb) Johnson, 1958	A	URB	M 180	M-180	MI	Driftwood	-	--	+
Humicola alopallonella	Meyers & Moore, 1960	A	KMPB	H 441	M-005	MI	Ni	-	-	+
Leptosphaeria australiensis	(Cribb & Cribb) GC Hughes, 1969	A	JK	4482/1	M-065	MI	Ni	-	-	+
Leptosphaeria obiones	(Crouan & Crouan) Saccardo, 1883	A	JK	3904	M-090	MI	*Spartina alterniflora*	-	-	+
Lignicola laevis	Höhnk, 1955	A	KMPB	H 36	M-016	MI	Ni	-	-	+
Lignicola laevis	Höhnk, 1955	A	PP	3239	M-196	MI	Wood	-	-	+
Lindra obtusa	Nakagiri & Tubaki, 1983	A	PP	4943	M-197	MI	Driftwood	-	-	+
Lulworthia lignoarenaria	Koch & Jones, 1984	A	PP	# 920	M-142	MI	Driftwood	-	-	+
Lulworthia sp.		A	KMPB	H 622	M-018	MI	Ni	-	-	+
Lulworthia sp.		A	JK	4298/1	M-068	MI	Wood/mangrove	-	-	+
Macrophoma sp.		A	JK	4342	M-069	MI	Proproot/Rhizophora	-	-	+

Table 3. Test results for fungi isolated from marine environments on the degradation of BIOPOL™, PHB homopolymer and triacetin.

Species	Authority	Taxon	Origin	Code	Rg Code	Marine	Substrate	BP	PHB	Ta
								degradation		
Marinosphaeria mangrovei	Hyde, 1988	A	PP	3820	M-198	MI	Mangrove	-	-	+
Microascus senegalensis	von Arx, 1975	A	JK	4288/2	M-071	MI	Wood/mangrove	-	-	+
Microascus senegalensis	von Arx, 1975	A	JK	4288/6	M-072	MI	Ni	-	-	+
Microascus trigonosporus	Emmons & Dodge	A	KMPB	H680	M-019	OM	Ni	-	-	+
Monodictys pelagica Teleomorph: *Nereiospora cristata*	(Johnson) Jones, 1963 (Kohlm.) Jones, Johnson & Moss, 1983	A	KMPB	H 106	M-006	MI	Ni	-	-	+
Monodictys pelagic Teleomorph: *Nereiospora cristata*	(Johnson) Jones, 1963 (Kohlm.) Jones, Johnson & Moss, 1983	A	KMPB	H 580	M-007	MI	Ni	-	-	+
Monodictys pelagic Teleomorph: *Nereiospora cristata*	(Johnson) Jones, 1963 (Kohlm.) Jones, Johnson & Moss, 1983	A	JK	516	M-070	MI	Piling	-	-	+
Monodictys sp.		A	KMPB	H 6745	M-052	MI	Seawater	-	-	+
Nais glitra	Crane & Shearer, 1986	A	PP	4892	M-208	MI	Mangrove	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	ATCC	34606	M-021	MI	Wood	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	JK	5071	M-154	MI	Proproot/Rhizophora	-	-	-
Nia vibrissa	Moore & Meyers, 1959	B	PP	2770	M-170	MI	Wood	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	PP	2772	M-171	MI	Wood	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	PP	1071	M-161	MI	Wood	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	PP	2765	M-167	MI	Wood	-	+	+
Nia vibrissa	Moore & Meyers, 1959	B	PP	2767	M-168	MI	Wood	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	PP	2768	M-169	MI	Ni	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	PP	1068	M-172	MI	Wood	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	JFO	32088	M-173	MI	Ni	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	JFO	32089	M-174	MI	Ni	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	ATCC	34606	M-175	MI	Ni	-	-	+
Nia vibrissa	Moore & Meyers, 1959	B	URB	OD 3	M-187	MI	Submerg. pine wood	-	-	+
Orbimyces spectabilis	Linder, 1944	A	PP	# 517	M-140	MI	Wood	-	-	-
Pestalozia sp.		A	PP	3641	M-157	MI	Driftwood	-	-	+
Periconia prolifica	Anastasiou, 1963	A	PP	3508	M-199	MI	Scots pine wood	-	-	+
Phialophora fastigiata	(Lagerberg & Melin) Conant, 1937	A	KMPB	H 708	M-041	MI	Sand/beach	-	-	+
Phialophora malorum Syn. *Cadophora malorum*	(Kidd & Beaumont) McColloch, 1944 (Kidd & Beaumont) W. Gams, 2000	A	KMPB	H 737b	M-042	MI	Sand/beach	-	-	+
Phoma sp.		A	KMPB	H 8	M-053	MI	Pine wood	-	-	+
Remispora hamata	(Höhnk) Kohlmeyer, 1961	A	KMPB	H 14	M-047	MI	Ni	-	-	-
Rhodosporidium bisporidii Syn. *Cystofilobasidium bisporidii*	Fell, Hunter & Tallman, 1973 (Fell, Hunt.&Tallm.)Oberw.&Bandoni, 1983	By	JF	ML 2498	M-099	OM	Ni	-	-	+
Rhodosporidium capitatum Syn. *Cystofilobasidium capitatum*	Fell, Hunter & Tallmann, 1973; (Fell,Hunt.&Tallm.)Oberw.& Bandoni,1983	By	JF	ML 2499	M-100	OM	Zooplankton	-	-	+
Rhodosporidium dacryoidum	Fell, Hunter & Tallmann, 1973	By	JF	ML 2505	M-102	OM	Seawater	-	-	+
Rhodosporidium diobovatum	Newell & Hunter, 1970	By	JF	# 2904	M-081	FM	Seawater	-	-	+
Rhodosporidium malvinellum	Fell & Hunter, 1970	By	JF	ML 2500	M-101	OM	Seawater	-	-	+
Rhodosporidium sphaerocarpum	Newell & Fell, 1970	By	JF	# 2920	M-080	OM	Seawater	-	-	+
Rhodosporidium sphaerocarpum	Newell & Fell, 1970	By	URM	M 184	M-184	MI	Ni	-	-	+
Rhodosporidium sphaerocarpum	Newell & Fell, 1970	By	URM	M 185	M-185	MI	Ni	-	+	+
Rhodosporidium toruloides	Banno, 1967	By	JF	ML 2965	M-108	FM	Seawater	-	-	+
Rhodotorula aurantiaca	(Saito) Lodder, 1934	By	JF	ML 2969	M-104	FM	Seawater	-	-	+

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Species	Authority	Taxon	Origin	Code	Rg Code	Marine	Substrate	BP	PHB	Ta
								degradation		
Rhodotorula glutinis	(Fresenius) Harrison, 1928	By	JF	ML 2919	M-106	FM	Seawater	-	-	+
Rhodotorula graminis	Di Menna, 1958	By	JF	ML 2925	M-107	FM	Seawater	-	-	+
Rhodotorula minuta	(Saito) Harrison, 1928	By	JF	# 2911	M-082	FM	Seawaater	-	-	+
Rhodotorula mucilaginosa Syn. *Rhodotorula rubra*	(A. Jörg.) Harrison, 1928; (Demme) Lodder, 1934	By	JF	" 2918	M-083	FM	Seawater	-	-	+
Savoryella paucispora	(Cribb & Cribb) Koch, 1982	A	JK	4478/9	M-073	MI	*Hibiscus tiliac.* wood	-	-	+
Sporobolomyces salmonicolor	(Fisch. & Breb.)Kluyv.& CB Niel, 1924	By	JF	# 2944	M-085	FM	Seawater	-	-	+
Trichocladium achrasporum	(Mayers & Moore) Dixon, 1968	A	JK	F 92	M-079	MI	Ni	-	-	+
Trichocladium achrasporum	(Mayers & Moore) Dixon, 1968	A	URB	M 177	M-177	MI	Wood	-	-	+
Varicosporina ramulosa	Meyers & Kohlmeyer, 1965	A	JK	113	M-074	MI	*Zostera marina*	-	-	-
Verruculina enalia	(Kohlm.)Kohlm.&Volkm.-Kohlm.,1990	A	PP	3019	M-192	MI	Mangrove wood	-	-	+
Verticillium lecanii Syn. *Lecanicillium lecanii*	(Zimmermann) Viegas, 1939; (Zimm.) R. Zare & W. Gams, 2001	A	KMPB	H 889	M-054	MI	Rhizosphaere	-	-	+
Zalerion maritimum Teleomorph: *Lulworthia uniseptata*	(Linder) Anastasiou, 1963; Nakagiri, 1984	A	JK	4779	M-160	MI	Driftwood	-	-	+
Zalerion maritimum Teleomorph: *Lulworthia uniseptata*	(Linder) Anastasiou, 1963; Nakagiri, 1984	A	PP	1329	M-163	MI	Wood	-	-	+
Zalerion maritimum Teleomorph: *Lulworthia uniseptata*	(Linder) Anastasiou, 1963; Nakagiri, 1984	A	URB	M 209	M-209	MI	Wood	-	-	+

Classification: A = Ascomycota, B = Basidiomycota, Ay = ascomycetous yeast, By = basisiomycetous yeast.

Origin: ATCC 0 American Type Culture Collection; JF = J. Fell, Miami USA; JFO = A. Nakagiri, Osaka, Japan; JK = J. Kohlmeyer, Moreheasd City, USA; PP = E.B.G. Jones, Portsmouth, UK; SC = S. Crow, Athens, USA; URB = Collection of the Botanical Institute of the University of Regensburg, Germany; URM = K.O. Stetter, Collection of the Microbiological Institute of the University of Regensburg, Germany.

Orig. code: Original code number of supplier of culture.

Rg. Code: Code number of the Regensburg University Collection.

Marine: Marine strains or marine isolates = MI, unless specified: OM = obligate marine, FM = facultative marine.

Substrate: Ni = No information available.

Degradation of: BIOPOL™, PHB = poly-β-hydroxybutyrate homopolymer, Ta = triacetin.

LITERATURE

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