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Reduction of Cell Lysate Viscosity during Processing of Poly(3-Hydroxyalkanoates) by Chromosomal Integration of the Staphylococcal Nuclease Gene in *Pseudomonas putida*

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Poly(3-hydroxyalkanoates) (PHAs) are biodegradable thermoplastics which are accumulated by many bacterial species in the form of intracellular granules and which are thought to serve as reserves of carbon and energy. *Pseudomonas putida* accumulates a polyester, composed of medium-side-chain 3-hydroxyalkanoic acids, which has excellent film-forming properties. Industrial processing of PHA involves purification of the PHA granules from high-cell-density cultures. After the fermentation process, cells are lysed by homogenization and PHA granules are purified by chemical treatment and repeated washings to yield a PHA latex. Unfortunately, the liberation of chromosomal DNA during lysis causes a dramatic increase in viscosity, which is problematic in the subsequent purification steps. Reduction of the viscosity is generally achieved by the supplementation of commercially available nuclease preparations or by heat treatment; however, both procedures add substantial costs to the process. As a solution to this problem, a nuclease-encoding gene from *Staphylococcus aureus* was integrated into the genomes of several PHA producers. Staphylococcal nuclease is readily expressed in PHA-producing *Pseudomonas* strains and is directed to the periplasm, and occasionally to the culture medium, without affecting PHA production or strain stability. During downstream processing, the viscosity of the lysate from a nuclease-integrated *Pseudomonas* strain was reduced to a level similar to that observed for the wild-type strain after treatment with commercial nuclease. The nuclease gene was also functionally integrated into the chromosomes of other PHA producers, including *Ralstonia eutropha*.

Polyhydroxyalkanoates (PHAs) are biodegradable and biocompatible polyesters that accumulate as intracellular inclusion bodies in a variety of bacteria (24, 27). Because these polymers are produced from renewable resources such as fatty acids and sugars, they provide a new resource for plastic materials and small molecules derived from their monomers (45). The properties of these polyesters range from stiff and brittle materials, such as poly(3-hydroxybutyrate) (PHB), to elastomers, such as poly(3-hydroxyoctanoate). The monomeric composition of a PHA depends on the bacterial strain, the culture conditions, and the carbon source used for growth, but generally, bacteria synthesizing PHAs can be subdivided into two groups. One group, including *Ralstonia eutropha*, produces short-side-chain PHAs with C_3 to C_5 monomers, while the second group, including *Pseudomonas putida*, synthesizes medium-side-chain PHAs with C_6 to C_{16} monomers (27).

PHAs can be recovered and purified from biomass by a number of different techniques. One technique involves extraction of the polymer from lyophilized cells with organic solvents (5). Most other techniques involve mechanical or chemical cell disruption followed by chemical or enzymatic treatment. During such processes, the PHA granules are released from the cells and are subsequently purified by repeated centrifugation and/or filtration steps. Cell disruption, however, also results in liberation of chromosomal DNA, which causes a rapid increase in the viscosity of the cell lysate, thereby impeding subsequent filtration and centrifugation (26, 39, 41). Since the efficiencies of filtration and centrifugation are inversely proportional to the viscosity and a high viscosity directly affects pumping, mixing, and heat transfer, quick removal of the chromosomal DNA is critical (3). Previously described methods for DNA degradation in PHA processes included the use of hypochlorite (4), heat treatment (8, 9), or enzyme cocktails (18). Even though these three methods may seem applicable in small-scale fermentation systems, they have some drawbacks for the envisioned 100 million-lb production scale for PHAs. Besides the limitations at the larger scale, these procedures have additional disadvantages, since hypochlorite causes limited hydrolysis of the PHA while heat treatment and the use of enzyme cocktails are costly.

To provide a commercially attractive solution to the viscosity problem, we integrated the staphylococcal nuclease gene into the chromosomes of different PHA producers. Staphylococcal nuclease has been shown to hydrolyze DNA and RNA to fragments of less than 100 nucleotides (2, 6). The described recombinant strains were stable in high-cell-density fermentations and during recovery of PHA granules. The viscosity of the lysates from such strains was reduced compared to the viscosity of a lysate from the wild-type strain.

MATERIALS AND METHODS

Bacterial strains and growth media. The strains used in this study are listed in Table 1. *Escherichia coli* and *P. putida* strains were routinely grown in Luria-Bertani medium or R medium (34). *R. eutropha* was grown in either Trypticase soy broth (Becton Dickinson, Cockeysville, Md.) or PCT medium (31) supplemented with 1% glucose. Media were supplemented with chloramphenicol (32 μg/ml), nalidixic acid (30 μg/ml), or kanamycin (50 μg/ml) as required. Benzonase was obtained from American International Chemical (Natick, Mass.).

Primers and DNA amplification. The *nuc* gene, encoding the nuclease from *Staphylococcus aureus*, was obtained by PCR, using plasmid pNuc1 (25) as a template. Reactions mixtures (50 μl) contained 10 pmol each of primers nucA (5′-TTCTCTAGAATTCAGGAGGTTTTTATGGCTATCAGTAATGTTTCG)

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TABLE 1. Strains and plasmids used in this study

Strain	Relevant characteristics	Reference or source
P. putida strains		
KT2442	*hsdR*; Rif^r	20
MBX918	KT2442 *nuc-kan*	This study
MBX919	KT2442 *nuc-kan*	This study
MBX920	KT2442 *nuc-kan*	This study
MBX921	KT2442 *nuc-kan*	This study
MBX922	KT2442 *nuc-kan*	This study
MBX923	KT2442 *nuc-kan*	This study
MBX924	KT2442 *rrn*::*nuc-kan*	This study
MBX925	KT2442 *nuc-kan*	This study
MBX926	KT2442 *nuc-kan*	This study
Pseudomonas sp. strains		
MBX978		MBX strain
MBX979	MBX978 *nuc-kan* (high-level nuclease activity)	This study
MBX980	MBX978 *nuc-kan*	This study
MBX981	MBX978 *nuc-kan* (low-level nuclease activity)	This study
MBX982	MBX978 *nuc-kan* (moderate nuclease activity)	This study
MBX983	MBX978 *nuc-kan*	This study
MBX984		This study
MBX985	MBX978 *nuc-kan* (moderate nuclease activity)	This study
MBX986	MBX978 *nuc-kan*	This study
MBX987	MBX978 *nuc-kan*	This study
R. eutropha strains		
NCIMB40124HD		NCIMB[a]
MBX917	NCIMB40124HD *nuc-kan*	This study
E. coli strains		
S17-1	*recA thi pro hsdR*$^-$*M*$^+$ RP4-2-Tc::Mu λpir; Sm^r	16
DH5αF$^-$	*recA1 hsdR17 endA1 thi-1 gyrA96 relA1*	NEB[b]
MBX245	LJ14 *rpoS*::Tn*10*; Nal^r	MBX strain
MBX988	MBX245 *nuc-kan*	This study

[a] National Center for Industrial Microorganisms and Bacteria.
[b] New England Biolabs, Beverly, Mass.

and nucB (5′-GCCGGTACCTTATTGACCTGAATCAGCGTTG) and the template in PCRmix (Gibco BRL, Gaithersburg, Md.), and reactions were performed in a thermocycler (Ericomp, San Diego, Calif.), using a program comprising 30 cycles of incubation at 95°C (30 s), at 55°C (45 s), and at 72°C (45 s). PCR products were gel purified and cloned into the pCR2.1 cloning vector (Invitrogen, Carlsbad, Calif.). The insert of the resulting plasmid, pCR2.1-nuc, was confirmed by DNA sequencing to be identical to the reported sequence of the *nuc* gene from *S. aureus* (37) (GenBank accession no. J01785).

Plasmid construction. pCR2.1-nuc was digested with *Eco*RI and *Acc*65I according to the manufacturer's (New England Biolabs, Beverly, Mass.) recommendations, and the *nuc* gene fragment was purified and cloned into the corresponding sites of pUC18Not (16). A promoterless, blunt-ended kanamycin gene from Tn*903* (obtained by PCR from pBGS18, using primers linkK1 [5′-TGCA TGCGATATCAATTGTCCAGCCAGAAAGTGAGG] and linkK2 [5′-ATTTA TTCAACAAAGCCGCC]), was inserted into the *Sma*I site to generate pMNX-nuc-kan. The *Not*I fragment containing the promoterless *nuc-kan* operon was then cloned into the *Not*I sites of the integration vector pUTkan (16), a process which deleted the original kanamycin resistance marker, generating pMUX-nuc-kan.

Transposon mutagenesis and selection of integrants. Plasmid pMUX-nuc-kan was transformed into *E. coli* S17-1 λpir and then conjugated into PHA-producing strains such as *P. putida*, *Pseudomonas* sp. strain MBX978, and *R. eutropha* as described elsewhere (16). *P. putida* and *Pseudomonas* sp. strain MBX978 integrants were selected on plates of minimal E_2 medium (22) containing 10 mM octanoate as the carbon source and kanamycin as the selective agent. Integrants were replica plated onto DNase agar plates (Difco Laboratories, Detroit, Mich.) supplemented with kanamycin, and clones that expressed nuclease were identified by the presence of zones of clearing around the colonies (37). For *R. eutropha*, integrants were selected on PCT medium supplemented with 1% glucose, nalidixic acid, and kanamycin. Integrants were subsequently identified by replica plating onto DNase agar plates supplemented with 10 g of Trypticase soy broth per liter and kanamycin. Integrants of *E. coli* MBX245 were selected on minimal E_2 plates supplemented with 10 mM octanoate, 0.5% corn steep liquor, kanamycin, and nalidixic acid. After replica plating of resulting colonies onto DNase agar plates and subsequent incubation, colonies exhibiting zones of clearing were selected.

DNA sequencing. Transposon insertion sites were determined from genomic fragments that contain the *nuc-kan* operon and adjacent chromosomal DNA. Chromosomal DNA was digested to completion with *Eco*RI and ligated into the corresponding site of pUC19 (36). After transformation of the ligation mixture into *E. coli* DH5α, kanamycin-resistant mutants were selected and the insertion site was determined, using the oligonucleotide kan-up3 (5′-CGCACTTGTGTA TAAGAGTC) as a primer. This primer allows the determination of the nucleotide sequence directly downstream of the insertion locus. Automated DNA sequencing was performed at Boston University Medical Center (Boston, Mass.).

Detection of nuclease activity. Nuclease expression was routinely examined by observing the appearance of zones of clearing around colonies grown on DNase agar plates (25). For convenient estimation of nuclease activity, agarose gel electrophoresis with high-molecular-weight DNA was used as follows. PHA-producing strains were grown in their corresponding minimal media, and at various times were removed 500-μl samples, to each of which was added 100 μl of chloroform to release periplasmic nuclease. After centrifugation, 16 μl of the aqueous supernatant was mixed with 4 μg of *P. putida* KT2442 genomic DNA, and the mixture was incubated at 37°C for 1 h after $CaCl_2$ was added to 1 mM (6). DNA was subsequently separated by agarose gel electrophoresis (36), and nuclease activity was assessed by determining the decrease in the molecular weight of the genomic DNA.

PHA analysis. PHA-containing cells (5 to 20 mg) were subjected to hydrolysis in dichloroethane-propanol-HCl (5:4:1) for 2 h at 100°C (35). Resulting propyl esters of hydroxyalkanoic acids were analyzed by gas chromatography as described previously (22).

Viscosity assay. *Pseudomonas* sp. strains MBX978 and MBX985 were grown in 20-liter computer-controlled fermentors (Applicon, Schiedam, The Netherlands) on R medium with a dissolved oxygen-controlled (DO-stat) octanoate feed (a detailed report on the fermentation procedures will be reported elsewhere). At the end of the fermentation, cultures were supplemented with 1 mM $CaCl_2$ and lysed by passage through a microfluidizer (model M110EH; Microfluidics International Corp., Newton, Mass.) operating at pressures ranging from 8,000 to 20,000 lb/in^2. The homogenized cultures were incubated at room temperature for 1 h, and the viscosities of the lysates were determined at room temperature with an LVF viscometer (Brookfield, Stoughton, Mass.). In control experiments, a

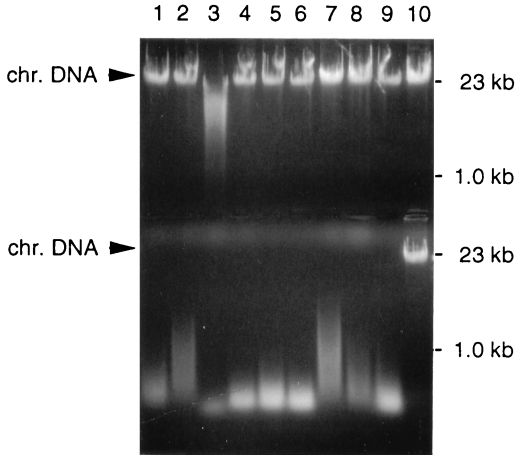

FIG. 1. Nuclease activity in nine *P. putida* nuclease integrants. *P. putida* KT2442 and derivatives with an integrated nuclease gene were grown in E_2–10 mM octanoate. Growth medium (top) and chloroform-permeabilized cell fractions representing the periplasm (bottom) were incubated with 4 μg of *P. putida* KT2442 genomic DNA at 37°C for 1 h. Lanes: 1, MBX926; 2, MBX925; 3, MBX924; 4, MBX923; 5, MBX922; 6, MBX921; 7, MBX920; 8, MBX919; 9, MBX918; 10, *P. putida* KT2442. chr. DNA, chromosomal DNA.

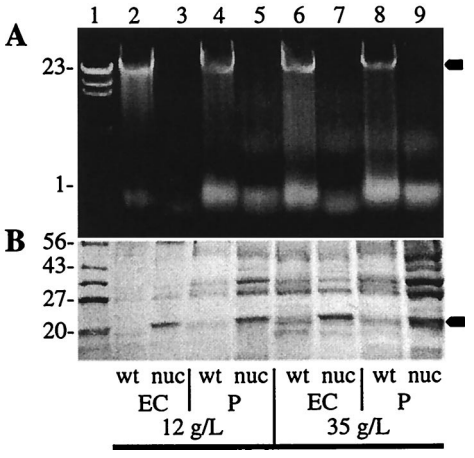

FIG. 2. Analysis of nuclease expression by *P. putida* KT2442 and *P. putida* MBX924 grown in a 20-liter fed-batch fermentation. Wild-type (wt) and nuclease-integrated (nuc) *Pseudomonas* strains were grown as described in the text, and at cell densities of 12 and 35 g/liter, samples were analyzed for nuclease activity (A) and protein (B) in the extracellular growth medium (EC) and periplasm (P). The arrows indicate chromosomal DNA (A) and the putative nuclease (B). Lane 1 contains molecular mass markers (either *Hin*dIII-digested λ DNA [A] or a combination of glutamic dehydrogenase [56 kDa], maltose binding protein [43 kDa], triosephosphate isomerase [27 kDa], and trypsin inhibitor [20 kDa] [B]).

commercial preparation of nuclease from *Serratia marcescens* (Benzonase; American International Chemical Inc.) was added.

SDS-PAGE. Cell extracts, obtained by sonicating cells from 50-ml cultures and resuspending them in 2 ml of lysis buffer (50 mM Tris-HCl [pH 8.0], 1 mM EDTA, 10 mM 2-mercaptoethanol, 5% glycerol), were subjected to sodium dodecyl sulfate (SDS)–12.5% polyacrylamide electrophoresis (PAGE) (Bio-Rad, Richmond, Calif.) as described elsewhere (36).

RESULTS

Nuclease integrants of *P. putida* KT2442. *P. putida* KT2442 strains that express staphylococcal nuclease were constructed by random integration of a *nuc-kan* cassette. Of 12,000 kanamycin-resistant integrants, 1,500 colonies were replica plated onto DNase agar plates to screen for the clones with the highest levels of nuclease expression. The presence of nuclease activity in both the extracellular medium and the periplasm of nine different isolates was subsequently determined (Fig. 1). Nuclease is primarily secreted into the periplasm, suggesting that the *P. putida* protein secretion apparatus recognizes the signal peptide of staphylococcal nuclease. Because the *nuc* gene in the transposon is not preceded by a promoter element, its expression is driven from promoter sequences located on the chromosome. This is expected to result in various levels of nuclease for different nuclease integrants, as was demonstrated by the low level of activity in MBX920 (lane 7), the high level of activity in MBX924 (lane 3), and intermediate levels of activity for the other strains. For *P. putida* MBX924, nuclease activity was detected in both the growth medium and the periplasm. The chromosomal DNA fragment containing the *nuc-kan* operon from *P. putida* MBX924 was cloned as a 4-kb *Eco*RI fragment into pUC19. The nucleotide sequence of the DNA downstream of the *nuc-kan* operon showed that the insertion was in a gene encoding a homolog of the 23S rRNA from *Pseudomonas aeruginosa* (96.4% identity over 362 nucleotides), suggesting that the corresponding promoter for this rRNA operon directs the transcription of *nuc* in MBX924. Unfortunately, a definite assignment of the *nuc* insertion site to this rRNA locus cannot be made because the complete sequence of the corresponding *P. putida* gene is unknown at this time.

P. putida MBX924 exhibited the highest level of nuclease activity among the *P. putida* integrants. This strain was therefore grown in a 20-liter fermentor to a high cell density to examine its growth behavior, stability, and nuclease secretion under fermentation conditions. Figure 2A shows the nuclease activity at two different cell densities, as observed by agarose gel electrophoresis, and again nuclease activity was present in both the periplasmic and extracellular fractions throughout the fermentation. Furthermore, by SDS-PAGE, a protein band corresponding to a molecular mass of 20 kDa was observed for both the periplasm and the growth medium of MBX924 at the different growth stages (Fig. 2B). The size of this protein corresponds well with the reported molecular mass of staphylococcal nuclease (7). In addition, this protein is not present in either the periplasm or the growth medium of the wild-type culture.

Construction of nuclease integrants of other PHA producers. By the use of similar methods, *nuc* was integrated into the chromosome of *Pseudomonas* sp. strain MBX978. For *Pseudomonas* sp. strain MBX978, 50 mutants were selected on DNase agar plates, of which 9 were grown in minimal E_2-octanoate medium and tested for relative nuclease activity levels by agarose gel electrophoresis. All nine clones secreted nuclease into the periplasm (results not shown).

Nuclease integrants for *R. eutropha* NCIMB40124HD and *E. coli* MBX245 were subsequently generated. *R. eutropha*, an exceptional PHB producer, was the strain of choice for commercial PHB production by ICI, Ltd. (5). *E. coli* generally does not synthesize PHAs but is regarded as a suitable host for improved recombinant PHA production processes (27). For *R. eutropha*, 10 random colonies were grown and tested for nuclease activity; only one (MBX917) exhibited nuclease activity. For *E. coli*, 75 colonies were screened; 4 mutants exhibited nuclease activity (data not shown). The transgenic nuclease-producing strains derived from both *E. coli* and *R. eutropha* secreted nuclease into the periplasm but not into the growth medium. Figure 3 shows a summary of the nuclease activities for the different transgenic nuclease-secreting species and their corresponding parent strains. These data indicate that trans-

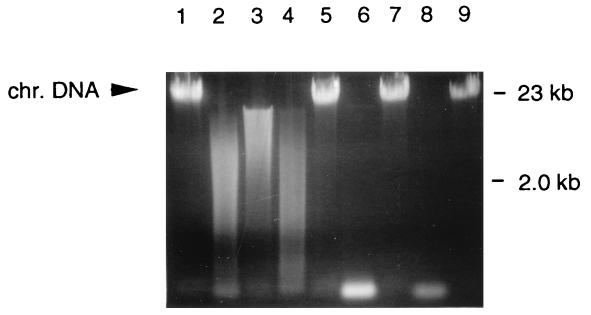

FIG. 3. Nuclease activity in wild-type and transgenic PHA producers with a chromosomally integrated nuclease gene. Chromosomal (chr.) DNA was treated with periplasmic fractions of *E. coli* MBX245 (lane 1), *E. coli* MBX988 (::*nuc-kan*) grown on R medium (lane 2) or Luria-Bertani medium (lane 3), *R. eutropha* MBX917 (::*nuc-kan*) (lane 4), *R. eutropha* NCIMB40124HD (lane 5), *Pseudomonas* sp. strain MBX985 (::*nuc-kan*) (lane 6), *Pseudomonas* sp. strain MBX978 (lane 7), *P. putida* MBX924 (::*nuc-kan*) (lane 8), or *P. putida* KT2442 (lane 9).

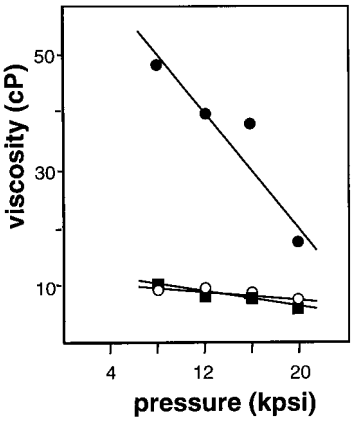

FIG. 4. Viscosities of cell lysates. Nuclease-expressing and wild-type *Pseudomonas* sp. strain MBX978 were grown in fed-batch mode to a density of approximately 60 g/liter. Cell suspensions were subsequently homogenized with or without the addition of Benzonase. The viscosity of the lysate was determined as a function of the operating pressure of the homogenizer. Closed circles, *Pseudomonas* sp. strain MBX978 without added Benzonase; open circles, *Pseudomonas* sp. strain MBX978 with added Benzonase; closed squares, nuclease integrant *Pseudomonas* sp. strain MBX985 without added Benzonase.

genic nuclease expression can be achieved by different PHA-producing strains and that it is a generally applicable procedure to prevent processing problems related to viscosity caused by chromosomal DNA.

Evaluation of nuclease integrants of *Pseudomonas* sp. strain MBX978 for PHA production. In order to successfully replace wild-type strains as PHA producers, the integrated strains should demonstrate the same stability and PHA productivity as the wild-type strain. All integrants derived from *Pseudomonas* sp. strain MBX978 were tested for PHA content, growth rate, and relative nuclease activity in E_2–10 mM octanoate medium before being analyzed in a large-scale fermentor for PHA production and processing. Table 2 lists the characteristic growth rates and PHA contents of these strains. Most of these nuclease integrants exhibited the same growth rate as the parental strain. Except for *Pseudomonas* sp. strain MBX984, the PHA contents and the compositions of the accumulated PHAs were similar. However, similar to what was observed for the nuclease integrants derived from *P. putida* KT2442 (Fig. 1), the nuclease levels in these strains differed. Because *Pseudomonas* sp. strain MBX985 exhibits a relatively high level of nuclease activity and growth characteristics similar to those of the wild-type strain, it was further evaluated for its ability to improve the PHA extraction process in a 20-liter fermentation-downstream processing protocol.

TABLE 2. PHA production by nuclease integrants of *Pseudomonas* sp. strain MBX978 in minimal E_2 medium with 10 mM octanoate as the carbon source

Strain	Nuclease activity[a]	A_{600}[b]	t_d[c] (h)	% PHA[d]		
				C_6	C_8	Total
MBX978	ND	3.7	1.5	4.2	29.2	33.4
MBX979	+++	3.6	1.6	3.8	26.0	29.8
MBX981	+	3.4	1.4	4.0	27.9	31.9
MBX982	+	3.5	1.6	4.0	27.1	31.1
MBX984	++	2.8	1.6	1.5	12.2	13.8
MBX985	++	3.8	1.4	4.1	28.3	32.4

[a] Relative activity was estimated by chromosomal DNA digestion followed by agarose gel electrophoresis as described in Materials and Methods. The sizes of resulting DNA fragments are noted as follows: 200 to 300 bp, +++; 400 to 1,000 bp, ++; 500 to 4,000 bp, +; or no degradation, ND.

[b] Optical density at 600 nm after 24 h of growth, at which point cells were harvested for PHA analysis.

[c] Doubling time (± 0.1 h).

[d] Accumulated levels of 3-hydroxyhexanoate (C_6), 3-hydroxyoctanoate (C_8), and total PHA as percentages of cell dry weight (total PHA is ±5%).

Pseudomonas sp. strain MBX978 and nuclease integrant *Pseudomonas* sp. strain MBX985 were grown in a 20-l fermentor operated in a fed-batch mode. Both fermentations reached a cell density of approximately 60 g/liter. The cells were homogenized, as described in Materials and Methods, to release the PHA. For the parent strain, homogenization took place in the presence or absence of Benzonase. The viscosities of the lysates as a function of the pressure used for homogenization are shown in Fig. 4. Already at the lowest homogenization pressure (8,000 lb/in^2), the viscosity of the *Pseudomonas* sp. strain MBX985 lysate was reduced to a level similar to that of the parent culture to which Benzonase had been added. Subsequent steps in the purification process of the PHA granules include centrifugation and/or filtration steps that involve chemical treatments with oxidizing agents and detergents (9, 39) that decrease the residual nuclease activity. The final nitrogen content of the PHA latex was less than 0.4%, as determined by the Kjeldahl method. These results confirm that the integrated nuclease gene of *Pseudomonas* sp. strain MBX985 was functionally expressed in high-cell-density fermentations and that the use of this strain eliminates the need to add nuclease preparations to reduce the viscosity of the cell lysate.

DISCUSSION

The nuclease gene from *S. aureus* was integrated into the genomes of several well-known PHA producers. Most pseudomonads from rRNA homology group I produce PHAs composed of medium-side-chain 3-hydroxy fatty acids when grown on fatty acids or on carbohydrates (15, 19, 20, 40). *R. eutropha* is a well-known producer of PHB and related short-side-chain PHAs (5); *E. coli* is a bacterium that is considered to be a potential recombinant PHA producer, and both short- and medium-side-chain PHAs are produced by this organism (23, 33, 42, 43). Here the effect of nuclease expression on ease of downstream processing was demonstrated for a *Pseudomonas* sp. strain MBX978 derivative, and nuclease-expressing strains were also derived from *P. putida* KT2442, *R. eutropha* NCIMB40124HD, and *E. coli* MBX245.

The production cost of a fermentation product depends to a large extent on the combined costs of fermentation and downstream processing (8). While molecular genetics has frequently been applied to improve the productivity (10, 11, 29) and fitness (17, 44) of a microorganism, its application to downstream processing is rather uncommon. In the aqueous processing of PHA-containing cells, it is necessary to lyse the cells, and this process is accompanied by a dramatic increase in sample viscosity, due to chromosomal DNA, that reduces the efficiency of the subsequent centrifugation, filtration, and washing steps (3). The PHA-producing strains that are described here express an endogenous nuclease whose use leads to significant cost savings. Such strains are useful in processes besides the production of PHAs, since many industrial fermentation processes deal with similar viscosity problems. The production of high-value pharmaceutical proteins frequently involves isolation of inclusion bodies, which should be essentially free of nucleic acids to allow efficient purification and formulation (14). Furthermore, we can expect a dramatic increase in the use of recombinant organisms for the production of chemicals, proteins, and polymers, and it is desirable to prevent proliferation of heterologous genes by inclusion of a procedure for DNA degradation.

The improvement of PHA-accumulating microorganisms for PHA production by integration of a nuclease-encoding gene is potentially of great economic value. Although future PHA production is envisioned to be a major agricultural process (30, 32, 45), the use of fermentation systems for the production of PHA latex as well as specialty PHAs will continue. Such specialty PHAs may contain monomers that are strictly derived from the feedstock and contain, for instance, aromatic, halogenated, or unsaturated functionalities (1, 12, 13, 21, 38), while the PHA latex can be used for making PHA films with potential applications for paper and food coating (28). The developments described here are therefore expected to contribute to the efficiency of future PHA production facilities.

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