Oil Bodies of Transgenic *Brassica napus* as a Source of Immobilized β-Glucuronidase

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ABSTRACT: The process of immobilizing enzymes is a major cost factor in the utilization of heterogeneous catalysts on an industrial scale. We have developed a new strategy, based on plant genetic manipulation, for the production of foreign peptides associated with the oil body in plant seeds. Seeds of transgenic rapeseed can be produced on a large scale at relatively low cost. Furthermore, oil bodies are readily isolated from seeds by floatation centrifugation. In this paper, we describe some physical and operational properties of an oil body—fusion protein complex and its suitability as a heterogeneous catalyst. Oil bodies from rapeseed, corn, and flax aggregate at pH 5, which facilitates their recovery by floatation. Oil bodies from transgenic rapeseed, carrying the reporter gene β-glucuronidase or the pharmaceutical peptide, hirudin, also aggregate in the same range. This aggregation is reversible. Oil bodies are resistant to a wide range of pH, with some lysis occurring (<10%) at the extremes. They are resistant to shearing forces, such as stirring. The thermal and pH stabilities, as well as the catalytic activity of β-glucuronidase expressed on the oil body surface, are comparable to those of free β-glucuronidase enzyme.
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KEY WORDS: β-Glucuronidase, immobilized enzyme, oil body, oleosin, transgenic plants.

Immobilized enzyme technology has applications in clinical, pharmaceutical, chemical, and food industries (1). Economic factors, such as the cost of enzymes, immobilization, capital investment and cleanup, as well as overall system performance, are determinants in utilization of immobilized enzymes (2). Conventional immobilization of enzymes employs a solid support matrix, such as acrylamide, cellulose and alginate, onto which the enzyme is attached by adsorption, entrapment, or covalent binding.

In recent years, rapid progress has been made in the area of plant genetic engineering. This was made possible by the development of plant transformation techniques by a variety of biological (*Agrobacterium*-mediated) and physical (e.g., biolistics, polyethylene glycol-mediated) methods. This research has made it possible to investigate the use of transgenic plants for large-scale production of foreign peptides and proteins (3–7). Farming costs associated with growing and harvesting of plants are relatively small compared to the scale-up costs associated with microbial, fungal, or mammalian cell cultures. Plant-based production systems, therefore, provide an attractive alternative for the production of enzymes or peptides.

Recently, our laboratory has developed a novel strategy for the production and purification of foreign proteins and peptides in seeds (8,9). This strategy involves the fusion of the protein of interest to oleosin. Oleosins are structural proteins that are tightly associated with the oil body, the natural oil storage organelle of the plant seed (10). The amino and carboxy termini of the oleosins are exposed on the surface of the oil body (9,10), while the highly conserved hydrophobic central domain is proposed to be embedded into the oil body matrix, securely anchoring the oleosin molecule to the oil body (Fig. 1). Oil bodies from a variety of oilseeds, including *Brassica napus*, are 0.5 to 2.5 μm in diameter (10).

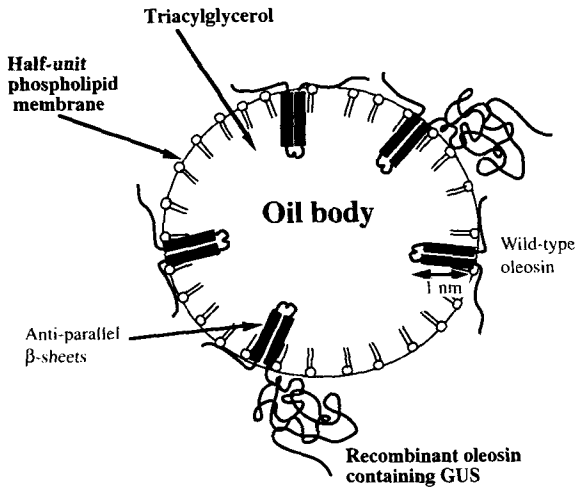

FIG. 1. Schematic representation of a transgenic oil body, based on the models of Huang (10) and van Rooijen and Moloney (8). The reporter gene β-glucuronidase (GUS) is expressed on the oil body surface as an oleosin—GUS fusion protein. *Brassica napus* oil bodies are 0.5–2.5 μm in diameter. The molecules are not drawn to scale.

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Oil bodies, and proteins associated with them, can be easily separated from the majority of other seed cell components by floatation centrifugation, which facilitates the purification of the desired protein. Our previous studies demonstrated that foreign proteins that are expressed as oleosin fusion proteins in transgenic plants of *B. napus* are correctly targeted into the oil body and remain on the oil body surface (8). When the foreign protein is fused to the oleosin *via* a linker that comprises a protease recognition site, this system allows for facilitated purification of the produced protein. Using this strategy, we have been able to produce and purify the blood anticoagulant hirudin and demonstrate its biological activity (9). Alternatively, if the protein is biologically active after fusion, the oil body complex can potentially be used as a heterogeneous catalyst. This approach completely bypasses the process of immobilization, which now takes place *in vivo.*

We have previously generated transgenic plants that express an enzymatically active oleosin-β-glucuronidase (GUS) fusion protein on the oil body surface (8). The bacterial enzyme GUS catalyzes the cleavage of a wide variety of β-glucuronides. Its absence in higher plants and its specific and sensitive activity assay render it an ideal reporter enzyme in plants. Here, we describe some of the physical properties of the oil body and the kinetic properties of the GUS enzyme attached to it by following the Guidelines for the Characterization of Immobilized Biocatalysts (11).

MATERIALS AND METHODS

Transgenic Brassica napus *(rapeseed) with an oleosin-β-glucuronidase gene.* Vector construction, introduction of the vector in *Agrobacterium,* plant transformation, and regeneration were previously described in van Rooijen and Moloney (12). The transgenic seeds used in this study are derived from a single plant transformed with pCGYOBPGUSD, called D10, selected for its high expression of GUS associated with the oil bodies. A schematic representation of an oil body that contains endogenous oleosin and oleosin-β-glucuronidase protein is shown in Figure 1.

Isolation of oil bodies. Oil bodies were isolated by floatation centrifugation as previously described (8). Dry, mature seeds were ground in Tricine buffer B (0.15 M Tricine-KOH pH 7.5, 10 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 0.6 M sucrose) with mortar and pestle for 5 min. The homogenate was filtered through a layer of miracloth and centrifuged at 16,250 × *g* for 15 min. The fat pad containing the oil bodies was removed, resuspended in fresh Tricine buffer B, overlaid with an equal volume of Tricine buffer A (0.15 M Tricine-KOH pH 7.5, 10 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 0.1 M sucrose) and centrifuged at 16,250 × *g* for 15 min. This procedure was repeated twice. Purified oil bodies were resuspended in Tricine buffer A for storage or in double distilled water for pH experiments.

Fractionation of oil body preparations. One mL of oil body suspension was centrifuged in a TLS-55 swinging bucket rotor in the TL-100 tabletop ultracentrifuge (Beckman, Mississauga, Ontario, Canada) at 100,000 × *g* for 1 h at 4°C. The fat pad containing the oil bodies was removed with a spatula and resuspended in 500 µL Tricine buffer A. The supernatant was transferred into a fresh tube. The tube containing the pellet was drained, and the pellet was resuspended in 100 µL Tricine buffer A. GUS activity was standardized to reflect the total activity of the fraction.

Absorbance measurements. Optical density at 650 nm (OD_{650}) was measured as follows: 5 to 10 µL oil body suspension in water was added to 1.5 mL buffer of a specific pH in a 1.5-mL cuvette. The cuvette was covered with parafilm, inverted three times, and absorbance was measured at 650 nm to yield t_0 value. The cuvettes were left for 18 h at room temperature with minimal disturbance and measured again for t_{18} value. Relative OD_{650} was calculated as (OD_{650} at t_{18}/OD_{650} at t_0) × 100%. The buffers used were KCl/HCl (pH 1.4–2.4), citric acid/Na_2HPO_4 (pH 2.8–7.6), and boric acid/NaOH (pH 8–10) (13). For the inversion experiments, described in the section *Effects of pH on oil body aggregation,* oil bodies were incubated in citric acid/Na_2HPO_4 buffers of pH2.6, 5.1, and 7.9, respectively, in a 50-mL flask. At $t = 0$, 1.5 mL of the suspension was taken for OD measurements in a 1.5-mL cuvette. After 18 h, the OD of the suspension in the cuvettes was measured again with minimal disturbance. The flasks were agitated, and the pH of the flask at 5.1 was decreased with concentrated HCl or increased with concentrated NaOH. Again, absorbance of 1.5-mL aliquots was measured at 650 nm at time 0 and 18 h.

Expression of bacterial β-glucuronidase. The GUS gene was cloned into the bacterial expression vector pKK233-2 (Clontech, Palo Alto, CA) and transformed into *Escherichia coli* strain DH5α. Bacteria carrying this plasmid were grown o/n in LB media with ampicillin (50 µg/mL). The cells were pelleted by centrifugation, washed with STE (100 mM NaCl, 10 mM Tris.Cl pH 7.5, 1 mM EDTA), and lysed with lysozyme (4 mg/mL) in GUS extraction buffer (50 mM sodium phosphate pH 7.0, 10 mM DTT, 1 mM EDTA, 0.1% sarkosyl, 0.1% Triton X-100) (14) at 37°C. After centrifugation, the nonviscous top layer was removed and used as a source of "free" enzyme.

GUS assay. The GUS assays were performed in GUS extraction buffer with 1 mM MUG (4-methyl umbelliferyl glucuronide; Jersey Lab Supply, Livingston, NJ) as described by Jefferson (14). The GUS enzyme catalyses the conversion of MUG to MU (4-methyl-umbelliferone). The accumulation of MU was measured on a Hitachi F-2000 fluorescence spectrophotometer (Tokyo, Japan) with excitation wavelength at 365 nm and emission at 455 nm.

Determination of K_m *and* V_{max} *of free and immobilized β-glucuronidase.* Dilutions of "free" GUS, produced in *E. coli,* and oil body-bound GUS, produced in *Brassica* seeds, were made to generate maximum fluorescence reading at 3-h incubation and 1 mM substrate. Reactions (500 µL) with increasing MUG concentrations were incubated at 37°C; 100-µL aliquots were taken at 5, 30, 90, and 180 min. To obtain the Lineweaver-Burk plot, slope values of generated trendlines

were plotted against substrate concentrations in reciprocals. K_m and V_{max} values of the reactions were calculated from the x and y intercepts of the plots, respectively.

RESULTS

Effect of pH on oil body aggregation. Oil bodies can be isolated by floatation centrifugation at relatively low g forces (15). Even though this separation is sufficient, complete recovery of the oil body fraction and ease of handling is hampered by easy disturbance of the fat pad. Oil bodies are negatively charged at neutral pH due to the negative and positive charges of the phospholipids and proteins, respectively (16). A decrease of the pH to 6.7 results in the loss of the negative charge and permits aggregation of the oil bodies. Further decrease of the pH results in the acquisition of a positive charge and a consequent loss of aggregation (16,17). The relative degree of aggregation can be monitored by OD_{650}. When oil bodies aggregate, they reach a floatation equilibrium faster than nonaggregated oil bodies. OD of the liquid in the lower portion of the cuvette, after 18 h with minimal disturbance, is inversely proportional to the degree of aggregation. Figure 2 shows the pH range at which oil bodies from various sources aggregate. Oil bodies from recombinant seeds, carrying oleosin—GUS fusion protein or oleosin—hirudin (9), aggregate in the same pH range as wild-type oil bodies, with a maximum aggregation at pH 5 (Fig. 2A). This suggests that the attached peptide at this level of expression does not influence the overall charge of the oil body. Also, wild-type oil bodies from corn and flax aggregate in the same pH range (Fig. 2B).

To reuse the oil bodies as a source of immobilized enzyme, disaggregation may be necessary. Results in Table 1 show that aggregation is in part reversible. Increase or decrease of the pH after initial aggregation at pH 5.1 resulted in dispersal of the aggregates and a corresponding increase of OD_{650}. This is in agreement with earlier reports (16,17).

Effect of pH on oil body stability. To assess the degree of damage to the oil bodies by the various pH treatments, oil bodies carrying the reporter gene GUS as an oleosin fusion were used. Oil bodies are surrounded by a half-unit membrane (18). Rupture of the membrane results in the release of the oil and an oil body "ghost", which can be separated from the intact oil body by high-speed centrifugation. At 100,000 × *g*, membranes from ruptured oil bodies may be pelleted, while intact oil bodies form a fat pad. GUS enzyme activity was assayed in the pellet, supernatant, and oil body fractions after the preparations were incubated at pH 2.6, 5.8, 7.8, and 8.4 for 18 h (Table 2): 2.3 and 7.2% of GUS activity was found in the pellet fraction after pH 7.8 and 8.4 treatment, respectively. GUS activity remained entirely associated with the oil body fraction after pH 5.8 treatment, suggesting that oil bodies are indeed less stable at higher pH values. Due to inactivation of GUS by pH 2.7, we were unable to measure the effect of this pH on the stability of the oil body. However, the presence of large membrane pellets in both pH 2.7 and pH 8.4-treated samples suggests that oil body lysis also occurs at pH 2.7 (Table 2).

The effect of stirring on oil body integrity. Resistance of the immobilized complex to shearing forces is an important parameter in the application of the system on an industrial

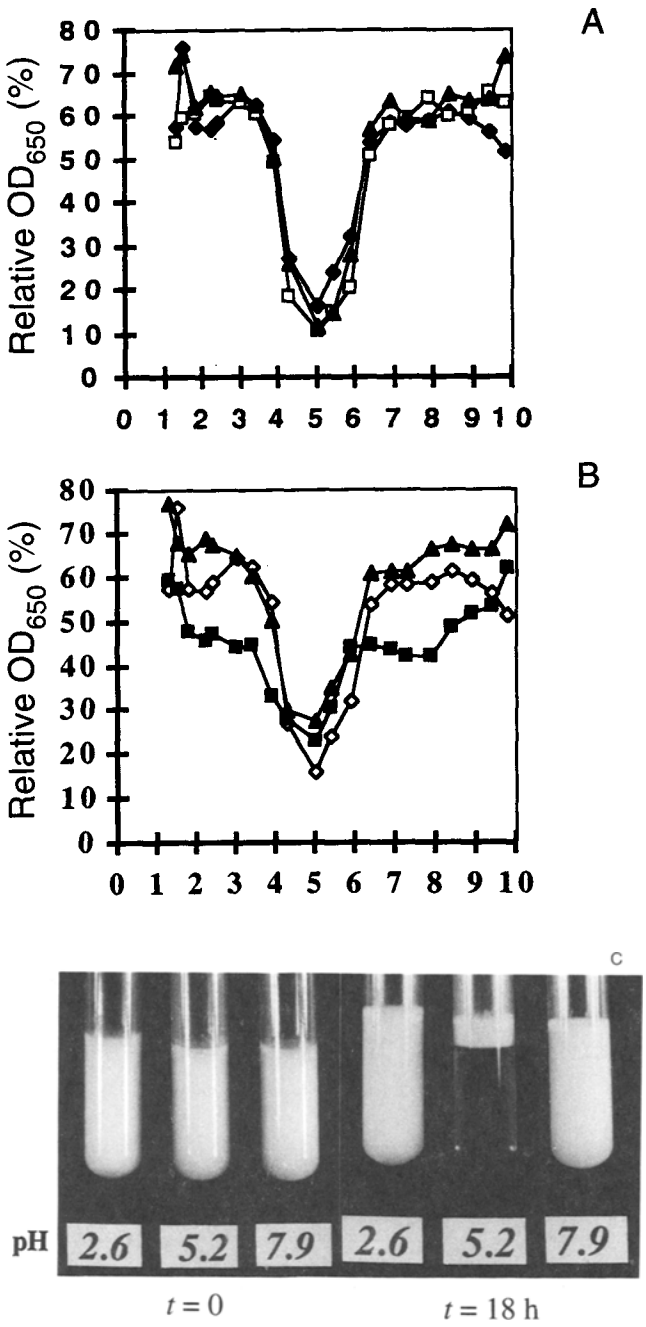

FIG. 2. Aggregation of oil bodies at varying pH values. Five to 10 µL of oil body suspensions in water were added to 1.5-mL pH buffer in a 1.5-mL cuvette. The buffers were HCl/KCl for pH 1.4 to 2.4, citric acid/Na_2HPO_4 for pH 2.8 to 7.6 and boric acid/NaOH for pH 8 to 10. Optical density (OD_{650}) was measured at time 0 and at 18 h. Relative OD_{650} was calculated as (OD_{650} at t_{18}/OD_{650} at t_0) × 100%. (A) Wild-type *Brassica napus* (rapeseed) and two transgenic lines, expressing hirudin and GUS, respectively; ◆, wild-type; □, GUS; ▲, hirudin. (B) wild-type rapeseed, corn, and flax oil bodies ◇, rapeseed; ■, flax; ▲, corn. (C) A high concentration of rapeseed oil bodies was added to citric acid/Na_2HPO_4 buffer of the indicated pH. Pictures were taken at time 0 and at 18 h.

TABLE 1
Inversion of Aggregation of Oil Bodies of *Brassica napus* at Various pH[a]

pH	Relative OD_{650} (%)			
	t_0	t_{18}	t'_0	t'_{18}
2.6	100	57	100	68
5.1	100	9	100	21[b] / 7 / 23[c]
7.9	100	54	100	63

[a]Wild-type rapeseed oil bodies were incubated in citric acid/Na_2HPO_4 buffers of pH 2.6, 5.1, and 7.9, respectively, in a 50-mL flask. At time t_0, 1.5 mL of the suspension was taken for optical density (OD) measurements in a 1.5-mL cuvette. After 18 h, the cuvettes were measured again with minimal disturbance (t_{18}). The flasks were then agitated, and the pH of the flask at 5.1, at which aggregation is maximal, was decreased with concentrated HCl to 2.6[b] or increased with concentrated NaOH to 7.9[c]. Absorbance of 1.5-mL aliquots was measured at 650 nm at time 0 (t'_0) and at 18 h (t'_{18}). Absorbance relative to t_0 and t'_0 is given.

scale. A suspension with a high oil body concentration was stirred on a magnetic stirrer at 4°C for 5 d at approximately 300 rpm. One-mL aliquots were taken at time intervals and kept at 4°C until the end of the experiment. Damage to the oil bodies was determined by assaying GUS activity in oil body, supernatant, and pellet fractions after 100,000 × *g* centrifugation. Throughout the experiment, GUS activity remained associated with the oil body fraction and at a constant level, with 0.1% of the activity in the pellet fraction. Damage to the GUS enzyme and the oil body did occur when the stirred volume of the suspension became so low that the magnetic stirrer was exposed above the liquid surface. After 5 d of stirring, total GUS activity decreased to 37% of the initial activity, with approximately half of the activity in the pellet fraction.

Properties of the oil body-associated GUS enzyme. Immobilization of an enzyme by conventional methods often results in altered properties of the enzyme (19,20). We determined the thermal and pH stabilities (Table 3) as well as the catalytic activity (Fig. 3) of oil body-immobilized GUS. Free and immobilized GUS samples were incubated for 2 h at various temperatures or pH buffers prior to the GUS assay. The assay was performed at 37°C and pH 7.5. As shown in Table 3, free and oil body-associated GUS exhibit comparable sensitivity to temperature and pH.

Catalytic parameters were determined by kinetic experiments, shown in Figure 3, interpreted in a Lineweaver-Burk plot. Free and oil body-associated GUS enzyme showed similar K_m of 272 and 276 μM, respectively, and V_{max}, 0.595 and 0.552 μM · min^{-1}. The size and density of the oil bodies at pH 7.5, which are the reaction conditions, are such that no significant partitioning occurred during the reaction.

DISCUSSION

Our previous studies demonstrated that foreign peptides expressed as oleosin fusion peptides in transgenic plants are cor-

TABLE 2
Relative GUS Activity and Protein Content in Fractions After pH Treatment and Fractionation Centrifugation[a]

pH	GUS activity				Protein[b]
	Total (%)	Oil body (%)	Supernatant (%)	Pellet (%)	Pellet (%)
2.7	0	0	0	0	1.6
5.8	100	94	n.d.[c]	0.1	0.3
7.8	100	85	1	2.3	1.5
8.4	100	67	4.3	7.2	2

[a]One mL of oil body suspension was centrifuged in a TLS-55 swinging bucket rotor in the TL-100 tabletop ultracentrifuge (Beckman) at 100,000 × *g* for 1 h at 4°C. The fat pad, containing intact oil bodies, was removed with a spatula and resuspended in 500 μL Tricine buffer A, with some loss of oil bodies occurring at this step. The supernatant was transferred into a fresh tube. The tube containing the pellet was drained, and the pellet was resuspended in 100 μL Tricine buffer A. The GUS activity was standardized to reflect the total activity of the fractions.
[b]Percent protein relative to total protein in oil body preparation prior to fractionation.
[c]Not determined.

TABLE 3
Effect of Temperature and pH on Free and Oil Body-Immobilized GUS[a]

Temp. (°C)	Free GUS (%)	Imm. GUS (%)	pH	Free GUS (%)	Imm. GUS (%)
4	100	100	4	0.4	0.9
20	98	94	5	17	15
28	91	91	7	100	100
37	92	88	8	108	94
45	96	75	9	69	64
55	58	52			
65	3	2			

[a]Samples were incubated at the indicated conditions for 2 h prior to the GUS assay. The assay was performed under standard conditions at 37°C and pH 7.5. Activity at 4°C and pH 7 was set at 100%.

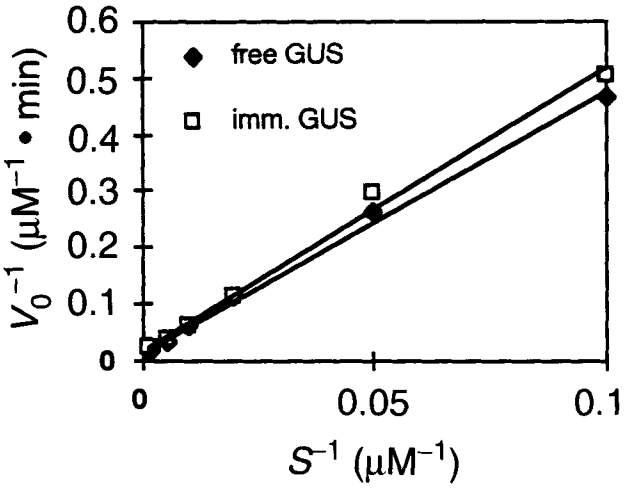

FIG. 3. Lineweaver-Burk plot of free and oil body-associated β-glucuronidase. K_m (272 and 276 μM) and V_{max} (0.595 and 0.552 μM . min^{-1}) values were calculated from the x- and y-intercepts of the trendline, respectively.

rectly targeted and expressed on the surface of the oil body (8,9). The scale and costs at which *B. napus* seeds are produced and the ease of oil body isolation make this an attractive system for the production of heterologous proteins, including enzymes. This paper describes properties of the oil body and its potential use as a natural immobilization matrix.

It is interesting that the addition of foreign polypeptides, such as GUS and hirudin, does not affect the pH prange of aggregation of the oil bodies. The predicted isoelectric point (pI) values of those peptides are 5.6 and 3.9, respectively. One possibility is that the ratio of the peptide the the rest of the oil body molecules is small and its charge contribution negligible. Alternatively, changes in the oil body composition, such as the free fatty acids (16), may compensate for the change of charge, to maintain a physiologically optimal pI. Despite divergence of C- and N-terminal oleosin sequences between species, flax and corn oil bodies also aggregate in the same pH range. This also suggests that a selective mechanism may exist to maintain an optimal pI of the oil body. Oil bodies are sensitive to low and high pH. They are resistant to the physical forces of stirring.

In the fusion protein, the foreign peptide is attached to the oil body by a peptide bond of the first amino acid. Covalent binding is the most secure method of immobilization. Conventional immobilization by cross-linking often results in partial loss of enzyme activity (19). Furthermore, the chemicals used for the cross-linking are often toxic, which limits its application in food processing (2). Our kinetic studies show that attachment of GUS to the oil body surface has no effect on the enzyme's apparent K_m nor its thermal and pH stability. It seems that, in this configuration, the enzyme is not affected by steric hindrance of the oil body.

We have previously shown that oil bodies expressing GUS can be recovered by centrifugation and reused as a heterogeneous catalyst (8). In addition, after the enzyme associated with the oil body reaches an activity that is no longer suitable for production, it is conceivable that the oil can be extracted from the oil bodies and used for industrial applications. Currently, we are investigating the wider applicability of the system to other enzymes with more immediate industrial relevance. These include xylanases, glycanases, and a variety of site-specific proteolytic enzymes.

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REFERENCES

1. Katchalski-Katzir, E., Immobilized Enzymes—Learning from Past Successes and Failures, *TIBTECH 11*:471–478 (1993).
2. Swaisgood, H.E., Immobilization of Enzymes and Some Applications in the Food Industry, in *Enzymes and Immobilized Cells in Biotechnology*, edited by Allen I. Laskin, The Benjamin/Cummings Publishing Company, Inc., 1985, pp. 1–24.
3. Vandekeckhove, J., J. Damme, M.V. Lijsebettens, J. Botterman, M. de Block, M. Vandewiele, A. De Clercq, J. Leemans, M. van Montagu, and E. Krebbers, Enkephalins Produced in Transgenic Plants Using Modified 2S Seed Storage Proteins, *Bio/Technol. 7*:929–932 (1989).
4. Sijmons, P.C., B.M.M. Dekker, B. Schrammeijer, T.C. Verwoerd, P.J.M. van den Elzen, A. Hoekema, M.J. Huisman, and L. Molendijk, Production of Correctly Processed Human Serum Albumin in Transgenic Plants, *Ibid. 8*:217–221 (1990).
5. Pen, J., A.J.J. van Ooyen, P.J.M. van den Elzen, K. Rietveld, and A. Hoekema, Direct Screening for High-Level Expression of an Introduced Alpha-Amylase Gene, *Plant Mol. Biol. 18*:1133—1139 (1992).
6. Pen, J., T.C. Verwoerd, P.A. van Paridon, R.F. Beudeker, P.J.M. van den Elzen, K. Geerse, J.D. van der Klis, H.A.J. Versteegh, A.J.J. van Ooyen, and A. Hoekema, Phytase-Containing Transgenic Seeds as a Novel Feed Additive for Improved Phosphorus Utilization, *Bio/Technol. 11*:811–814 (1993).
7. Moloney, M.M., "Molecular Farming" in Plants: Achievements and Prospects, *Biotechnol. and Biotechnol. Eq. 9*:3–9 (1995).
8. van Rooijen, G.J.H., and M.M. Moloney, Plant Seed Oil Bodies as Carriers for Foreign Proteins, *Bio/Technol. 13*:72–77 (1995).
9. Parmenter, D.L., J.G. Boothe, G.J.H. van Rooijen, E.C. Yeung, and M.M. Moloney, Production of Biologically Active Hirudin in Plant Seeds Using Oleosin Partitioning Technology, *Plant Mol. Biol. 29*:1167–1180 (1995).
10. Huang, A.H.C., Oil Bodies and Oleosins in Seeds, *Ann. Rev. Plant Physiol. and Plant Mol. Biol. 43*:177–200 (1992).
11. Buchholtz, K., and J. Klein, Characterization of Immobilized Biocatalysts, *Meth. Enzymol. 135 (part B)*:3–30 (1987).
12. van Rooijen, G.J.H., and M.M. Moloney, Structural Requirements of Oleosin Domains for Subcellular Targeting to the Oil Body, *Plant Physiol. 109*:1353–1361 (1995).
13. McKenzie, H.A., and R.M.C. Dawson, pH, Buffers and Physiological Media, in *Data for Biochemical Research*, edited by R.M.C. Dawson, D.C. Elliott, W.H. Elliott, and K.M. Jones, Oxford University Press, 2nd edn, 1969, pp. 475–508.
14. Jefferson, R.A., Assaying Chimeric Genes in Plants: The GUS Gene Fusion System, *Plant Mol. Biol. Rep. 5*:387–405 (1987).
15. Holbrook, L.A., G.J.H. van Rooijen, R.W. Wilen, and M.M. Moloney, Oil Body Proteins in Microspore-Derived Embryos of *Brassica napus, Plant Physiol. 97*:1051–1058 (1991).

16. Tzen, J.T.C., G.C. Lee, and A.H.C. Huang, Characterization of the Charged Components and Their Topology on the Surface of Plant Seed Oil Bodies, *J. Biol. Chem. 267*:15626–15634 (1992).
17. Slack, C.R., W.S. Bertaud, B.D. Shaw, R. Holland, J. Browse, and H. Wright, Some Studies on the Composition and Surface Properties of Oil Bodies from the Seed Cotyledons of Safflower (*Carthamus tinctorius*) and Linseed (*Linum ustatissimum*), *Biochem. J. 190*:551–561 (1980).
18. Yatsu, I.Y., and T.J. Jacks, Spherosome Membranes; Half Unit Membranes, *Plant Physiol. 49*:937–943 (1972).
19. Hu, Z.-C., R.A. Korus, and K.E. Stormo, Characterization of Immobilized Enzymes in Polyurethane Foams in a Dynamic Bed Reactor, *Appl. Microbiol. Biotechnol. 39*:289–295 (1993).
20. Heichal-Segal, O., S. Rappoport, and S. Braun, Immobilization in Alginate Silicate Sol-Gel Matrix Protects Beta-Glucosidase Against Thermal and Chemical Denaturation, *Bio/Technol. 13*:798–800 (1995).

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