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## *Mycobacterium smegmatis* acyltransferase catalyzes the synthesis of esters and polyesters†

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In the present work, *Mycobacterium smegmatis* acyltransferase (MsAcT) was successfully immobilized onto polypropylene beads and the selectivity was investigated both in esterification and polycondensation reactions. All the syntheses were carried out under solventless conditions at room temperature to better comply with today's green chemistry principles. Therefore, ester synthesis was performed according to the planned full-factorial design of experiments (DoE) investigation to study MsAcT selectivity towards ester functional groups (vinyl, ethyl, and methyl), alcohol carbon chain length (C<sub>4</sub>, C<sub>8</sub>, C<sub>12</sub>) and ester carbon chain length (C<sub>2</sub>, C<sub>4</sub>, C<sub>6</sub>). The results clearly showed MsAcT selectivity towards vinyl esters (vinyl- > ethyl- > methyl-) and short-chain compounds (C<sub>2</sub> esters and C<sub>4</sub> alcohol). Moreover, to confirm the obtained DoE model in ester synthesis, the immobilized MsAcT formulation was used to perform polycondensation reactions using bio-based diesters and diols. Higher conversion rates were obtained using ethylene glycol when compared to 1,4-butanediol in polyester synthesis with divinyl adipate as the diesters and at different time-lengths of applied vacuum (20 mbar). This resulted in agreement with the selectivity of MsAcT in esterification reactions. Furthermore, divinyl succinate (DVS) was used in polycondensations and all the resulting oligomers were analyzed *via* GPC and LC-MS.

## 1 Introduction

Since the very first *in vitro* enzymatically catalyzed polyester synthesis carried out originally in 1984 by Okumura *et al.*<sup>1</sup> and then in 1993 by two independent groups,<sup>2,3</sup> lipases, and in

### Sustainability spotlight

The need for greener sustainable technologies to produce both small molecules and polymers highlighted once again how biocatalysts can play a pivotal role in humanity's fight against climate change. In this work, the acyltransferase from *Mycobacterium smegmatis* was for the first time used to prepare short esters (that could be used as additives or flavoring ingredients) and short oligoesters from biomass-derived monomers using a solventless synthetic approach. The work relates to the UN sustainability goal numbers 4, 9, 12 and 13.

general  $\alpha/\beta$  hydrolases, have always been representing the most sought-after choice for polycondensation and ring-opening polymerization (ROP) reactions.<sup>4</sup> Furthermore, the same superfamily of enzymes, which includes proteases and esterases, have been widely investigated in the past decades and found exceptional for the synthesis of different short-esters and flavor esters.<sup>5-7</sup>

Recently, a new and promising acyltransferase from *Mycobacterium smegmatis* (MsAcT) has been fully characterized,<sup>8</sup> attracting a lot of interest for its outstanding activity in an extensive range of reactions, shedding light on the possibility of becoming the next big player in the biotransformation scenario.<sup>9</sup>

This enzyme possesses a catalytic triad (Ser11, Asp192 and His195) common to the  $\alpha/\beta$  hydrolase superfamily, but has unusual architecture that allows its two-step catalytic mechanism to take place in water. In fact, MsAcT, unlike many cofactor-dependent acyltransferases, is able to hydrolyze both the acyl donor and the final product therefore favoring the condensation reaction over the hydrolysis.<sup>10</sup>

Many researchers have exploited this extraordinary feature exploring methods to synthesize short esters,<sup>11</sup> *N*-acylation of amines and *trans*-amidation reaction<sup>12</sup> utilizing both batch<sup>11,13</sup> and flow systems.<sup>14</sup> Moreover, several immobilization strategies such as those on acid-functionalized multiwalled carbon nanotubes,<sup>15</sup> single-walled carbon nanotubes,<sup>16</sup> and on activated glyoxyl agarose<sup>14</sup> were also developed to further enhance MsAcT's stability and reusability.

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Interestingly, many groups have recently aimed their effort at engineered MsAcT with different purposes: among others, Finnveden and co-workers<sup>17</sup> designed single point (L12A) and double point (T93A/F154A) mutants to expand the acyl donor specificity, enabling longer substrates to accommodate the active site; first Godehard *et al.*<sup>18</sup> and then Jost *et al.*<sup>19</sup> successfully created libraries of single and double variants with different specificities and selectivities, obtaining biocatalysts with increased acyl transferase to hydrolysis ratio. Moreover, Contente and colleagues<sup>20</sup> reported a strategic single point mutation (S11C) in the catalytic triad able to extend the enzyme activity towards a wider set of substrates, enabling the acceptance of thiols and secondary amines (while vinyl ester was employed as the acylating agent).

In this work, the goal was to investigate transesterification reactions catalyzed by MsAcT in the absence of water based on a design of experiments (DoE). This strategy was led by the fact that most organic compounds employed for industrial applications are not water soluble and therefore some alternative enzymatic synthetic strategies might be of interest to produce more hydrophobic structures. Furthermore, the possibility of performing synthesis at room temperature and in bulk (*i.e.*, solventless) fulfills two of today's green chemistry principles<sup>21</sup> (number 5 and 6) regarding a more sustainable way of catalyzing condensation reactions, hence reducing the process' environmental impact by lowering energy consumption and avoiding the use of common petrol-based solvents such as hexane or toluene.<sup>22</sup>

To the best of our knowledge, this work represents the first study where MsAcT was investigated related to the synthesis of short oligoesters. As already pointed out by Cannazza *et al.*,<sup>9</sup> in the past, only a few efforts were made in using this biocatalyst for the biotransformation/synthesis of macromolecules. The study by Finnveden *et al.*<sup>17</sup> probably represents the only available example in the literature connecting MsAcT to polymer biotechnology since it reports a method for the selective mono-substitution of symmetric dicarboxylic esters (divinyl adipate), having as a scope the possibility of producing multifunctional vinyl ester monomers.

## 2 Results and discussion

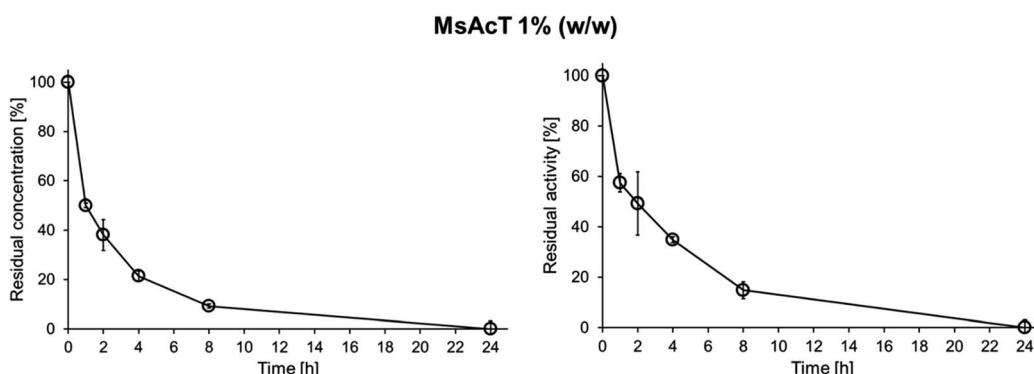
### 2.1 MsAcT immobilization and characterization

MsAcT was immobilized onto the Accurel MP 1000 polypropylene beads following an already established protocol (see the ESI, Section 2.3†). The final immobilized formulation consisted of 1% w/w of the biocatalyst on the amount of solid support, according to the following formula:

$$\text{MsAcT 1\% (w/w)} = \frac{10 \text{ mg MsAcT}}{1000 \text{ mg Polypropylene beads}}$$

Therefore, the enzyme derived from the purification was diluted to a concentration of  $0.286 \text{ mg}_{\text{MsAcT}} \text{ mL}^{-1}$  in 0.1 M  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer having a pH of 8.0 (in a total volume of 35 mL). The enzyme dilution was stirred for 24 h at 25 °C using a blood rotator set at 20 rpm with 1 g of the activated Accurel MP 1000 to ensure proper adsorption of the acyltransferase on the hydrophobic polypropylene beads. The results of the immobilization, given as residual concentration and activity (%), were calculated on the supernatant by dividing every timepoint by the starting point of the immobilization procedure (0 h timepoint). The outcomes are shown in Fig. 1 ( $\geq 99\%$  bound protein,  $\geq 99\%$  lost activity of the supernatant).

Already after 8 h of MsAcT immobilization, less than 10% of residual protein concentration was detected in the supernatant, meaning that  $>90\%$  of the enzyme was successfully adsorbed onto the solid support. Almost the same percentage was found for the residual activity, with around 15% of activity left, confirming the 85–90% enzyme immobilization rate after 8 h. Finally, after 24 h, the percentage of immobilized biocatalyst increased to  $\geq 99\%$ , according to both the protein concentration and enzyme activity, indicating that all the acyltransferase was properly adsorbed onto the polypropylene beads. This result confirms once more MsAcT's ease of immobilization and versatility, since from its first immobilization in 2010 on carbon nanotubes,<sup>15,16</sup> it has been successfully immobilized onto various solid supports by many different groups.<sup>14,23</sup> Moreover, the immobilization profile of MsAcT 1% w/w aligns very well with other 1% immobilization formulations on



**Fig. 1** Immobilization of MsAcT onto Accurel MP 1000 (polypropylene) beads in 0.1 M  $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$  buffer at pH 8. Samples were withdrawn at 0, 1, 2, 4, 8 and 24 h. Remaining protein concentration (left) and *para*-nitrophenyl acetate activity (right) from the supernatant of the immobilization procedure of MsAcT 1% w/w. The decrease of such parameters is directly proportional to the amount of protein adsorbed onto the solid support. The figure shows the mean  $\pm$  SD.



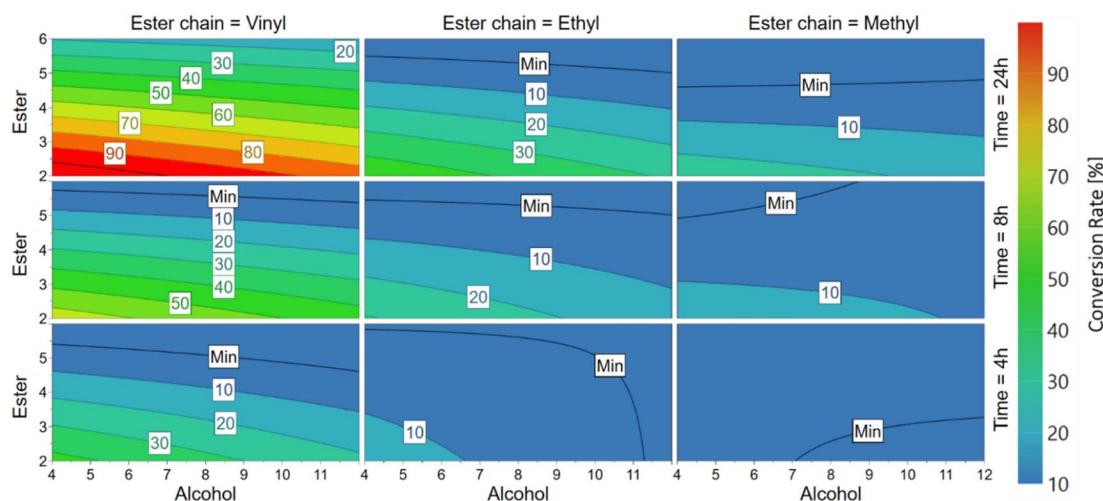


Fig. 2 4D-contour response of *Mycobacterium smegmatis* acyltransferase (MsAcT) in ester synthesis at 25 °C. The graph columns display a fixed ester chain (vinyl, ethyl, methyl) while the rows show a fixed reaction time (4, 8, 24 h). The ester and the alcohol carbon chain length vary on the Y and X axes, respectively. The color scale indicates percentual conversion, as shown in the legend at the right end side of the figure.

propylene beads (Accurel MP 1000) reported by the Pellis' group,<sup>24,25</sup> where most of the tested lipases and cutinases appeared to be >90% adsorbed onto the solid support after 8 h, while the complete immobilization was achieved after 24 h. These data suggest an enzyme immobilization rate on polypropylene beads that seems to be independent of their hydrophobicity (Gravy index of MsAcT +0.147 and CaLB +0.037 polar, HiC -0.034 non-polar) and of their molecular weight (CaLB 33 kDa, HiC 22 kDa, MsAcT 23 kDa). The obtained MsAcT formulation was further tested for ester and polyester synthesis.

## 2.2 Enzymatic synthesis of esters

Upon immobilization, the MsAcT preparation was used to carry out the full factorial DoE (detailed in the ESI, Table S1†) with the aim of elucidating the selectivity of the enzyme related to: (1) the ester functional group (vinyl, ethyl, or methyl), (2) the ester chain length (from C<sub>2</sub> to C<sub>6</sub>) and (3) the alcohol chain length (from C<sub>4</sub> to C<sub>12</sub>). The optimal temperature for this enzyme was not further investigated in the DoE study, since the peculiarity of being active at room temperature (25 °C) was already reported by several groups for transesterification or transacylation reactions on small molecules.<sup>8,26,27</sup> Moreover, the possibility of performing such reactions in bulk and at low operational temperatures fully aligns with the green chemistry principles of a more sustainable way of catalyzing condensation reactions, both in terms of energy consumption and avoidance of toxic solvents. Therefore, the reactions were carried out for 24 h at 25 °C and 400 rpm in bulk, with timepoint withdrawals at 4, 8 and 24 h. The response measured for every experiment was the conversion (%) of the ester (calculated by GC-FID, see ESI Section 2.9†). After performing each reaction from the DoE, the data were elaborated into a 4D contour response model, where the factors are shown in the axis while the response is represented by different colors in the plot (Fig. 2).

Quite unexpectedly, regarding the preferred ester functional group, MsAcT showed high conversion when a vinyl ester was

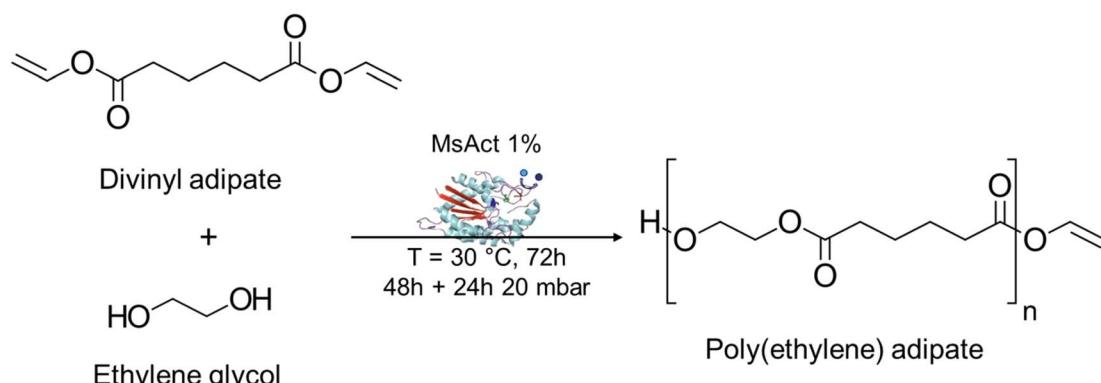
used, leading to conversions up to 4-times higher when compared to the ethyl ester (100% vs. 24% for the reaction of vinyl acetate and ethyl acetate with 1-octanol after 24 h, respectively). The advantage of using vinyl esters was already reported by several authors that observed enhanced enantioselectivities and reaction rates when using the lipase B from *Candida antarctica* (in apolar solvents such as *n*-hexane and toluene) for catalyzing the resolution of aryl aliphatic carboxylic acids.<sup>28</sup> Moreover, de Leeuw *et al.*<sup>29</sup> observed higher conversion rates for MsAcT in the synthesis of aromatic cyanohydrins using vinyl acetate rather than ethyl acetate as the acyl donor, confirming the reactivity profile. On the other hand, the enzyme activity appeared to be very limited towards methyl esters, showing the best conversion with methyl acetate and 1-butanol (25% after 24 h reaction). These results align well with the literature, since in the work performed by Finnveden *et al.*,<sup>17</sup> only very limited (<4%) conversion yields of methyl esters with 1-octanol catalyzed by wild-type MsAcT were observed after 6 h of reaction time.

Regarding the selectivity towards monomers with various carbon chain lengths, MsAcT showed a higher conversion rate towards short-chain alcohols and esters. This can be easily observed in the top part of the 4D-contour plot, where the highest conversions were obtained for all the different ester chains with the smallest carbon compound (acetate, C<sub>2</sub>). Furthermore, regarding alcohols, the conversions for vinyl acetate and 1-butanol, 1-octanol and 1-dodecanol after 8 h reaction clearly showed the enzyme selectivity towards short-chained alcohols, since 77%, 49% and 47% conversions were obtained, respectively. The same preference pattern of 1-butanol > 1-octanol > 1-dodecanol was maintained in the conversions after 8 h both with ethyl acetate (46%, 17%, 16%) and methyl acetate (22%, 15%, 14%), confirming the enzyme's preference towards short-chained alcohols and esters. The same trend was observed once more by Finnveden *et al.*,<sup>17</sup> where the highest conversion for MsAcT wild-type in divinyl adipate transacylation reactions was reported

**Table 1** Polycondensation of DVA with EG or BDO catalyzed by 30% (w/w) of immobilized MsAcT at 30 °C for 72 h at different vacuum (20 mbar) duration times

Nº	Diol	Reaction time	Vacuum	$M_n^a$	$M_w^a$	$D^a$	$M_o$	DP <sup>c</sup>	Conv. <sup>b</sup> [%]
1	EG	72 h	—	500	500	1.01	172.2	2.93	48
2			24 h	500	550	1.01		3.02	57
3			48 h	400	400	1.04		2.18	15
4	BDO	168 h	—	400	450	1.08	200.2	2.18	58
5			72 h	—	500	1.01		2.59	27
6			24 h	500	550	1.18		2.41	42
7			48 h	400	400	1.11		1.84	14

<sup>a</sup> Calculated via GPC calibrated with low molecular weight polystyrene standards 250–70000 Da. <sup>b</sup> Calculated via <sup>1</sup>H-NMR by comparing the ratio between the signal methylene groups adjacent to –OH of EG/BDO and the methylene groups of DVA (assumed as constant). <sup>c</sup> Degree of polymerization (DP) =  $M_n$ /molecular weight of the repeating unit ( $M_o$ ).



**Fig. 3** Example of the most successful enzymatic polycondensation reaction catalysed by MsAcT. 2 mmol of the monomers and 30% of the immobilized enzyme (1% w/w MsAcT per beads) on the total monomer weight reacted at 30 °C at 1000 mbar for 48 h to form oligomers and then at 20 mbar for 24 h to combine and elongate the polymer chains. In the figure, divinyl adipate was reacted with ethylene glycol to obtain poly(ethylene adipate) oligomers.

with butanediol vinyl ether ( $C_4$ ) as the acyl acceptor, reaching around 80% of DVA conversion after 28 h, while a lower conversion of around 60% was obtained with 1-octanol ( $C_8$ ), confirming this enzyme selectivity towards small chain compounds. Notably, in all the reactions carried out with a  $C_6$  ester (namely vinyl, ethyl or methyl hexanoate), no conversion was observed after 24 h, except for vinyl hexanoate and 1-butanol where only a meagre 5% was obtained, highlighting once more MsAcT poor reactivity with long chain esters. These results are in good agreement with the work reported by Hendil-Forssell,<sup>30</sup> who observed that MsAcT wild-type is able to accommodate esters only up to  $C_4$  as the acyl donors in its active site and by Godehard *et al.*,<sup>18</sup> who detected no MsAcT wild-type activity using *p*-nitrophenyl hexanoate as the acyl donor with 10 mM benzyl alcohol as the acyl acceptor.

In this full-factorial design investigation, our model showed that MsAcT expressed the highest conversion using vinyl acetate ( $C_2$ ) and 1-butanol ( $C_4$ ), therefore leading to the conclusion that this biocatalyst selectivity is towards vinyl esters (vinyl > ethyl > methyl) and short-chained alcohols and esters ( $C_2 > C_4 > C_6$  for esters,  $C_4 > C_8 > C_{12}$  for alcohols), while poor results were achieved using long-chain compounds (no conversion observed with almost all  $C_6$  esters).

### 2.3 Polyester synthesis

To verify and confirm the previously obtained MsAcT 4D contour model in the short-ester synthesis, polycondensation reactions were carried out using various bio-based monomers as the building blocks. Divinyl adipate was adopted as the model diester as it is commercially available, and a first screening was carried out to investigate MsAcT selectivity towards diols. The immobilized enzyme (1% w/w MsAcT per beads) concentration in the reaction mixture was first tested at 10% (w/w) on the total weight of monomers but very limited conversions were obtained (see ESI Table S3†). Therefore, a higher concentration of 30% was adopted for the polyester synthesis. To elucidate whether MsAcT accommodates better short-chained diols or not, polycondensations of divinyl adipate with ethylene glycol (EG,  $C_2$ ) and 1,4-butanediol (BDO,  $C_4$ ) were carried out at 30 °C, as it is shown in Table 1 (entries 1 and 5). Moreover, different time-lengths of applied vacuum (20 mbar) were used to investigate the best reaction conditions for MsAcT in polyester synthesis (Fig. 3).

The conversion of the diol was higher in polycondensations with DVA with EG than for BDO (48% and 27%, respectively) after 72 h and without applying a vacuum (reactions 1 and 5).

The same trend was observed in all the other reactions with the same vacuum settings: with 24 h of applied vacuum, 57% and 42% conversions for EG and BDO were observed, respectively ( $M_w = 550$  Da for both entries 2 and 6), while after 48 h vacuum, meagre values of 15% and 14% were achieved for EG and BDO ( $M_w = 400$  Da for both entries 3 and 7), confirming MsAcT selectivity towards short-chained compounds. The molecular masses of the resulting polymers partially confirmed the trend, since the highest  $M_w$  and degree of polymerization were obtained using EG after 24 h of vacuum ( $M_w = 550$  Da, DP = 3.02, reaction 2), while a slightly lower DP was shown with BDO (DP = 2.41, reaction 6). In order to verify if a longer reaction time could result in higher yields, a 168 h reaction was performed at 1000 mbar (entry 4), achieving 58% conversion and, as expected, a low molecular polyester ( $M_w = 450$ ). These results, obtained using MsAcT as the biocatalyst, are somehow limited when compared to previous studies in which well-established lipases were used. For instance, Uyama *et al.*<sup>31</sup> reported the synthesis of poly(ethylene adipate) starting from DVA and EG using the lipase from *Pseudomonas cepacia* at 45 °C for 48 h in diisopropyl ether, obtaining a polyester with  $M_n = 6000$  Da. Moreover, Russell and co-workers<sup>32</sup> used Novozym-435 (CaLB immobilized on acrylic resin beads) for the solventless reaction of DVA and BDO at 50 °C, resulting in a polymer having a  $M_w$  of 23 200 Da. In this study, since one of MsAcT's advantages lies in the ability to be active at room temperature (25–30 °C), the polycondensations were performed at 30 °C in the bulk, thus presenting very interesting reaction conditions for the enzymatic polyester synthesis.

As the observed differences in molecular masses of the polyesters catalyzed by MsAcT were minimal and the obtained molecular weights were rather low, an LC-MS analysis was carried out. These analyses confirmed that prolonging the reaction time (see the ESI, Fig. S3 and S5†) and applying a vacuum (see the ESI, Fig. S4 and S6†) are useful to obtain higher molecular weight oligomers (up to trimers) while for the other reactions only shorter oligomers were obtained. When changing the divinyl ester from DVA to divinyl succinate (DVS, see ESI Table S4†) a monomer conversion drop (<14% under the best reaction conditions) was observed. This lower reactivity of DVS might be due to the proximity of the two carbonyl groups that might somehow hinder the accessibility and interaction of the monomer with the enzyme's active site.

### 3 Conclusions

*Mycobacterium smegmatis* acyltransferase (MsAcT) was successfully immobilized *via* adsorption onto polypropylene beads. To study its selectivity in ester synthesis, a full-factorial design of experiment (DoE) was implemented. The results showed the acyltransferase selectivity towards vinyl esters (vinyl- > ethyl- > methyl-) and short-chained alcohols and esters ( $C_2 > C_4 > C_6$  for esters,  $C_4 > C_8 > C_{12}$  for alcohols). In particular, the results obtained after 8 h using vinyl acetate ( $C_2$  ester) and 1-butanol, 1-octanol and 1-dodecanol clearly underlined the selectivity trend, since 77%, 49% and 47% conversions were obtained, respectively. Moreover, MsAcT showed poor results using long-

chain compounds (no conversion observed with almost all  $C_6$  esters) thus confirming its preference for short-chained compounds. Divinyl adipate (DVA) and two different diols, ethylene glycol (EG) and 1,4-butanediol (BDO), were used as bio-based building blocks for MsAcT-catalyzed polycondensation reactions. MsAcT showed a higher conversion when using EG rather than BDO, achieving 47% and 27% conversion respectively, confirming the previously observed short ester synthesis trend. The best results were obtained after 24 h at 20 mbar using EG, achieving 57% conversion and  $M_w = 550$  Da, DP = 3.02, while with BDO, 42% conversion,  $M_w = 550$  Da and a slightly lower DP = 2.41 were achieved. LC-MS analysis confirmed the obtained results that a longer reaction period and reduced pressure were suitable to obtain longer oligomers, up to trimers. On the other side, polycondensation reactions carried out using divinyl succinate (DVS) as the diesters showed very limited results (only 14% conversion under the best conditions), hence revealing a potential steric hindrance by the proximity of the carbonyl carbons of DVS towards the MsAcT active site. In conclusion, this work represents the first study in which MsAcT was directly used to catalyze a polycondensation reaction, shedding light on the possibility of using this biocatalyst in the polymer biotechnology field.

### Author contributions

F. F. and I. V. immobilized the enzyme, carried out the ester and polyester synthesis and performed the NMR and the GPC analysis. S. D. carried out the divinyl succinate syntheses. L. N. expressed and purified the enzyme. V. M. R. carried out the LC-MS analysis. F. F. and A. P. wrote the manuscript. M. L. C., L. M., G. M. G. and A. P. supervised the work. M. L. C., L. M. and A. P. acquired the funding. The manuscript was revised and approved by all authors before submission.

### Conflicts of interest

The authors declare no competing financial interests.

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### References

- 1 S. Okumura, M. Iwai and Y. Tominaga, *Agric. Biol. Chem.*, 1984, **48**, 2805–2808.
- 2 D. Knani, *Enzymatic Polyesterification in Organic Media*, 1993, vol. 31, pp. 1221–1232.
- 3 H. Uyama and S. Kobayashi, *Chem. Lett.*, 1993, **22**, 1149–1150.
- 4 S. Kobayashi, *Proc. Jpn. Acad. B: Phys. Biol.*, 2010, **86**, 338–365.



5 S. Hari Krishna and N. G. Karanth, *Catal. Rev.: Sci. Eng.*, 2002, **44**, 499–591.

6 N. R. Khan and V. K. Rathod, *Process Biochem.*, 2015, **50**, 1793–1806.

7 A. G. A. SÁ, A. C. de Meneses, P. H. H. de Araújo and D. de Oliveira, *Trends Food Sci. Technol.*, 2017, **69**, 95–105.

8 I. Mathews, M. Soltis, M. Saldajeno, G. Ganshaw, R. Sala, W. Weyler, M. A. Cervin, G. Whited and R. Bott, *Biochemistry*, 2007, **46**, 8969–8979.

9 P. Cannazza, S. Donzella, A. Pellis and M. L. Contente, *Biotechnol. Adv.*, 2022, **59**, 107985.

10 M. Kazemi, X. Sheng, W. Kroutil and F. Himo, *ACS Catal.*, 2018, **8**, 10698–10706.

11 L. Wiermans, S. Hofzumahaus, C. Schotten, L. Weigand, M. Schallmey, A. Schallmey and P. D. De María, *ChemCatChem*, 2013, **5**, 3719–3724.

12 V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471–479.

13 M. L. Contente, A. Pinto, F. Molinari and F. Paradisi, *Adv. Synth. Catal.*, 2018, **360**, 4814–4819.

14 M. L. Contente, S. Farris, L. Tamborini, F. Molinari and F. Paradisi, *Green Chem.*, 2019, **21**, 3263–3266.

15 C. Z. Dinu, G. Zhu, S. S. Bale, G. Anand, P. J. Reeder, K. Sanford, G. Whited, R. S. Kane and J. S. Dordick, *Adv. Funct. Mater.*, 2010, **20**, 392–398.

16 C. Z. Dinu, I. V. Borkar, S. S. Bale, A. S. Campbell, R. S. Kane and J. S. Dordick, *J. Mol. Catal. B: Enzym.*, 2012, **75**, 20–26.

17 M. Finnveden, S. Semlitsch, O. He and M. Martinelle, *Catal. Sci. Technol.*, 2019, **9**, 4920–4927.

18 S. P. Godehard, C. P. S. Badenhorst, H. Müller and U. T. Bornscheuer, *ACS Catal.*, 2020, **10**, 7552–7562.

19 E. Jost, M. Kazemi, V. Mrkonjić, F. Himo, C. K. Winkler and W. Kroutil, *ACS Catal.*, 2020, **10**, 10500–10507.

20 M. L. Contente, D. Roura Padrosa, F. Molinari and F. Paradisi, *Nat. Catal.*, 2020, **3**, 1020–1026.

21 P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*, 2000.

22 A. Pellis, F. P. Byrne, J. Sherwood, M. Vastano, J. W. Comerford and T. J. Farmer, *Green Chem.*, 2019, **21**, 1686–1694.

23 M. H. Sipponen, M. Farooq, J. Koivisto, A. Pellis, J. Seitsonen and M. Österberg, *Nat. Commun.*, 2018, **9**, 1–7.

24 F. Fabbri, F. A. Bertolini, G. M. Guebitz and A. Pellis, *Int. J. Mol. Sci.*, 2021, **22**(16), 8493.

25 S. Weinberger, A. Pellis, J. W. Comerford, T. J. Farmer and G. M. Guebitz, *Catalysts*, 2018, **8**(9), 369.

26 I. C. Perdomo, S. Gianolio, A. Pinto, D. Romano, M. L. Contente, F. Paradisi and F. Molinari, *J. Agric. Food Chem.*, 2019, **67**, 6517–6522.

27 K. Szymańska, K. Odrozak, A. Zniszczoł, G. Torrelo, V. Resch, U. Hanefeld and A. B. Jarzębski, *Catal. Sci. Technol.*, 2016, **6**, 4882–4888.

28 H. Yang, E. Henke and U. T. Bornscheuer, *J. Org. Chem.*, 1999, **64**, 1709–1712.

29 N. de Leeuw, G. Torrelo, C. Bisterfeld, V. Resch, L. Mestrom, E. Straulino, L. van der Weel and U. Hanefeld, *Adv. Synth. Catal.*, 2018, **360**, 242–249.

30 P. Hendil-forssell, *Rational Engineering of Esterases for Improved Amidase Specificity in Amide Synthesis and hydrolysis Stockholm 2016*, 2016.

31 H. Uyama, S. Yaguchi and S. Kobayashi, *Polym. J.*, 1999, **31**, 380–383.

32 A. K. Chaudhary, B. J. Kline, E. J. Beckman and A. J. Russell, *Am. Chem. Soc., Div. Polym. Chem., Prepr.*, 1997, **38**, 396–397.

