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Synthesis of alkyl coumarate esters by celite-bound lipase of *Bacillus licheniformis* SCD11501

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ABSTRACT

Coumaric acid and its derivatives are known to inhibit UV-induced melanogenesis, skin erythema, angiogenesis, platelet accumulation and osteoclastogenesis besides regulation of bone formation, anti-oxidant and anti-microbial activities. The present study is a novel piece of work in which the syntheses of a series of alkyl coumarates (methyl coumarate, ethyl coumarate, propyl coumarate and butyl coumarate) have been achieved using celite-immobilized lipase of *Bacillus licheniformis* strain SCD11501 in a water-free medium at 55 °C under shaking in a chemical reactor. The maximum yield(s) of coumaric acid based esters *i.e.* methyl coumarate (69.0%), ethyl coumarate (63.1%), *n*-propyl coumarate (59.8%) and *n*-butyl coumarate (55.1%) using celite-immobilized lipase could be achieved after optimizing various reaction parameters such as incubation time, incubation temperature, relative molar concentration of reactants, biocatalyst concentration and amount of molecular sieves added to the reaction system. Molecular sieves had an important effect on the ester synthesis resulting in an enhanced yield. Maximum yield was recorded for methyl coumarate (69.0%) possibly because methyl group causes less stearic hindrance to the catalytic site of the lipase due to which it becomes more accessible for immobilized lipase to undergo esterification. The characterization of synthesized esters was done through FTIR spectroscopy and ^{1}H NMR spectra.

1. Introduction

Lipases (triacylglycerol acylhydrolases; EC 3.1.1.3) constitute a group of enzymes defined as carboxylesterases that catalyse the hydrolysis of long-chain acylglycerols at the lipid–water interface [1]. Lipase is an important class of hydrolytic enzyme with innumerable applications and industrial potential. Lipases are produced by animals, plants and microorganisms [2]. Immobilization of enzymes is one of the useful techniques to improve the application of enzymes at the industrial level [3]. For successful immobilization, the matrix must has the mesh size large enough to allow molecules of the substrate and of the reaction product to diffuse in and out and to keep the enzyme entrapped in the matrix, too [4]. Immobilized enzymes can provide an easily separable and reusable system (together with enhanced product recovery) which is often also more resistant to deactivation by heat and/or denaturing solvents as compared to the parent free enzyme [5].

4-Coumaric acid (4-CA) and its derivatives possess wide spectrum of anti-microbial and anti-oxidant activities [6]. It is also a common dietary polyphenolic antioxidant that induces pro-oxidant effects in human [7]. Coumaric acid (CA) can be obtained from basic hydrolysis of coumarin through a reaction process consisting of opening the lactone ring and *cis–trans isomerization*. Coumarin derivatives also have diverse biological properties such as enzyme inhibition, hypotoxicity, anti-carcinogenic and anti-coagulant activity [8]. CA is known to suppress UV-induced skin erythema and melanogenesis, inhibit angiogenesis, prevent platelet aggregation, inhibit osteoclastogenesis and stimulate bone formation. Through its free radical scavenging ability, 4-CA prevents oxidative damage to bio-molecules and renders organoprotection against drug-induced toxicities [9]. 4-CA: Coenzyme A ligase (4CL) is involved in monolignol biosynthesis for lignification in plant cell walls. It ligates coenzyme A (CoA) with hydroxycinnamic acids, such as 4-coumaric and caffeic acids, into hydroxycinnamoyl-CoA thioesters [10]. CA, a common dietary polyphenolic antioxidant, can dose-dependently induce pro-oxidant effects in human endothelial cells [7]. 4-CA formulated in a cream permeated skin tissue ex vivo and its topical application mitigated UVB-induced inflammatory erythema and skin pigmentation in vivo in human skin [11]. In animals and human the alkyl

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coumarates are synthesized through the phenylpropanoid pathway. The beneficial effects of alkyl coumarates have been attributed to their antioxidant activity particular against oxidative attacks by their radical-scavanging activity.

Alkyl coumarate esters inhibit the oxidation of low-density lipoproteins more efficiently and exhibited a higher antiradical activity than coumaric acid, a natural antioxidant. In addition, alkyl coumarates find extensive applications in chemical, pharmaceutical and cosmetics industries [12]. Alkyl coumarates have also been reported to inhibit several key molecular targets like histone-deacetylase, mitogen-activated protein kinases, cancer and immune disorders. Because of many imporatnet biological properties of alkyl coumarates, the present work was undertaken to optimize the reaction process parameters to efficiently synthesize different C-chain length alkyl esters of coumaric acid in a water-free medium employing celite-bound lipase of *Bacillus licheniformis* SCD11501.

2. Materials and methods

2.1. Materials

Celite 545 (S.D. Fine-Chem Ltd., Hyderabad, India); *p*-nitrophenyl acetate (*p*-NPA), *p*-nitrophenyl benzoate (*p*-NPB), *p*-nitrophenyl caprylate (*p*-NPC), *p*-nitrophenyl formate (*p*-NPF), *p*-nitrophenyl laurate (*p*-NPL), *p*-nitrophenyl palmitate (*p*-NPP) and glutaraldehyde (Lancaster Synthesis, England); molecular sieves (3 Å × 1.5 mm), methanol, ethanol, *n*-propanol and *n*-butanol (MERCK, Mumbai, India); Tris buffer and coumaric acid (Himedia Laboratory, Ltd., Mumbai, India) were procured from various commercial suppliers. All chemicals were of analytical grade and were used as received. A chemical reactor with stirring and heating (MiniBlock^{TM}, China) was used to perform ester synthesis using celite-immobilized lipase of *B. licheniformis* SCD11501.

2.2. Microorganism

An aerobic, rod-shaped (cocco-bacillary), thermophilic endospore forming bacterium of genus *Bacillus* was originally isolated from hot springs of Tatapani, District: Mandi (Himachal Pradesh), India. This bacterium was identified *B. licheniformis* strain SCD11501 (GenBank Accession Number JN998712.1) by Xcelris Labs Ltd., Ahmedabad-380054, India on the basis of 16S RNA sequence analysis.

2.3. Purification and immobilization of lipase of Bacillus licheniformis strain SCD11501

An extracellular solvent- and thermo-tolerant lipase produced by *B. licheniformis* strain SCD11501 was purified (37.0 U/mg protein) to homogeneity by successive techniques of salting out using ammonium sulphate, dialysis and DEAE anion-exchange chromatography to 10.5-fold with an overall yield of 8.4% experimental data not provided here). To avoid mixing of biocatalyst with the product, the lipase was immobilized by physical adsorption (~95% binding of protein/lipase) onto Celite-545 matrix and immobilization of protein onto the matrix was stabilized by glutaraldehyde treatment/cross-linking. The celite-bound lipase was completely dehydrated under vacuum in a freeze drier for 2 h and this celite-bound lipase (63.9 U/g celite matrix) was used thereafter to perform bio-catalytic reactions in a chemical reactor under stirring. The lipase activity of the celite-bound lipase-treated with glutaraldehyde was comparatively higher than that of celite-bound biocatalyst without glutaraldehyde treatment [13].

2.3.1. Unit of lipase activity

The unit (U) of enzyme activity was defined as μmole(s) of *p*-nitrophenol released from *p*-NPP per min by one ml of free enzyme or one gram of celite-immobilized enzyme (weight of matrix included) under standard assay conditions.

2.4. Esterification reaction

Coumaric acid esters were synthesized by using 1 M alcohol (methanol, ethanol, *n*-propanol or *n*-butanol), 1 M coumaric acid and celite-bound purified lipase (63.9 U/g, hydrolysis reaction activity) taken in Teflon coated glass-vials (20 ml capacity). The reaction was performed at 55 °C for 10 h in a chemical reactor under stirring. The syntheses (Fig. 1) of the methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate was optimized by studying the effect of various physico-chemical parameters such as incubation time, reaction temperature, relative molar concentration of reactants, biocatalyst concentration and concentration of molecular sieves added to the reaction system using celite-bound lipase. The formed esters were separated in different test tubes on the basis of their solubility in hot-water using a separating funnel. The coumaric acid was soluble in hot water while the formed esters (methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate formed in different test tubes) were insoluble in hot water and were separated out using a separating funnel. The amount of ester synthesized in each case was determined and represented as % yield and characterized by FTIR and NMR techniques.

2.5. Optimization of reaction conditions for the synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

Esterification was carried out by reacting coumaric acid with methanol, ethanol, *n*-propanol or *n*-butanol separately in different ratios in the presence of celite-bound lipase at different temperatures for different time periods in a chemical reactor.

2.5.1. Effect of incubation time on synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

The reaction mixture (2 g) comprised of celite-immobilized lipase (10 mg); coumaric acid (1 M): alcohol such as methanol, ethanol, *n*-propanol or *n*-butanol (1 M) taken in different glass vials in a water-free system. The glass vials were incubated at 55 °C in a chemical reactor for 4, 6, 8, 10, 12 and 14 h under shaking. The amount of ester synthesized in each case was determined and represented as % yield in each case.

2.5.2. Effect of reaction temperature on the synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

The effect(s) of reaction temperature (40, 45, 50, 55 and 60 °C) on the synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate were studied for 8 h under shaking. The reaction mixture (2 g) comprised of celite-immobilized lipase (10 mg); coumaric acid (1 M): alcohol such as methanol, ethanol, *n*-propanol or *n*-butanol (1 M) taken in different glass vials in a water-free system. The amount of esters synthesized was determined and represented as % yield in each case.

2.5.3. Effect of relative molar concentration of reactants on the synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

The concentration of one of the reactants (coumaic acid) was kept constant at 1 M and the concentration of the other

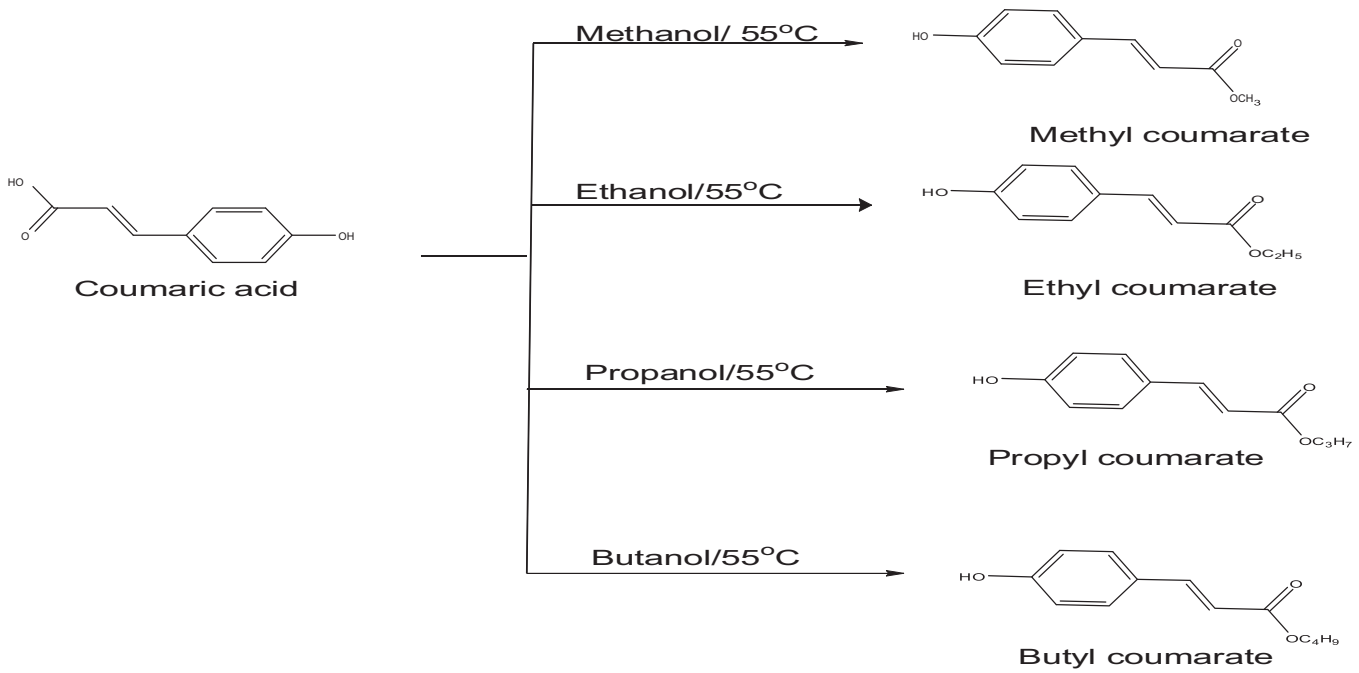

Fig. 1. Scheme of the synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate.

reactant (methanol, ethanol, *n*-propanol or *n*-butanol) was varied (0.25 to 4 M) in the in a water-free system. The reaction mixture (2 g) comprised of celite-immobilized lipase (10 mg); coumaric acid (1 M): alcohol taken in different glass vials and esterification was carried out at 55 °C for optimized time (8 h) under continuous shaking. The amount of esters synthesized was determined and represented as % yield in each case.

2.5.4. Effect of amount of biocatalyst on synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

The syntheses of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate were studied by taking different amount of immobilized lipase (0.5–3% of acid weight) in reaction mixture (2 g) containing 1 M of coumaic acid: 1 M methanol, ethanol, *n*-propanol or *n*-butanol in a water-free system at 55 °C for 8 h under shaking. The amount of esters synthesized was determined and represented as % yield in each case.

2.5.5. Effect of molecular sieves on the synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

Molecular sieves (1% to 5% of reaction volume) were added to the reaction system to study their effect on the synthesis of alkyl coumarate using immobilized lipase (20 mg) in a reaction mixture (2 g) containing 1 M of coumaric acid: 1 M methanol, ethanol, *n*-propanol or *n*-butanol in a water-free system at 55 °C for 8 h under shaking. The synthesized ester in each case was determined and % yield was recorded.

2.5.6. Characterization of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

Synthesized esters *i.e.* methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate were characterized by FTIR (Fourier transform infrared spectroscopy) and NMR (Nuclear magnetic resonance) to get evidence of the formation of reaction product(s). FTIR spectrum was recorded on Perkin Elmer spectrophotometer in transmittance mode in KBr and NMR spectroscopy (INOVA 400 MHz spectrophotometer) using TMS as internal standard in $CHCl_3$.

3. Results and discussion

3.1. Optimization of reaction conditions for the synthesis of ethyl coumarate, methyl coumarate, n-propyl coumarate and n-butyl coumarate

Esterification was carried out by reacting coumaric acid and methanol, ethanol, *n*-propanol or *n*-butanol in different molar ratios in the presence of celite-bound lipase at different temperatures for different time periods in a chemical reactor.

3.2. Effect of incubation time on synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

The effect of reaction time on synthesis of esters of coumaric acid using celite-immobilized lipase was studied at a temperature of 55 °C under continuous shaking (Fig. 2) upto 14 h. Maximum yield of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate (64.3%, 60.6%, 54.4% and 49.1%, respectively) was recorded at 8 h. The synthesis of coumaric acid ester(s) was time dependant and methyl coumarate (64.3%) was produced maximally after 8 h of reaction. Thereafter is seems there was a loss of activity of lipase that might be on account of extended exposure of celite-bound biocatalyst because of heat-denaturation leading to decreased esterification. In a previous study, reaction time of 6 h at 45 °C for immobilized-lipase was considered optimum for the synthesis of butyl ferulate [14]. Earlier, a maximum molar conversion of methyl butyrate and octyl acetate has been obtained at reaction times of 14 and 12 h. After the specified time intervals (12 h for octyl acetate and 14 h for methyl butyrate) the molar conversion was relatively constant, which might be due to the attainment of reactions at the equilibrium [15].

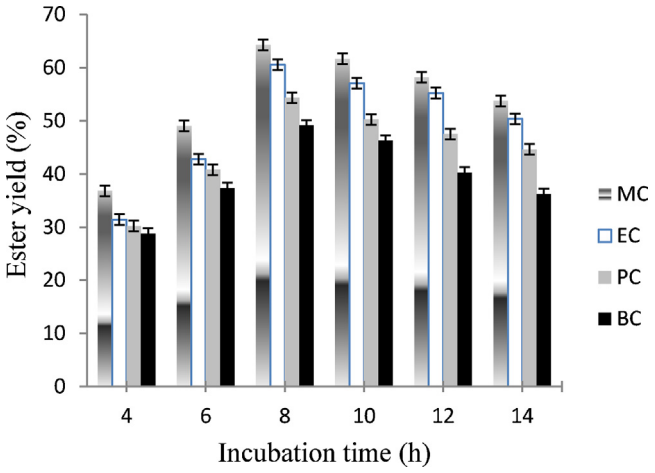

Fig. 2. Effect of incubation time on synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate.

3.3. Effect of reaction temperature on the synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

An increase in temperature of reaction mixture might interfere with the porosity, hydrophobic character, diffusion of the reactants and/or products at the catalytic site of enzyme as well as catalytic activity of the enzyme. The reaction temperature above or below 55 °C decreased the production of esters (Fig. 3) of coumaric acid in the solvent-free reaction system. This might be on account of denaturation of the lipase as well as alteration in the 3-D structure of celite-bound lipase. The recorded yields of synthesized esters such as methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate were 65.3%, 61.5%, 57.8% and 52.6%, respectively at 55 °C in 8 h under continuous shaking. Any marked increase or decrease in temperature of reaction mixture might interfere with the biological activity of the enzyme added to the reaction system [16]. Little information is available about the use of lipase in the synthesis of alkyl esters of coumaric acid in literature. Previously, optimum temperature for butyl ferulate (62 mM) synthesis in DMSO using silica-bound lipase was found to be 45 °C [14]. Generally, thermostable enzymes have a more rigid and packed

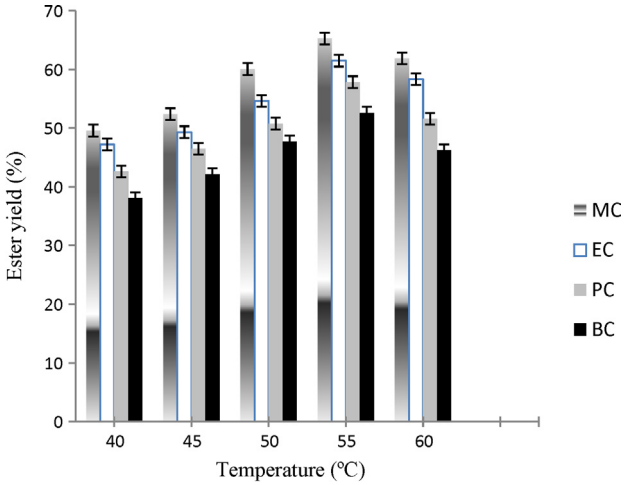

Fig. 3. Effect of reaction temperature on the synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate.

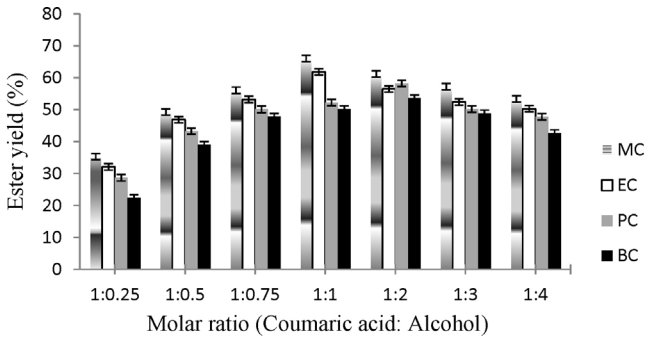

Fig. 4. Effect of relative molar concentration of reactants on the synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate.

conformational structure which is stabilized when immobilized on hydrophobic supports such as silica and celite. Hence, the immobilized thermostable lipases provide a higher conversion rate; minimal risk of microbial contamination; higher solubility of the substrates and lower viscosity of the reaction medium. Thermostability is dependent on the structure of the enzyme, the environment (solvent), pH, temperature, the presence of additives (organic solvents, ions) and type of immobilization. In the present study, the *B. licheniformis* SCD11501 that was used as a source of lipase was found to possess a GC content of 55% on the basis of its 16S rRNA nucleotide sequence thereby indicating the thermophilic nature of the organism used as a source of lipase. A maximum increase in the yield of esters of coumaric acid was recorded at a temperature of 55 °C thereafter the yield declined.

3.4. Effect of relative molar concentration of reactants on the synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

The esterification was performed by varying the molar concentration (0.25, 0.5, 0.75, 1, 2, 3 or 4 M) of selected alcohol (methanol, ethanol, *n*-propanol or *n*-butanol) while the concentration of coumaric acid was kept constant (1 M) in a water-free system. The molar ratio of 1:1 for alcohol and coumaric was found to be optimum for the synthesis of methyl coumarate (66.0%)

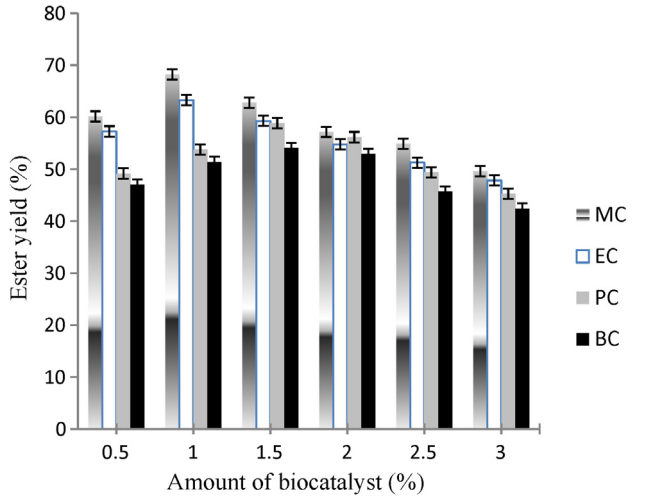

Fig. 5. Effect of amount of biocatalyst on synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate.

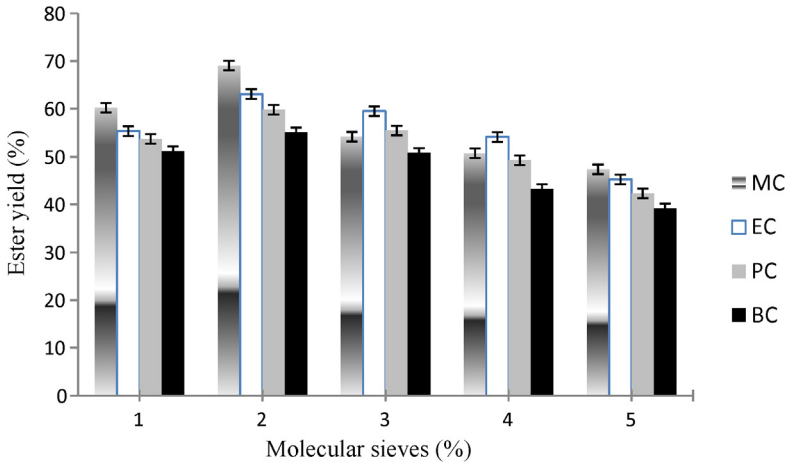

Fig. 6. Effect of molecular sieves (3 Å × 1.5 mm) on synthesis of methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate.

and ethyl coumarate (61.9%) while maximum yield for *n*-propyl coumarate (58.2%) and *n*-butyl coumarate (53.6%) was observed at 1:2 molar concentrations of the reactants at 55 °C in 8 h under continuous shaking (Fig. 4). Previously, it has been reported that optimal synthesis of butyl acetate by lipase from *Bacillus coagulans*, immobilized on nylon-6 was achieved when ester and alcohol were used in equimolar ratio (100 mM) in the reaction mixture [17]. In a recent study, the synthesis of ethyl ferulate was carried out in DMSO at equimolar concentration of ethanol and ferulic acid by celite-bound lipase [18]. Previously, maximum yield of biodiesel was attained after 12 h at the stoichiometric molar ratio of 3:1 of methanol and butyric acid using a bacterial lipase [19].

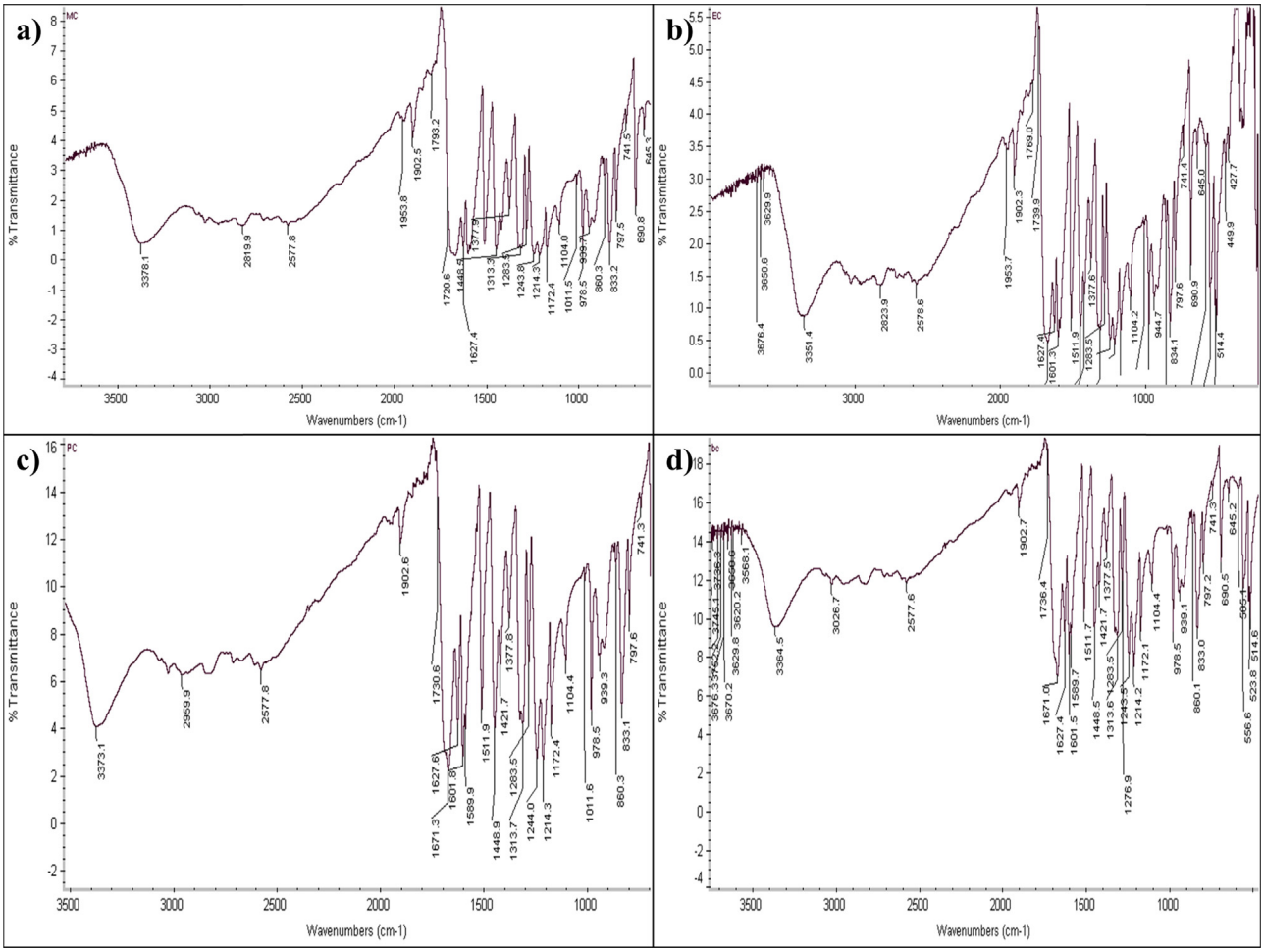

Fig. 7. FTIR results (a) methyl coumarate, (b) ethyl coumarate, (c) *n*-propyl coumarate and (d) *n*-butyl coumarate.

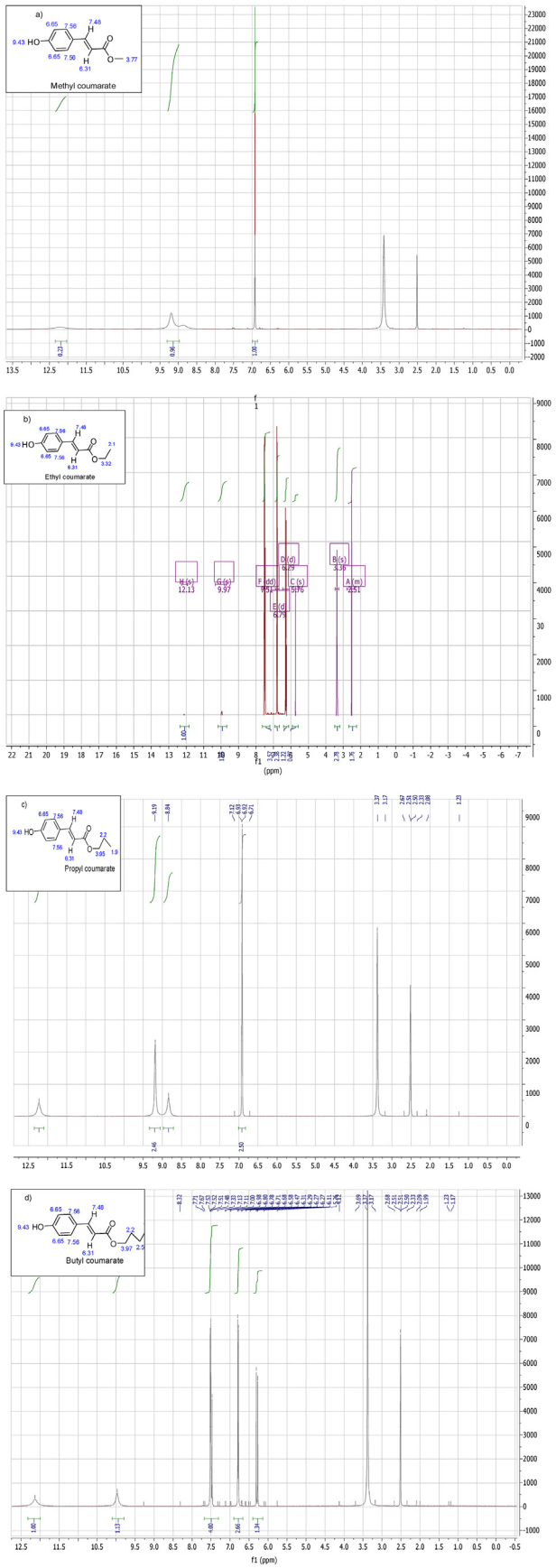

Fig. 8. NMR spectrum of (a) methyl coumarate, (b) ethyl coumarate, (c) *n*-propyl coumarate and (d) *n*-butyl coumarate.

3.5. Effect of amount of biocatalyst on synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

Enzyme concentration is known to influence the transesterification behavior. To establish the optimal amount of celite-immobilized lipase that could be used for efficient esterification reaction, different quantities of celite-bound-lipase (0.5–3%; w/v) were used and ester synthesis was monitored by FTIR and NMR. The maximum yield of methyl coumarate (68.2%) and ethyl coumarate (63.3%) was detected at 1% concentration of biocatalyst (Fig. 5). However, a concentration of 1.5% was optimal for maximum yield of ester in case of propyl coumarate (58.8%) and butyl coumarate (54.0%). In a recent study, 10 mg/mL (*i.e.* 1 g/mL) of celite-bound lipase was found to give an optimum yield of ethyl ferulate in DMSO [18].

3.6. Effect of molecular sieves on synthesis of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

When the reaction mixture was treated with varying amounts (1% to 5% of reaction volume of 2 g) of molecular sieves to remove traces of water molecules produced as by-product of the esterification reaction at 8 h at 55 °C, a relatively higher amount of methyl coumarate (69.0%), ethyl coumarate (63.1%), *n*-propyl coumarate (59.8%) and *n*-butyl coumarate (55.1%) was recorded when the molecular sieves were used at a concentration of 2% of reaction mixture (Fig. 6). Any further addition of molecular sieves to the reaction mixture prompted a decreased in the amount of ester synthesized. Molecular sieves, a class of synthetic water scavengers often improve the ester yield by withdrawing water molecules, produced as by-product in the esterification reaction thus promoting the forward reaction. Esterification is generally a water limited reaction [20] and excess of water activity inhibits the forward catalytic reaction in a water restricted/organic medium. The formation of water also causes aggregation of support particles resulting in a decrease in the rate of enzyme activity. Esterification of isopropyl alcohol and ferulic acid by silica-immobilized lipase in the absence of a water scavenger/molecular sieves exhibited approximately 84% esterification [21]. The addition of molecular sieves usually improved the equilibrium conversion [18], yet in many cases negative effects such as the formation of diester and degradation of unstable substrates have also been reported [22]. However, in the present study, addition of molecular sieves to the reaction system modulated the ester yields.

3.7. Characterization of methyl coumarate, ethyl coumarate, n-propyl coumarate and n-butyl coumarate

Characterization of the coumaric acid esters (methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate) was carried out by FTIR spectroscopy (Nicollet 5700) and ^{1}H NMR spectra (INOVA 400 MHz spectrophotometer) in deuterated chloroform ($CDCl_3$) solution with internal standard TMS (0 ppm) and chemical shifts were recorded in parts per million (δ/ppm). FTIR spectrum of methyl coumarate clearly showed a sharp peak at 1730 cm^{-1} which was due to –C=O group of ester and when compared with the spectrum of coumaric acid this peak of ester was not present in it which clearly confirmed that the lipase-catalyzed esterification reaction had occurred (Fig. 7 a). The other peak(s) at value 2829.5 cm^{-1} clearly confirm C–H stretching which was due to methyl group presented in methyl coumarate. Again in next three spectrum of ethyl, propyl and butyl coumarate the characteristic peaks of ester with value 1730.2, 1739.9, 1736.6 cm^{-1} respectively confirmed the ester synthesis as these were the peaks due to –C=O stretching of a ester

Table 1
Characteristic peaks of various esters formed from coumaric acid.

S. No.	Compound	FTIR characteristics peaks		^{1}H NMR peaks value in	
		Functional groups	Values in (cm^{-1})	Functional groups	Values in ppm
1.	Coumaric acid	–C=O, –OH	1693,3500	–COOH, benzene ring	12.05, 7.5, 6.5
2.	Methyl coumarate	–C=O, –O–C–O, –C–H	1730,1215.5, 2829.5	–C=O, –C–H, benzene ring	3.89, 6.95, 7.5
3.	Ethyl coumarate	–C=O, –O–C–O, –C–H	1730.21,172.32,939.9	–C–O–C_2H_5, benzene ring	2.3, 3.92, 6.95, 7.5
4.	*n*-Propyl coumarate	–C=O, –O–C–O, –C–H	1739.91,192.52,832.5	–C–O–C_3H_7, benzene ring	2.2, 2.3, 3.5, 6.95, 7.5
5.	*n*-Butyl coumarate	–C=O, –O–C–O, –C–H	1736.3,1172.5,2991.2	–C–O–C_4H_9, benzene ring	1.5, 2.2, 2.3,3.5, 6.95, 7.1

group (Fig. 7b–d). The peaks at value near 2700–2900 cm^{-1} were due to C–H stretching of ethyl, propyl and butyl group presented in the respective esters.

The ^{1}H NMR spectrum of coumarate series (methyl coumarate, ethyl coumarate, *n*- propyl coumarate and *n*-butyl coumarate) were compiled (Fig. 8). Various peaks corresponding to methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate, respectively were recorded (Fig. 8a–d; Table 1). Spectrum of methyl coumarate has peaks with (δ/ppm) 3.95 (singlet, 3H of ester group attached with CH_3) and 6.95 (doublet, 1 H of benzene ring; Fig. 8a). As the chain length of the ester increased the corresponding peaks due to the methylene and methyl protons of the alkyl group also appeared with δ/ppm values in the range 3.2–1.9 confirming the synthesis of different esters with various alcohols. For ethyl coumarate the signals due to –C_2H_5 and benzene ring attained value at 2.3 (–CH_3), 3.92 (–CH_2), and 6.95 ppm (Fig. 8b). Similarly, for *n*-propyl and *n*-butyl coumarate the peaks due to the absorption by different protons of –C_3H_7 and –C_4H_9 δ values at 2.3 (–CH_3), 2.2 (–CH_2), 3.92 (–CH_2) and 6.95 ppm and 1.5 (–CH_2), 2.2 (–CH_2), 2.3 (–CH_2), 3.92 (–CH_2), 6.95, respectively, were observed (Fig. 8c and d).

4. Conclusion

The aim of the present study was to synthesis the esters of chosen alcohol and coumaric acid (methyl coumarate, ethyl coumarate, *n*-propyl coumarate and *n*-butyl coumarate) by employing celite-immobilized lipase of a thermophilic *B. licheniformis strain* SCD11501 as a biocatalyst. Esterification was successfully carried out by reacting coumaric acid and methanol or ethanol or *n*-propanol or *n*-butanol in different ratios in the presence of celite-immobilized lipase at different temperatures for different time periods in a chemical reactor. A good yield(s) of methyl coumarate (69.0%), ethyl coumarate (63.1%), *n*-propyl coumarate (59.8%) and *n*-butyl coumarate (55.1%) using immobilized lipase could be achieved after sequentially optimizing various reaction parameters. The maximum yield of methyl coumarate was 55 °C in 8 h at 1% biocatalyst concentration because methyl group is likely to cause less stearic hindrance and hence seems to be easily accessible to immobilized lipase to undergo esterification. Molecular sieves had a moderate effect on the ester syntheses resulting in enhanced yield(s) at a concentration of 2%. The characterization of synthesized esters was done through FTIR spectroscopy and ^{1}H NMR spectra.

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