Although the above means and methods allow to produce isobutene from 3-methylcrotonic acid, there is still a need for improvements, in particular as regards a further increase in efficiency of the process so as to make it more suitable for industrial purposes.

The present application addresses this need by providing the embodiments as defined in the claims.

Summary

In one aspect, the present disclosure refers to a variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is derived and having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 55% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 434, 7, 17, 27, 33, 35, 45, 46, 48, 51, 140, 144, 183, 184, 185, 222, 227, 284, 285, 286, 287, 288, 289, 290, 292, 321, 322, 327, 329, 330, 331, 337, 338, 355, 365, 378, 380, 384, 387, 388, 389, 391, 392, 393, 394, 395, 419, 424, 425, 428, 429, 431, 432, 436, 437, 438, 439, 443, 444, 446, 447, 448, 449, 451, 453, 457, 458, 459, 460, 462, 464, 465, 467, 468, 470 and 471 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions. In another aspect, the present disclosure refers to a nucleic acid molecule encoding the MDC variant as described herein. In another aspect, the present disclosure refers to a vector comprising the nucleic acid molecule as described herein. In another aspect, the present disclosure refers to a host cell comprising the nucleic acid molecule as described herein or the vector as described herein. In another aspect, the present disclosure refers to the use of the MDC variant as described herein or the host cell as described herein for the conversion of 3-methylcrotonic acid into isobutene. In another aspect, the present disclosure refers to a method for producing isobutene from 3-methylcrotonic acid by incubating 3-methylcrotonic acid with the MDC variant as described herein. In another aspect, the present disclosure refers to a composition comprising a variant of an MDC as described herein, the nucleic acid molecule as described herein, the vector as described herein or the host cell as described herein. In another aspect, the present disclosure refers to a composition comprising a variant of an MDC as described herein, the nucleic acid molecule as described herein, the

vector as described herein or the host cell as described herein and 3-methylcrotonic acid.

Detailed Description

Thus, the present invention provides a variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is derived as defined in the claims.
An improved enzyme variant or an enzyme variant capable of catalyzing a reaction with increased activity is defined as an enzyme variant which differs from the wildtype enzyme and which catalyzes the conversion of 3-methylcrotonic acid into isobutene so that the specific activity of the enzyme variant is higher than the specific activity of the wildtype enzyme for at least one given concentration of a 3-methylcrotonic acid (preferably any 3-methylcrotonic acid higher than 0 M and up to 1 M). A specific activity is defined as the number of moles of substrate converted to moles of product by unit of time by mole of enzyme. K_{cat} (turnover number) is the specific activity at saturating concentration of substrate.

In the context of the present invention, an “improved activity” means that the activity of the enzyme in question is at least 10%, preferably at least 20%, more preferably at least 30% or 50%, even more preferably at least 70% or 80% and particularly preferred at least 90% or 100% higher than that of the enzyme from which the variant is derived, preferably higher than that of the enzyme represented by SEQ ID NO:1. In even more preferred embodiments the improved activity may be at least 150%, at least 200%, at least 300%, at least 750% or at least 1000% higher than that of the corresponding enzyme from which the variant is derived, preferably higher than that of the enzyme represented by SEQ ID NO:1. In a particularly preferred embodiment, the activity is measured by using an assay with purified enzyme and chemically synthesized substrates, as described below. The improved activity of a variant can be measured as a higher isobutene production in a given time under defined conditions, compared with the parent enzyme. This improved activity can result from a higher turnover number, e.g. a higher kcat value. It can also result from a lower Km value. It can also

CLAIMS

1. A variant of a 3-methylcrotonic acid decarboxylase (MDC) showing an improved activity in converting 3-methylcrotonic acid into isobutene over the corresponding MDC from which it is derived and having an amino acid sequence as shown in SEQ ID NO:1 or an amino acid sequence having at least 55% sequence identity to SEQ ID NO:1, in which one or more amino acid residues at a position selected from the group consisting of positions 434, 7, 17, 27, 33, 35, 45, 46, 48, 51, 140, 144, 183, 184, 185, 222, 227, 284, 285, 286, 287, 288, 289, 290, 292, 321, 322, 327, 329, 330, 331, 337, 338, 355, 365, 378, 380, 384, 387, 388, 389, 391, 392, 393, 394, 395, 419, 424, 425, 428, 429, 431, 432, 436, 437, 438, 439, 443, 444, 446, 447, 448, 449, 451, 453, 457, 458, 459, 460, 462, 464, 465, 467, 468, 470 and 471 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions, are substituted with another amino acid residue or deleted or wherein an insertion has been effected at one or more of these positions.

2. The MDC variant of claim 1, wherein
   (1) an amino acid residue at position 7 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or
   (2) an amino acid residue at position 17 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or
   (3) an amino acid residue at position 27 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or
   (4) an amino acid residue at position 33 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or
   (5) an amino acid residue at position 35 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or
   (6) an amino acid residue at position 45 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or glutamine; and/or

(7) an amino acid residue at position 46 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or isoleucine; and/or

(8) an amino acid residue at position 48 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or

(9) an amino acid residue at position 51 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate; and/or

(10) an amino acid residue at position 140 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or

(11) an amino acid residue at position 144 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or

(12) an amino acid residue at position 183 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or

(13) an amino acid residue at position 184 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or serine; and/or

(14) an amino acid residue at position 185 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or

(15) an amino acid residue at position 122 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

(16) an amino acid residue at position 227 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or tyrosine; and/or

(17) an amino acid residue at position 284 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate or glutamate or a proline is inserted; and/or

(18) an amino acid residue at position 285 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, histidine, isoleucine, leucine, methionine, proline, serine, threonine or valine; and/or

(19) an amino acid residue at position 286 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, aspartate, glycine, histidine, asparagine, serine or tryptophan; and/or

(20) an amino acid residue at position 287 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, lysine, proline, glutamine, arginine, serine, threonine or valine or a serine is inserted; and/or

(21) an amino acid residue at position 288 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or threonine; and/or

(22) an amino acid residue at position 289 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, histidine, methionine, glutamine, arginine, tryptophan or tyrosine; and/or

(23) an amino acid residue at position 290 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamate; and/or

(24) an amino acid residue at position 292 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or valine; and/or

(25) an amino acid residue at position 321 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine or valine; and/or

(26) an amino acid residue at position 322 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or

(27) an amino acid residue at position 327 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or

(28) an amino acid residue at position 329 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or

(29) an amino acid residue at position 330 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

(30) an amino acid residue at position 331 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or

(30) an amino acid residue at position 331 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or

(31) an amino acid residue at position 337 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with threonine; and/or

(32) an amino acid residue at position 338 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or

(33) an amino acid residue at position 355 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine, threonine or valine; and/or

(34) an amino acid residue at position 365 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, methionine, serine or threonine; and/or

(35) an amino acid residue at position 378 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or

(36) an amino acid residue at position 380 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

(37) an amino acid residue at position 384 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glycine, leucine, serine or threonine; and/or

(38) an amino acid residue at position 387 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or

(39) an amino acid residue at position 388 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine, glutamine or serine; and/or

(40) an amino acid residue at position 389 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, histidine or glutamine; and/or

(41) an amino acid residue at position 391 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine or leucine; and/or

(42) an amino acid residue at position 392 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or

(43) an amino acid residue at position 393 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or

(44) an amino acid residue at position 394 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, glycine, histidine, leucine, glutamine or arginine; and/or

(45) an amino acid residue at position 395 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

(46) an amino acid residue at position 419 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or proline; and/or

(47) at position 424 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, an alanine, aspartate, leucine, serine or tryptophan is inserted; and/or

(48) at position 425 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, a tryptophan is inserted.

(49) an amino acid residue at position 428 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine or threonine; and/or

(50) an amino acid residue at position 429 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartate or valine or a glycine or an asparagine is inserted; and/or

(51) an amino acid residue at position 431 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, lysine, methionine, asparagine, proline, threonine or valine or a glycine, leucine or asparagine is inserted; and/or

(52) an amino acid residue at position 432 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, phenylalanine or tryptophan; and/or

(53) an amino acid residue at position 434 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, methionine, valine or tryptophan; and/or

(54) an amino acid residue at position 436 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, methionine, asparagine, glutamine, threonine or tryptophan; and/or

(55) an amino acid residue at position 437 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, glycine, isoleucine, leucine, methionine, threonine or valine; and/or

(56) an amino acid residue at position 438 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine, methionine or tryptophan or an aspartate is inserted; and/or

(57) an amino acid residue at position 439 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine or proline; and/or

(58) an amino acid residue at position 443 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine or an alanine, glycine, leucine, arginine, threonine or valine is inserted; and/or

(59) an amino acid residue at position 444 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, isoleucine, leucine, methionine, asparagine, arginine, threonine or valine or a glutamate, glycine, histidine, lysine, leucine, asparagine, proline, arginine, serine, threonine or valine is inserted; and/or

(60) an amino acid residue at position 446 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate, glycine or proline; and/or

(61) an amino acid residue at position 447 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, histidine, isoleucine, threonine or tryptophan or a lysine, leucine, proline or tyrosine is inserted; and/or

(62) an amino acid residue at position 448 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine, glutamate, threonine or valine or a serine is inserted; and/or

(63) an amino acid residue at position 449 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine or tryptophan valine or a serine is inserted; and/or

(64) an amino acid residue at position 451 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or

(65) an amino acid residue at position 453 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or

(66) an amino acid residue at position 457 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or

(67) an amino acid residue at position 458 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine, glutamate, isoleucine, leucine, methionine, glutamine or arginine or a glycine is inserted; and/or

(68) an amino acid residue at position 459 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, leucine, proline, arginine or threonine; and/or

(69) an amino acid residue at position 460 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted glycine, histidine, isoleucine, leucine, methionine, valine or tyrosine; and/or

(70) an amino acid residue at position 462 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, cysteine, isoleucine, proline, arginine, threonine or valine or an alanine is inserted; and/or

(71) an amino acid residue at position 464 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with proline; and/or

(72) an amino acid residue at position 465 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, isoleucine or valine; and/or

(73) an amino acid residue at position 467 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tryptophan; and/or

(74) an amino acid residue at position 468 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or

(75) an amino acid residue at position 470 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine, serine or threonine; and/or

(76) an amino acid residue at position 471 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine.

3. The MDC variant of claim 1 or 2, wherein said variant furthermore shows at least one modification at positions 6, 15, 25, 39, 43, 72, 89, 126,141,189, 223, 224, 272, 283, 332, 333, 336, 361, 366, 368, 379, 382, 385, 418, 421, 422, 423, 426, 430, 440, 441, 445, 461, 463, 469 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions.

4. The MDC variant of any one of claims 1 to 3, wherein said variant furthermore shows at least one modification at positions 6, 15, 25, 39, 43, 72, 89, 126,141,189, 223, 224, 272, 283, 332, 333, 336, 361, 366, 368, 379, 382, 385, 418, 421, 422, 423, 426, 430, 440, 441, 445, 461, 463, 469 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to any of these positions is an MDC variant, wherein

(1) an amino acid residue at position 6 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or

(2) an amino acid residue at position 15 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine or leucine; and/or

(3) an amino acid residue at position 25 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with leucine; and/or

(4) an amino acid residue at position 39 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glycine; and/or

(5) an amino acid residue at position 43 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamate; and/or

(6) an amino acid residue at position 72 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine; and/or

(7) an amino acid residue at position 89 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with asparagine; and/or

(8) an amino acid residue at position 126 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine; and/or

(9) an amino acid residue at position 141 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine, leucine, glutamine, serine or tyrosine; and/or

(10) an amino acid residue at position 189 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine; and/or

(11) an amino acid residue at position 223 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with tyrosine; and/or

(12) an amino acid residue at position 224 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, proline or glutamine; and/or

(13) an amino acid residue at position 272 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine, lysine, leucine or arginine; and/or

(14) an amino acid residue at position 283 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine, phenylalanine, histidine, leucine, glutamine, serine, threonine or valine; and/or

(15) an amino acid residue at position 332 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, arginine or serine; and/or

(16) an amino acid residue at position 333 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with methionine; and/or

(17) an amino acid residue at position 336 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, asparagine or glutamine; and/or

(18) an amino acid residue at position 361 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with lysine; and/or

(19) an amino acid residue at position 3665 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine; and/or

(20) an amino acid residue at position 368 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with serine; and/or

(21) an amino acid residue at position 379 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with isoleucine; and/or

(22) an amino acid residue at position 382 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine or leucine; and/or

(23) an amino acid residue at position 385 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate, phenylalanine, glycine, leucine, proline, arginine, serine, threonine or tryptophan; and/or

(24) an amino acid residue at position 418 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with valine; and/or

(25) an amino acid residue at position 421 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with phenylalanine, lysine, leucine, threonine, valine or tryptophan or an aspartate, glutamate, glycine, isoleucine, proline, arginine serine or theronine is inserted; and/or

(26) an amino acid residue at position 422 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate, phenylalanine, glycine, histidine, leucine, glutamine, serine or valine or a lysine is inserted; and/or

(27) an amino acid residue at position 423 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with cysteine or glycine; and/or

(28) an amino acid residue at position 426 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with histidine, isoleucine, leucine, threonine, valine, tryptophan or tyrosine or a leucine is inserted; and/or

(29) an amino acid residue at position 430 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, lysine, asparagine, proline, arginine, serine, threonine or valine or a cysteine, glycine, histidine, isoleucine, leucine, asparagine, glutamine, arginine or serine is inserted; and/or

(30) an amino acid residue at position 440 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate or isoleucine; and/or

(31) an amino acid residue at position 441 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with aspartate, glycine, histidine, isoleucine, leucine, proline, threonine or valine; and/or

(32) an amino acid residue at position 445 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, glutamine or threonine or a glutamine is inserted; and/or

(33) an amino acid residue at position 461 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartate, glutamate, glycine, asparagine, serine, threonine, valine or tyrosine; and/or

(34) an amino acid residue at position 463 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with glutamate, phenylalanine, glycine, isoleucine, leucine, methionine, proline, serine, threonine or valine; and/or

(35) an amino acid residue at position 469 in the amino acid sequence shown in SEQ ID NO:1 or at a position corresponding to this position, is deleted or substituted with alanine, aspartate, glutamate, histidine, asparagine, serine or threonine.

5. A nucleic acid molecule encoding the MDC variant of any one of claims 1 to 4.

6. A vector comprising the nucleic acid molecule of claim 5.

7. A host cell comprising the nucleic acid molecule of claim 5 or the vector of claim 6.

8. Use of the MDC variant of any one of claims 1 to 4 or the host cell of claim 7 for the conversion of 3-methylcrotonic acid into isobutene.

9. A method for producing isobutene from 3-methylcrotonic acid by incubating 3-methylcrotonic acid with the MDC variant of any one of claims 1 to 4.

10. The method of claim 9, wherein the enzymatic conversion is carried out in vitro.

11. A composition comprising a variant of an MDC of any one of claims 1 to 4, the nucleic acid molecule of claim 5, the vector of claim 6 or the host cell of claim 7.

12. A composition comprising a variant of an MDC of any one of claims 1 to 4, the nucleic acid molecule of claim 5, the vector of claim 6 or the host cell of claim 7 and 3-methylcrotonic acid.