Claims

1. A method for the production of a methacrylate comprising

   a) providing a genetically modified microorganism with increased tolerance to a C_3-C_{12} methacrylate ester compared to the wild type microorganism in a fermentation medium and

   b) growing the microorganism under conditions whereby a C_3-C_{12} methacrylate ester is produced;

   wherein the genetically modified microorganism comprises a mutation in one or more of the following nucleic acid sequences: a *soxR* (redox-sensitive transcriptional activator) nucleic acid sequence, an *acrR* (acriflavine resistance regulator) nucleic acid sequence, a *rob* (right origin-binding) nucleic acid sequence and/or a *marR* (multiple antibiotic resistance protein) nucleic acid sequence.

2. The method according to claim 1 wherein the microorganism is tolerant to at least 20% v/v C_3-C_{12} methacrylate ester when grown in a liquid medium at about 37°C.

3. The method according to claim 1 or 2 wherein said microorganism is a bacterium.

4. The method according to a preceding claim wherein said microorganism is selected from the family of *Enterobacteriaceae*.

5. The method according to a preceding claim wherein said bacterium is selected from the genus *Escherichia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Serratia*, *Erwinia*, *Salmonella*, *Morganella*, *Brevibacterium*, *Corynebacterium*, *Microbacterium Alicyclobacillus*, *Bacillus*, *Hydrogenobacter*, *Methanococcus*, *Acetobacter*, *Acinetobacter*, *Agrobacterium*, *Axorhizobium*, *Azotobacter*, *Anaplasma*, *Bacteroides*, *Bartonella*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Calymmatobacterium*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Clostridium*, *Coxiella*, *Cupriavidus*, *Ehrlichia*, *Enterococcus*, *Francisella*, *Fusobacterium*, *Gardnerella*, *Haemophilus*, *Helicobacter*, *Methanobacterium*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pasteurella*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Pseudomonas*, *Rhizobium*, *Rickettsia*, *Rochalimaea*, *Rothia*, *Shigella*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, *Treponema*, *Vibrio*, *Wolbachia*, *Yersinia.*

6. The method according to claim 5 wherein the bacterium is *E. coli*.

7. The method according to a preceding claim wherein the genetically modified microorganism comprises a mutation in one or more nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance systems.

~~8. The method according to a preceding claim wherein the genetically modified microorganism comprises a mutation in one or more of the following nucleic acid sequences: a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.~~

~~9~~8. The method according to any of claims ~~8~~ 1 to 7 wherein the *soxR* nucleic acid sequence comprises SEQ ID NO. 1 or a homologue thereof, the *acrR* nucleic acid sequence comprises SEQ ID NO. 2 or a homologue thereof, the *rob* nucleic acid sequence comprises SEQ ID NO. 5 or a homologue thereof and the *marR* nucleic acid sequence comprises SEQ ID NO. 7 or a homologue thereof.

~~10~~9. The method according to any of claims 1 to ~~8 or 9~~ wherein the mutant *soxR* nucleic acid sequence encodes a mutant SoxR protein with a mutation in the DNA binding domain or in the FE-S cluster domain.

~~11~~10. The method according to claim ~~10~~ 9 wherein the mutation is selected from one of the following: a substitution of R20 with another amino acid, a substitution of R20 with another amino acid, deletion of residue 146, or a truncation at residue 139 in SoxR as set forth in SEQ ID NO.2.

~~12~~11. The method according to any of claims claim ~~8~~ 1 to ~~11~~ 10 wherein the mutant *acrR* nucleic acid sequence encodes a mutant AcrR protein with a mutation in the DNA binding domain or in the ligand binding domain.

~~13~~12. The method according to claim ~~12~~ 11 wherein the mutation is selected from one of the following: a substitution of V29 with another amino acid, a frameshift mutation at T32, A191 or position 49 in AcrR as set forth in SEQ ID NO. 3.

~~14~~13. The method according to claims 8 to ~~13~~ 12 wherein the mutant *rob* nucleic acid sequence encodes a mutant Rob protein with a mutation in the N-terminal DNA binding domain or the C-terminal domain.

~~15~~14. The method according to claim ~~14~~ 13 wherein the mutation is selected from one of the following: a substitution of A70 or a substitution of R156 with another amino acid in Rob as set forth in SEQ ID NO. 5.

~~16~~15. The method according to any of claims ~~8~~ 1 to ~~15~~ 14 wherein the mutant *marR* nucleic acid sequence encodes a MarR protein with a mutation in the DNA binding domain.

~~17~~16. The method according to claim ~~16~~ 15 wherein the mutation is a substitution of V84 with another amino acid in MarR as set forth in SEQ ID NO. 7.

~~18~~17. The method according to a preceding claim wherein the genetically modified microorganism comprises a mutation in an acrR nucleic acid sequence and one or more of the following nucleic acid sequences: a soxR nucleic acid sequence, a rob nucleic acid sequence and/or a marR nucleic acid sequence.

~~19~~18. The method according to a preceding claim wherein the genetically modified organism comprises one or more further mutation~~, for example selected from Table 1 a or 1b~~.

~~20~~19. The method according to claim ~~19~~ 18 wherein the one or more further mutation is in a nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance systems.

~~21~~20. The method according to a preceding claim wherein the microorganism further comprises a vector or construct encoding an enzyme of the C_3-C_{12} methacrylate ester biosynthetic pathway.

~~22~~21. The method according to a preceding claim wherein the microorganism further comprises or a vector or construct to enhance production of a C_3-C_{12} methacrylate ester.

~~23~~22. The method according to a preceding claim comprising the steps of removing said C_3-C_{12} methacrylate ester from the fermentation medium and transesterifying the removed C_3-C_{12} methacrylate ester with methanol.

~~24~~23. The method according to a preceding claim wherein said C_3-C_{12} methacrylate ester is butyl methacrylate (BMA).

~~25~~24. A method for increasing the tolerance of a microorganism to a C_3-C_{12} methacrylate ester comprising introducing a mutation into a nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance systems; wherein the genetically modified microorganism comprises a mutation in one or more of the following nucleic acid sequences: a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.

~~26~~25. A method for growing or maintaining a microorganism in the presence of a C_3-C_{12} methacrylate ester comprising providing a genetically modified microorganism with increased tolerance to a C_3-

C_{12} methacrylate ester compared to a wild type microorganism in a fermentation medium under conditions whereby a C_3-C_{12} methacrylate ester is produced; wherein the genetically modified microorganism comprises a mutation in one or more of the following nucleic acid sequences: a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.

~~27~~26. A fermentation medium comprising a C_3-C_{12} methacrylate ester and a genetically modified microorganism with increased tolerance to a C_3-C_{12} methacrylate ester compared to a wild type microorganism; wherein the genetically modified microorganism comprises a mutation in one or more of the following nucleic acid sequences: a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.

~~28~~27. A use of a genetically modified microorganism with increased tolerance to a C_3-C_{12} methacrylate ester compared to a wild type microorganism for producing a C_3-C_{12} methacrylate ester; wherein the genetically modified microorganism comprises a mutation in one or more of the following nucleic acid sequences: a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.

~~29~~28. The use according to claim ~~28~~ 27 wherein said microorganism is selected from the family of *Enterobacteriaceae*.

~~30~~29. The use according to claim ~~28~~ 27 or ~~29~~ 28 wherein said bacterium is selected from the genus *Escherichia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Serratia*, *Erwinia*, *Salmonella*, *Morganella*, *Brevibacterium*, *Corynebacterium*, *Microbacterium Alicyclobacillus*, *Bacillus*, *Hydrogenobacter*, *Methanococcus*, *Acetobacter*, *Acinetobacter*, *Agrobacterium*, *Axorhizobium*, *Azotobacter*, *Anaplasma*, *Bacteroides*, *Bartonella*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Calymmatobacterium*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Clostridium*, *Coxiella*, *Cupriavidus*, *Ehrlichia*, *Enterococcus*, *Francisella*, *Fusobacterium*, *Gardnerella*, *Haemophilus*, *Helicobacter*, *Methanobacterium*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pasteurella*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Pseudomonas*, *Rhizobium*, *Rickettsia*, *Rochalimaea*, *Rothia*, *Shigella*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, *Treponema*, *Vibrio*, *Wolbachia*, *Yersinia*

~~31~~30. The use according to claim ~~30~~ 29 wherein the bacterium is *E. coli*.

~~32~~31. The use according to any of claims ~~28~~ 27 to ~~31~~ 29 wherein genetically modified microorganism comprises a mutation in one or more nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance system.

~~33. The use according to any of claims 28 to 32 wherein the genetically modified microorganism comprises a mutation in a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.~~

~~34~~32. The use according to any of claims ~~33~~ 27 to 31 wherein the *soxR* nucleic acid sequence comprises SEQ ID NO. 1 or a homologue thereof, the *acrR* nucleic acid sequence comprises SEQ ID NO. 2 or a homologue thereof, the *rob* nucleic acid sequence comprises SEQ ID NO. 5 or a homologue thereof and the *marR* nucleic acid sequence comprises SEQ ID NO. 7 or a homologue thereof.

~~35~~33. The use according to claim ~~34~~ 32 wherein the mutant *soxR* nucleic acid sequence encodes a mutant SoxR protein with a mutation in the DNA binding domain or in the FE-S cluster domain.

~~36~~34. The use according to claim ~~35~~ 33 wherein the mutation is selected from one of the following: a substitution of R20 with another amino acid, a substitution of R20 with another amino acid, deletion of residue 146 or a truncation at residue 139 in SoxR as set forth in SEQ ID NO.2.

~~37~~35. The use according to any of claims ~~33~~ 27 to ~~36~~ 34 wherein the mutant *acrR* nucleic acid sequence encodes a mutant AcrR protein with a mutation in the DNA binding domain or in the ligand binding domain.

~~38~~36. The use according to claim ~~37~~ 35 wherein the mutation is selected from one of the following: a substitution of V29 with another amino acid, a frameshift mutation at T32, A191 or position 49 in AcrR as set forth in SEQ ID NO. 3.

~~39~~37. The use according to any of claims ~~33~~ 27 to ~~38~~ 36 wherein the mutant *rob* nucleic acid sequence encodes a mutant Rob protein with a mutation in the N-terminal DNA binding domain or the C-terminal domain.

~~40~~38. The use according to claim ~~39~~ 37 wherein the mutation is a substitution of A70 or a substitution of R156 with another amino acid in Rob as set forth in SEQ ID NO. 5.

~~41~~39. The use according to any of claims ~~33~~ 27 to ~~40~~ 38 wherein the mutant *marR* nucleic acid sequence encodes a MarR protein with a mutation in the DNA binding domain.

~~42~~40. The use according to claim ~~41~~ 39 wherein the mutation is a substitution of V84 with another amino acid in MarR as set forth in SEQ ID NO. 7.

4341. The use according to any of claims ~~28~~ 27 to ~~42~~ 40 wherein the genetically modified organism comprises one or more further mutation.

4442. The use according to claim ~~43~~ 41 wherein the one or more further mutation is in a nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance system.

4543. A genetically modified microorganism wherein said microorganism comprises one of the following mutations: a) a mutation in a *marR* nucleic acid sequence encoding a mutant protein wherein V84 is substituted with another amino acid; b) a mutation in a *Rob* nucleic acid sequence encoding a mutant protein wherein A70 or R156 is substituted with another amino acid; c) a mutation in a *soxR* nucleic acid sequence encoding a mutant protein wherein R20 is substituted with another amino acid, residue 146 is deleted or the protein is truncated at residue 139 or d) a mutation in an *acrR* nucleic acid sequence encoding a mutant protein a frameshift mutation at T32, A191 or position 49 in AcrR or e) a mutation in two or more nucleic acids selected from the following: a *soxR*, *acrR*, *marR* and/or a *rob* nucleic acid or a homolog thereof wherein said nucleic acid encodes a mutant protein.

4644. A genetically modified microorganism according to claim ~~45~~ 43 wherein said microorganism is selected from the family of *Enterobacteriaceae*.

4745. A genetically modified microorganism according to claim ~~45~~ 43 or ~~46~~ 44 wherein said bacterium is selected from the genus *Escherichia*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Serratia*, *Erwinia*, *Salmonella*, *Morganella*, *Brevibacterium*, *Corynebacterium*, *Microbacterium Alicyclobacillus*, *Bacillus*, *Hydrogenobacter*, *Methanococcus*, *Acetobacter*, *Acinetobacter*, *Agrobacterium*, *Axorhizobium*, *Azotobacter*, *Anaplasma*, *Bacteroides*, *Bartonella*, *Bordetella*, *Borrelia*, *Brucella*, *Burkholderia*, *Calymmatobacterium*, *Campylobacter*, *Chlamydia*, *Chlamydophila*, *Clostridium*, *Coxiella*, *Cupriavidus, Ehrlichia*, *Enterococcus*, *Francisella*, *Fusobacterium*, *Gardnerella*, *Haemophilus*, *Helicobacter*, *Methanobacterium*, *Micrococcus*, *Moraxella*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pasteurella*, *Peptostreptococcus*, *Porphyromonas*, *Prevotella*, *Pseudomonas*, *Rhizobium*, *Rickettsia*, *Rochalimaea*, *Rothia*, *Shigella*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, *Treponema*, *Vibrio*, *Wolbachia*, *Yersinia.*

4846. A genetically modified microorganism according to a claim ~~47~~ 45 wherein the bacterium is *E. coli*.

4947. A genetically modified microorganism according to any of claims ~~45~~ 43 to ~~48~~ 46 wherein the genetically modified microorganism comprises a mutation in one or more nucleic acid encoding a

protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance system.

~~50. A genetically modified microorganism according to any of claims 45 to 49 wherein the genetically modified microorganism comprises a mutation in a *soxR* nucleic acid sequence, an *acrR* nucleic acid sequence, a *rob* nucleic acid sequence and/or a *marR* nucleic acid sequence.~~

~~51~~48. A genetically modified microorganism according to any of claims ~~50~~ 43 to 47 wherein the *soxR* nucleic acid sequence comprises SEQ ID NO. 1 or a homologue thereof, the *acrR* nucleic acid sequence comprises SEQ ID NO. 2 or a homologue thereof, the *rob* nucleic acid sequence comprises SEQ ID NO. 5 or a homologue thereof and the *marR* nucleic acid sequence comprises SEQ ID NO. 7 or a homologue thereof.

~~52~~49. A genetically modified microorganism of any of claims ~~50 or 51~~43 to 48 wherein the mutant *soxR* nucleic acid sequence encodes a mutant SoxR protein with a mutation in the DNA binding domain or in the FE-S cluster domain.

~~53~~50. A genetically modified microorganism according to claim ~~52~~ 49 wherein the mutation is selected from one of the following: a substitution of R20 with another amino acid, a substitution of R20 with another amino acid, deletion of residue 146, or a truncation at residue 139 in SoxR as set forth in SEQ ID NO. 2.

~~54~~51. A genetically modified microorganism according to any of claims ~~50 to 53~~43 to 50 wherein the mutant *acrR* nucleic acid sequence encodes a mutant AcrR protein with a mutation in the DNA binding domain or in the ligand binding domain.

~~55~~52. A genetically modified microorganism according to claim ~~54~~ 51 wherein the mutation is selected from one of the following: a substitution of V29 with another amino acid, a frameshift mutation at T32, A191 or position 49 in AcrR as set forth in SEQ ID NO. 3.

~~56~~53. A genetically modified microorganism according to any of claims ~~50~~ 43 to ~~55~~ 52 wherein the mutant *rob* nucleic acid sequence encodes a mutant Rob protein with a mutation in the N-terminal DNA binding domain or the C-terminal domain.

~~57~~54. A genetically modified microorganism according to claim ~~56~~ 53 wherein the mutation is a substitution of A70 or a substitution of R156 with another amino acid in Rob as set forth in SEQ ID NO. 5.

~~58~~55. A genetically modified microorganism according to any of claims ~~50 43~~ to ~~57 54~~ wherein the mutant *marR* nucleic acid sequence encodes a MarR protein with a mutation in the DNA binding domain.

~~59~~56. A genetically modified microorganism according to claim ~~58 55~~ wherein the mutation is a substitution of V84 with another amino acid in MarR as set forth in SEQ ID NO. 7.

~~60~~57. A genetically modified microorganism according to any of claims ~~45 43~~ to ~~59 56~~ wherein the genetically modified organism comprises one or more further mutation ~~selected from Table 1 a or 1b~~ in a nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance system.

~~61. A genetically modified microorganism according to claim 60 wherein the one or more further mutation is in a nucleic acid encoding a protein that regulates or forms part of the oxidative stress response and the bacterial multidrug resistance system.~~

~~62~~58. A genetically modified microorganism according to any of claims ~~45 43~~ to ~~61 57~~ further comprising a genetic construct or vector to enhance production of a C_3-C_{12} methacrylate ester.

~~63~~59. The use of an isolated genetically modified microorganism according to any of claims ~~45 43~~ to ~~62 58~~ for producing a C_3-C_{12} methacrylate ester.

~~64~~60. A method for the isolation of a methacrylate tolerant microorganism comprising:

a) providing a microorganism in a fermentation medium

b) contacting the microorganism with a methacrylate; and

c) isolating the viable microorganism of step (b)

wherein the viable microorganism is tolerant to at least 20% v/v a methacrylate when grown in liquid medium at about 37°C.

~~65~~61. A methacrylate tolerant microorganism obtained or obtainable by a method of claim ~~64~~60.