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 any of claims 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.

9. The method according to any of claims 1 to 8 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.

10. The method according to claim 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.

11. The method according to any of claims claim 1 to 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.

12. The method according to claim 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.

13. The method according to claims 8 to 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.

14. The method according to claim 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.

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

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

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.

18. The method according to a preceding claim wherein the genetically modified organism comprises one or more further mutation.

19. The method according to claim 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.

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.

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.

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.

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

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.

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.

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.

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.

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

29. The use according to claim 27 or 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*

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

31. The use according to any of claims 27 to 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.

32. The use according to any of claims 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.

33. The use according to claim 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.

34. The use according to claim 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.

35. The use according to any of claims 27 to 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.

36. The use according to claim 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.

37. The use according to any of claims 27 to 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.

38. The use according to claim 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.

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

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

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

42. The use according to claim 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.

43. 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.

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

45. A genetically modified microorganism according to claim 43 or 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.*

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

47. A genetically modified microorganism according to any of claims 43 to 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.

48. A genetically modified microorganism according to any of claims 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.

49. A genetically modified microorganism of any of claims 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.

50. A genetically modified microorganism according to claim 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.

51. A genetically modified microorganism according to any of claims 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.

52. A genetically modified microorganism according to claim 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.

53. A genetically modified microorganism according to any of claims 43 to 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.

54. A genetically modified microorganism according to claim 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.

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

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

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

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

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

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.

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