Supplementary Material

Heavy-metal-resistant microorganisms in deep-sea sediments disturbed by mining activity: an application towards the development of an experimental in vitro systems.

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**List of Contents:**

**Table S1. Sampling location details**

**Table S2. 16S rRNA BLASTN analysis results**

**Table S3. Combinations of phenotypical test giving a unique bacterial identification**

**Table S4. Mn cell trafficking putative genes sequences accession number list**

**Table S1. Sampling location details**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Probe Nr. | Date  (UTC) | Latitude  (N) | Longitude  (W) | Water depth  (m) | Core length  (cm) |
| SO240-14MUC | 11.05.15 | 13°10,528' | 118°10,108' | 4332 | 10 |
| SO240-95MUC | 05.06.15 | 11°49,262' | 117°13,197' | 4150 | - |

**Table S2. 16S rRNA BLASTN analysis results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sample | Laboratory  Reference | GenBank  accession Nr. | Isolation depth  (cm) | Closest phylogenetic relative  (Accession no., organism) | Homology (%) |
| 14 MUC | SO240BG01 | MK254646 | Water | *NR\_043546.1, Arthrobacter subterraneus* strain M1406 | 99 |
| SO240BG02 | MK254647 | 6 -7 | *NR\_116685.1, Dietzia maris* strain DSM 43672 | 99 |
| SO240BG03 | MK254648 | 8 - 9 | 99 |
| SO240BG04 | MK254649 | 0 - 1 | *NR\_028741.1, Erythrobacter citreus* strain RE35F/1 | 99 |
| SO240BG05 | MK254650 | 1 - 2 | 99 |
| SO240BG06 | MK254651 | 2 - 3 | 99 |
| SO240BG07 | MK254652 | 5 - 6 | 99 |
| SO240BG08 | MK254653 | 7 - 8 | 99 |
| SO240BG09 | MK254654 | Water | NR\_042063.1, *Halomonas aquamarina* strain DSM 30161 | 99 |
| SO240BG10 | MK254655 | 0 - 1 | NR\_042066.1, *Halomonas meridiana* strain DSM 5425 | 99 |
| SO240BG11 | MK254656 | 1 - 2 | 99 |
| SO240BG12 | MK254657 | 2 - 3 | 99 |
| SO240BG13 | MK254658 | 3 - 4 | 99 |
| SO240BG14 | MK254659 | 4 - 5 | 99 |
| SO240BG15 | MK254660 | 6 - 7 | 99 |
| SO240BG16 | MK254661 | 7 - 8 | 99 |
| SO240BG17 | MK254662 | 3 - 4 | NR\_028924.1, *Kocuria polaris* strain CMS 76or | 99 |
| SO240BG18 | MK254663 | 5 - 6 | 99 |
| SO240BG19 | MK254664 | 0 - 1 | NR\_114307.1, *Loktanella cinnabarina* strain LL-001 | 99 |
| SO240BG20 | MK254665 | Water | NR\_025799.1, *Marinobacter flavimaris* isolate: D1-1M | 99 |
| SO240BG21 | MK254666 | 1 - 2 | NR\_125458.1, *Pseudoalteromonas shioyasakiensis* strain SE3 | 99 |
| SO240BG22 | MK254667 | 2 - 3 | 99 |
| SO240BG23 | MK254668 | 3 - 4 | 99 |
| SO240BG24 | MK254669 | 4 - 5 | 99 |
| SO240BG25 | MK254670 | 5 - 6 | 99 |
| SO240BG26 | MK254671 | 6 - 7 | 99 |
| SO240BG27 | MK254672 | 8 - 9 | 99 |
| SO240BG28 | MK254673 | 9 -10 | 99 |
| SO240BG29 | MK254674 | 1 - 2 | NR\_041715.1, *Pseudomonas stutzeri* strain 13635O | 99 |
| SO240BG30 | MK254675 | 3 - 4 | 99 |
| SO240BG31 | MK254676 | 7 - 8 | 99 |
| 95 MUC | SO240BG32 | MK254677 | Nodule 1 - 2 | AM110948.1, *Bacillus subtilis* isolate B-3141 | 99 |
| SO240BG33 | MK254678 | Nodule 1 - 2 | *NR\_028741.1, Erythrobacter citreus* strain RE35F/1 | 99 |
| SO240BG34 | MK254679 | Nodule 0 - 1 | NR\_042063.1, Halomonas aquamarina strain DSM 30161 | 99 |
| SO240BG35 | MK254680 | Nodule 0 - 1 | NR\_027219.1, Halomonas axialensis strain Althf1 | 99 |
| SO240BG36 | MK254681 | Nodule 1 - 2 | 99 |
| SO240BG37 | MK254682 | Nodule 1 - 2 | NR\_042066.1, Halomonas meridiana strain DSM 5425 | 99 |
| SO240BG38 | MK254683 | Nodule 0 - 1 | NR\_041715.1, Pseudomonas stutzeri strain ATCC 17588 | 99 |
| SO240BG39 | MK254684 | Nodule 1 - 2 | 99 |
| SO240BG40 | MK254685 | Nodule 1 - 2 | KU904404.1, Rhodococcus erythropolis strain JA30 | 99 |

**Table S3. Combinations of phenotypical test giving a unique bacterial identification**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test** | **Arthrobacter subterraneus**  **(SO240BG6)** | **Bacillus subtilis**  **(SO240BG40)** | **Dietzia Maris**  **(SO240BG8)** | **Dietzia Maris**  **(SO240BG10)** | **Erythrobacter citreus**  **(SO240BG22)** |
|
| **General** | Motility & Pigmentation  Gram & Pigmentation | Gram & Pigmentation  Gram & Motility |  |  | Pigmentation |
| **API-20NE** |  |  |  |  | Malic acid |
| **API-20NE + general** | Nitrite reduction & Pigmentation  D-mannose & Pigmentation  Capric acid & Pigmentation  Adipic acid & Pigmentation  Trisodium citrate & Pigmentation |  |  |  | D-glucose & Motility  D-mannitol & Motility  D-maltose & Motility  Potassium gluconate & Motility |
| **API-ZYM** |  | Esterase/ Alkaline phosphatase  α-Glucosidase & Alkaline phosphatase  Esterase lipase & Alkaline phosphatase |  | Valine arylamidase & N-acetyl-glucosamine  Cystine arylamidase & N-acetyl-glucosamine  Valine arylamidase & Lipase  Cystine arylamidase & Valine arylamidase  Acid phosphatase & Valine arylamidase  α-Glucosidase & Valine arylamidase  N-acetyl-β-glucosaminidase & Valine arylamidase | α-Glucosidase & Cystine arylamidase  N-acetyl-β-glucosaminidase & Potassium gluconate |
| **API-ZYM + general** | Esterase &Pigmentation  Lipase & Pigmentation  α-Glucosidase & Pigmentation  Adipic acid & Gram  Esterase & Gram | Alkaline phosphatase & Gram  α-Glucosidase & Gram | Trisodium citrate & Pigmentation  Valine arylamidase & Pigmentation  Cystine arylamidase & Pigmentation | Valine arylamidase & Pigmentation  Cystine arylamidase & Pigmentation  Valine arylamidase & Motility  Valine arylamidase & Gram  Cystine arylamidase & Gram | α-Glucosidase & Motility |
| **20NE +ZYM** | N-acetyl-glucosamine & D-mannose  Adipic acid & D-mannose  Adipic acid & D-maltose  Adipic acid & Potassium gluconate  Esterase & Adipic acid |  |  | Valine arylamidase & D-glucose  Valine arylamidase & D-mannose  Cystine arylamidase & D-mannose  Valine arylamidase & D-mannitol  Valine arylamidase & D-maltose  Valine arylamidase & Potassium gluconate  Valine arylamidase & Adipic acid  Trisodium citrate & Pigmentation | Lipase & D-glucose  Valine arylamidase & D-glucose  Cystine arylamidase & D-glucose  Acid phosphatase & D-glucose  N-acetyl-β-glucosaminidase & D-glucose  Cystine arylamidase & L-arabinose  Lipase & D-mannitol  Cystine arylamidase & D-mannitol  Cystine arylamidase & D-maltose/Potassium gluconate  Cystine arylamidase & Adipic acid |

**Table S3. Continued**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Test** | **Halomonas aquamarina**  **(SO240BG31)** | **Halomonas axialensis**  **(SO240BG23)** | **Halomonas meridiana**  **(SO240BG3)** | **Halomonas meridiana**  **(SO240BG14)** | **Kocuria polaris**  **(SO240BG21)** | **Loktanella cinnabarina**  **(SO240BG12)** |
| **General** | Pigmentation |  |  |  | Pigmentation | Motility & Pigmentation |
| **API-20NE** | D-mannitol & D-glucose  Capric acid & Hydrolysis of Esculin  D-maltose & D-mannitol  Capric acid & D-mannitol  Adipic acid & D-mannitol  Trisodium citrate & D-mannitol |  |  |  |  |  |
| **API-20NE + general** |  |  |  |  |  |  |
| **API-ZYM** | Trypsin & Lipase |  |  |  |  | Leucine-arylamidase  α-Mannosidase  β-Glucuronidase  β-Glucosidase & Lipase  β-Galactosidase  Trypsin & Cystine arylamidase  α-chymotripsin & Cystine arylamidase  Acid phosphatase & Cystine arylamidase  β-Glucosidase & Cystine arylamidase  α-chymotripsin & Trypsin  β-Glucosidase & Trypsin  Acid phosphatase & α-chymotripsin  β-Glucosidase & α-chymotripsin  β-Glucosidase & Acid phosphatase |
| **API-ZYM + general** |  |  |  |  |  | Cystine arylamidase & Pigmentation  β-Glucosidase & Gram  Cystine arylamidase & Pigmentation  β-Glucosidase & Pigmentation  Alkaline phosphatase & Motility  Trypsin & Motility  α-chymotripsin & Motility |
| **20NE +ZYM** | Trypsin & L-arabinose  Trypsin & D-mannitol  α-Glucosidase & D-mannitol |  |  | Alkaline phosphatase & Nitrite reduction  Esterase/Esterase lipase & Nitrite reduction  Acid phosphatase & Nitrite reduction |  | β-Glucosidase & Nitrite reduction  Cystine arylamidase & Hydrolysis of Esculin  Trypsin & Hydrolysis of Esculin  β-Glucosidase & L-arabinose  Cystine arylamidase & Capric acid  α-chymotripsin & Capric acid  α-chymotripsin & Adipic acid  Cystine arylamidase & Trisodium citrate  α-chymotripsin & Trisodium citrate  β-Glucosidase & Urease  β-Glucosidase & Phenylacetic acid |

**Table S3. Continued**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Test** | **Marinobacter flavimaris**  **(SO240BG9)** | **Pseudoalteromonas shioyasakiensis**  **(SO240BG4)** | **Pseudoalteromonas shioyasakiensis**  **(SO240BG20)** | **Pseudomonas stutzeri**  **(SO240BG43)** | **Rhodococcus erythropolis**  **(SO240BG28)** |
| **General** |  |  |  | Motility & Pigmentation  Gram & Pigmentation | Pigmentation |
| **API-20NE** | Malic acid & D-glucose | D-maltose & Hydrolysis of Esculin  D-maltose & Hydrolysis of Gelatin  D-maltose & D-glucose  D-maltose & L-arabinose  D-maltose & D-mannitol |  |  | Urease  Phenylacetic acid  L-arabinose & Nitrite reduction |
| **API-20NE + general** | D-glucose & Pigmentation  D-mannitol & Pigmentation  D-glucose & Motility |  |  | Nitrite reduction & Pigmentation  D-mannose & Pigmentation  Capric acid & Pigmentation  Adipic acid & Pigmentation  Trisodium citrate & Pigmentation | Nitrite reduction & Motility  Lipase & Motility  Nitrite reduction & Gram |
| **API-ZYM** | D-glucose & Pigmentation  D-mannitol & Pigmentation  D-glucose & Motility  Acid phosphatase & Lipase  N-acetyl-β-glucosaminidase & Lipase  Cystine arylamidase & Valine arylamidase  Acid phosphatase & Valine arylamidase  α-Glucosidase & Valine arylamidase  N-acetyl-β-glucosaminidase & Valine arylamidase  N-acetyl-β-glucosaminidase & α-Glucosidase | α-chymotripsin & Leucine-arylamidase  α-chymotripsin & α-Mannosidase  α-chymotripsin & β-Glucuronidase  α-chymotripsin & β-Galactosidase  α-chymotripsin & Cystine arylamidase  α-chymotripsin & Trypsin  Acid phosphatase & α-chymotripsin  β-Glucosidase & α-chymotripsin  α-chymotripsin & Alkaline phosphatase  α-chymotripsin & Esterase  α-chymotripsin & Esterase lipase | α-Glucosidase & N-acetyl-glucosamine |  | β-Glucosidase & N-acetyl-glucosamine  β-Glucosidase & Alkaline phosphatase  β-Glucosidase & Esterase  β-Glucosidase & Esterase lipase  β-Glucosidase & Lipase  β-Glucosidase & Leucine-arylamidase  β-Glucosidase & α-Mannosidase  β-Glucosidase & β-Glucuronidase  β-Glucosidase & β-Galactosidase  β-Glucosidase & Cystine arylamidase  β-Glucosidase & Trypsin  β-Glucosidase & α-chymotripsin  β-Glucosidase & Acid phosphatase  N-acetyl-β-glucosaminidase & β-Glucosidase |
| **API-ZYM + general** | Valine arylamidase & Pigmentation  Valine arylamidase & Motility  Valine arylamidase & Gram | α-chymotripsin & Motility |  | Esterase/Esterase lipase & Pigmentation  Lipase & Pigmentation  Acid phosphatase & Pigmentation  α-Glucosidase & Pigmentation | Lipase & Motility  Lipase & Gram  β-Glucosidase & Gram |
| **20NE +ZYM** | Lipase & D-glucose  Valine arylamidase & D-glucose  Cystine arylamidase & D-glucose  Acid phosphatase & D-glucose  N-acetyl-β-glucosaminidase & D-glucose  Valine arylamidase & L-arabinose  N-acetyl-β-glucosaminidase & L-arabinose  Valine arylamidase & D-mannose  Valine arylamidase & D-mannitol  Acid phosphatase & D-mannitol  N-acetyl-β-glucosaminidase & D-mannitol  Lipase & Adipic acid  Valine arylamidase & Adipic acid  Lipase & Trisodium citrate | α-chymotripsin & Capric acid  α-chymotripsin & Adipic acid  α-chymotripsin & Trisodium citrate  α-chymotripsin & D-maltose | Lipase & D-mannose  α-Glucosidase & D-mannose |  | β-Glucosidase & Nitrite reduction  β-Glucosidase & Hydrolysis of Gelatin  β-Glucosidase & L-arabinose |

**Table S4. Mn cell trafficking putative genes sequences accession number list**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | | Accession number | | |
| Related to | **Conserved Domain** | ***Dietzia maris*** | ***Loktanella cinnabarina*** | ***Rhodococcus Erythropolis*** |
| Cation diffusion facilitator (CDF) | **FieF** | LVFF01000032.1 | BATB01000030.1 BATB01000021.1 BATB01000017.1 | ACNO01000036.1 ACNO01000069.1 ACNO01000076.1 ACNO01000072.1 ACNO01000004.1 |
| P-type ATPase | **ZntA** | LVFF01000044.1 | BATB01000042.1 BATB01000021.1 BATB01000003.1 | ACNO01000116.1 ACNO01000104.1 ACNO01000057.1 (2hits) |
| Transcriptional regulator | **MntR** | LVFF01000053.1 | NQWH01000082.1 | ACNO01000032.1 |