**Summary of changes made in AGORA version 1.03, released 29.01.2019**

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**Additions in the updated version**

In the present update, the 818 AGORA were expanded by several pathways and further curated against newly available experimental data. Moreover, an extensive correction of predictions that disagree with experimental data as well as quality control/quality assurance of reconstruction properties was performed with the help of a test suite (publication in preparation).

Experimental data from several recent publications (Table 1) was retrieved and served as the input for the data-driven curation and expansion of pathways in AGORA. First, 54 AGORA reconstructions were refined based on defined growth media reported by Tramontano et al [1] for the corresponding 54 organisms (Supplementary Table 1). Moreover, an improved pipeline was used to resolve false positive predictions of required nutrients in all reconstructions with available experimental data (see below). Growth on the media was enabled by combining the gap-filling reactions proposed by Tramontano et al. with gap-filling performed by the AGORA pipeline. Second, a comparative genomic analysis of a recently described pathway for aromatic amino acid degradation [2] was performed and the corresponding reactions were added to AGORA. Third, comparative genomic data was retrieved from a recent study on putrefaction pathways in gut microbes [3] and the corresponding reactions were reconstructed. Fourth, the 12α-hydroxysteroid dehydrogenase reaction, and the trans-4-hydroxy-L-proline dehydratase reaction were added based on a comparative genomic analysis of the respective, recently described genes [4, 5]. Fifth, novel carbon sources were added based on recent experimental evidence [6]. Sixth, 5-aminovalerate fermentation to valerate was reconstructed for *Clostridium viride* DSM 6836 [7]. Seventh, experimental data on B-vitamin secretion as well as secretion of vitamin K and GABA was gathered [8-23] and secretion of these compounds was enabled in the corresponding reconstructions. Finally, we recently performed a comparative genomic analysis of mucin degradation pathways in the gut microbiome [24]. The corresponding pathways were reconstructed with great biochemical detail.

For all added pathways (Table 1), it was also ensured that the added reactions could carry flux in every reconstruction.

**Table 1**: Pathways that were added in the new version, and supporting references.

|  |  |  |
| --- | --- | --- |
| **Pathway added** | **Number of analyzed AGORA organisms carrying pathway** | **Reference** |
| Putrefaction pathways | 159 | [3] |
| Aromatic amino acid degradation | 14 | [2] |
| 12α-hydroxysteroid dehydrogenase reaction | 37 | [4] |
| trans-4-hydroxy-L-proline dehydratase reaction | 21 | [5] |
| 5-aminovalerate fermentation to valerate | 1 | [7] |
| Mucin degradation pathway reconstructed through comparative genomic analysis | 233 | [24] |
| Experimentally determined carbon sources | 12 | [6] |
| B-vitamin, vitamin K, and GABA secretion based on experimental evidence | 124 | [8-23] |

**Testing and quality control/quality assurance of the reconstructions**

A COBRA Toolbox-based test suite for the AGORA reconstructions (publication in preparation) was created that systematically accesses the capability of each reconstruction to capture known metabolic traits of the organism and determines features of the reconstructions that indicate their quality, e.g., mass and charge balance, blocked reactions, futile cycles, and leaking metabolites. The tested features are summarized in Table 2. The comparison with experimental data (e.g., carbon sources, fermentation products) and comparative genomics (e.g., aromatic amino acid degradation) was carried out as follows: The experimental or comparative genomics data serves as the input and the capability of each corresponding AGORA reconstruction to take up or produce the corresponding metabolite is tested. True positives indicate that the strain is known to take up or produce the metabolite and the corresponding AGORA reconstruction can also take or secrete the metabolite. False negatives indicate that the strain is known to have this capability but the corresponding reconstruction does not capture the trait. For growth requirements, two types of experimental information were available: nutrients that are known to be required by the organism in question, and nutrients that are known to not be required. This allowed us to additionally determine true negatives and false positives for growth requirements (Figure 1).



**Figure 1: Schema of the four outcomes when comparing *in vivo* findings and *in silico* predictions of growth requirements for an organism.**

Tests for reconstruction properties that determine biochemical and thermodynamic feasibility and quality of the reconstruction (e.g., biomass production, ATP production, mass-charge balance, blocked reactions) were carried out using established COBRA Toolbox functions (Table 2). All models in AGORA 1.03 produced biomass and reasonable amounts of ATP on the Western Diet (Figure 2).

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**Figure 2: Model properties computed for the current version (AGORA 1.03) and the previous version (AGORA 1.02) on a simulated Western diet. a) Anaerobic biomass production, b) aerobic biomass production, c) anaerobic ATP production, d) aerobic ATP production.**

**Table 2**: Summary of reconstruction features analyzed in the test suite for AGORA that was used to access the predictive potential of the reconstructions.

|  |  |  |
| --- | --- | --- |
| **Feature** | **Input data** | **COBRA Toolbox function** |
| Mass and charge balance | Reconstructions | checkMassChargeBalance |
| Leaking metabolites | Reconstructions | fastLeakTest |
| Blocked reactions | Reconstructions | identifyBlockedRxns |
| ATP production on Western diet | Reconstructions | optimizeCbModel |
| Biomass on Western diet | Reconstructions | optimizeCbModel |
| Carbon source usage | Reconstructions, experimental data | In preparation |
| Fermentation products | Reconstructions, experimental data | In preparation |
| Growth requirements | Reconstructions, experimental data | In preparation |
| Growth on defined medium according to experimental data | Reconstructions, experimental data | In preparation |
| B-vitamin biosynthesis | Reconstructions, comparative genomics, experimental data | In preparation |
| B-vitamin secretion | Reconstructions, experimental data | In preparation |
| 4-hydroxyproline dehydration | Reconstructions, comparative genomics | In preparation |
| Bile acid deconjugation and conversion | Reconstructions, comparative genomics | In preparation |
| Putrefaction | Reconstructions, comparative genomics | In preparation |
| Aromatic amino acid degradation | Reconstructions, comparative genomics, experimental data | In preparation |
| Mucin degradation | Reconstructions, comparative genomics | In preparation |



**Figure 3: Comparison of false negative predictions for a) fermentation products, b) carbon sources, c) bile acids synthesized, and d) false negative predictions nutrient requirements in the current version (AGORA 1.03) and the previous version (AGORA 1.02).**

The tests were carried out for the 818 reconstructions in the recent version (1.03) and the previous version (1.02). When performing the tests, a number of remaining false negative predictions for carbon sources and fermentation products, and false positives/ false negatives for growth requirements were found. Extensive curation of the corresponding AGORA reconstructions was performed to ensure that the corresponding reconstructions could take up all known carbon sources, produce all known fermentation products, and match known growth requirements. It was noted that many false negatives for growth requirements could not be corrected. This is due to an organism requiring a nutrient despite the biosynthesis pathway for the nutrient being present in its genome. Such discrepancies are challenging to curate against even in fully manually curated reconstructions [25, 26]. As a result of the extensive curation of growth requirements, all 279 AGORA 1.03 models with available experimental data were able to grow on the respective experimentally determined media compared with only 129 models in AGORA 1.02.

Overall, as a result of the additional curation, the number of true positives was increased and the number of false negatives was decreased in version 1.03 compared with version 1.02 (Figure 3, Table 3). The sensitivity for all pathways was 1 (Table 3) demonstrating that all false negative predictions with the exception of false negative growth requirements were eliminated in AGORA 1.03.

**Table 3**: Comparison in predictive potential in the current version (1.03) compared with the previous version (1.02) of AGORA. n.d.=not determined.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Feature** | **Sensitivity** | **Specificity** | **Accuracy** | **Sensitivity** | **Specificity** | **Accuracy** |
|  | **AGORA 1.03 (01/2019)** | **AGORA 1.02 (02/2018)** |
| **Aromatic amino acid degradation** | 1.00 | n.d. | n.d. | 0.20 | n.d. | n.d. |
| **Bile acid biosynthesis** | 1.00 | n.d. | n.d. | 0.87 | n.d. | n.d. |
| **Carbon sources** | 1.00 | n.d. | n.d. | 0.98 | n.d. | n.d. |
| **Fermentation products** | 1.00 | n.d. | n.d. | 0.99 | n.d. | n.d. |
| **Nutrient requirements** | 0.54 | 1.00 | 0.99 | 0.55 | 0.97 | 0.96 |
| **Putrefaction pathways** | 1.00 | n.d. | n.d. | 0.24 | n.d. | n.d. |
| **Vitamin secretion** | 1.00 | n.d. | n.d. | 0.07 | n.d. | n.d. |

**Availability of AGORA version 1.03**

Due to the large number of reactions added with the mucin degradation subsystem, two versions of the refined reconstructions (AGORA 1.03) are provided: one with and one without the mucin degradation subsystem. Both are available in SBML format at <https://www.vmh.life/#downloadview>).

Both versions are provided without dietary constraints. To enable users to simulate fluxes in AGORA on a diet, a tutorial has been created

(<https://github.com/opencobra/COBRA.tutorials/tree/develop/analysis/simulateAGORAGrowthInDiets>).

**References**

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**Supplementary Table 1**: List of the 54 AGORA reconstructions that were curated against defined media determined for the corresponding organisms by Tramontano et al. [1].

|  |  |  |
| --- | --- | --- |
| **AGORA Reconstruction ID** | **Strain** | **NCBI Taxonomy ID** |
| Akkermansia\_muciniphila\_ATCC\_BAA\_835 | Akkermansia muciniphila ATCC BAA-835 | 349741 |
| Actinomyces\_odontolyticus\_ATCC\_17982 | Actinomyces odontolyticus ATCC 17982 | 411466 |
| Alistipes\_putredinis\_DSM\_17216 | Alistipes putredinis DSM 17216 | 445970 |
| Alistipes\_shahii\_WAL\_8301 | Alistipes shahii WAL 8301 | 717959 |
| Bifidobacterium\_adolescentis\_ATCC\_15703 | Bifidobacterium adolescentis ATCC 15703 | 367928 |
| Bifidobacterium\_animalis\_lactis\_Bi\_07 | Bifidobacterium animalis lactis Bi-07 | 742729 |
| Bifidobacterium\_animalis\_lactis\_Bl\_04\_ATCC\_SD5219 | Bifidobacterium animalis lactis Bl-04, ATCC SD5219 | 580050 |
| Bacteroides\_caccae\_ATCC\_43185 | Bacteroides caccae ATCC 43185 | 411901 |
| Bacteroides\_clarus\_YIT\_12056 | Bacteroides clarus YIT 12056 | 762984 |
| Bacteroides\_coprocola\_M16\_DSM\_17136 | Bacteroides coprocola M16, DSM 17136 | 310298 |
| Butyrivibrio\_crossotus\_DSM\_2876 | Butyrivibrio crossotus DSM 2876 | 511680 |
| Bacteroides\_dorei\_DSM\_17855 | Bacteroides dorei DSM 17855 | 483217 |
| Bacteroides\_eggerthii\_DSM\_20697 | Bacteroides eggerthii DSM 20697 | 483216 |
| Bacteroides\_fragilis\_NCTC\_9343 | Bacteroides fragilis NCTC 9343 | 272559 |
| Bacteroides\_fragilis\_3\_1\_12 | Bacteroides fragilis 3\_1\_12 | 457424 |
| Blautia\_hansenii\_VPI\_C7\_24\_DSM\_20583 | Blautia hansenii VPI C7-24, DSM 20583 | 1322 |
| Bifidobacterium\_longum\_infantis\_ATCC\_15697 | Bifidobacterium longum infantis ATCC 15697 | 1682 |
| Blautia\_obeum\_ATCC\_29174 | Blautia obeum ATCC 29174 | 411459 |
| Bacteroides\_ovatus\_ATCC\_8483 | Bacteroides ovatus ATCC 8483 | 411476 |
| Bacteroides\_thetaiotaomicron\_VPI\_5482 | Bacteroides thetaiotaomicron VPI-5482 | 226186 |
| Bacteroides\_uniformis\_ATCC\_8492 | Bacteroides uniformis ATCC 8492 | 411479 |
| Bacteroides\_vulgatus\_ATCC\_8482 | Bacteroides vulgatus ATCC 8482 | 435590 |
| Collinsella\_aerofaciens\_ATCC\_25986 | Collinsella aerofaciens ATCC 25986 | 411903 |
| Clostridium\_bolteae\_ATCC\_BAA\_613 | Clostridium bolteae ATCC BAA-613 | 411902 |
| Coprococcus\_comes\_ATCC\_27758 | Coprococcus comes ATCC 27758 | 470146 |
| Clostridium\_leptum\_DSM\_753 | Clostridium leptum DSM 753 | 428125 |
| Clostridium\_perfringens\_ATCC\_13124 | Clostridium perfringens ATCC 13124 | 195103 |
| Clostridium\_ramosum\_VPI\_0427\_DSM\_1402 | Clostridium ramosum VPI 0427, DSM 1402 | 1547 |
| Dorea\_formicigenerans\_ATCC\_27755 | Dorea formicigenerans ATCC 27755 | 411461 |
| Desulfovibrio\_piger\_ATCC\_29098 | Desulfovibrio piger ATCC 29098 | 411464 |
| Escherichia\_coli\_UTI89\_UPEC | Escherichia coli UTI89 (UPEC) | 364106 |
| Eubacterium\_eligens\_ATCC\_27750 | Eubacterium eligens ATCC 27750 | 515620 |
| Eggerthella\_lenta\_DSM\_2243 | Eggerthella lenta DSM 2243 | 479437 |
| Eubacterium\_siraeum\_DSM\_15702 | Eubacterium siraeum DSM 15702 | 428128 |
| Lactobacillus\_acidophilus\_NCFM | Lactobacillus acidophilus NCFM | 272621 |
| Lactobacillus\_fermentum\_ATCC\_14931 | Lactobacillus fermentum ATCC 14931 | 525325 |
| Lactobacillus\_gasseri\_ATCC\_33323 | Lactobacillus gasseri ATCC 33323 | 324831 |
| Lactococcus\_lactis\_subsp\_lactis\_Il1403 | Lactococcus lactis subsp. lactis Il1403 | 272623 |
| Lactobacillus\_plantarum\_WCFS1 | Lactobacillus plantarum WCFS1 | 220668 |
| Lactobacillus\_salivarius\_HO66\_ATCC\_11741 | Lactobacillus salivarius HO66, ATCC 11741 | 1624 |
| Lactobacillus\_vaginalis\_ATCC\_49540 | Lactobacillus vaginalis ATCC 49540 | 1423814 |
| Odoribacter\_splanchnicus\_1651\_6\_DSM\_20712 | Odoribacter splanchnicus 1651/6, DSM 20712 | 28118 |
| Pseudoflavonifractor\_capillosus\_strain\_ATCC\_29799 | Pseudoflavonifractor capillosus strain ATCC 29799 | 411467 |
| Prevotella\_copri\_CB7\_DSM\_18205 | Prevotella copri CB7, DSM 18205 | 165179 |
| Parabacteroides\_distasonis\_ATCC\_8503 | Parabacteroides distasonis ATCC 8503 | 435591 |
| Prevotella\_melaninogenica\_ATCC\_25845 | Prevotella melaninogenica ATCC 25845 | 553174 |
| Parabacteroides\_merdae\_ATCC\_43184 | Parabacteroides merdae ATCC 43184 | 411477 |
| Ruminococcus\_gnavus\_ATCC\_29149 | Ruminococcus gnavus ATCC 29149 | 411470 |
| Roseburia\_hominis\_A2\_183 | Roseburia hominis A2-183 | 585394 |
| Roseburia\_intestinalis\_L1\_82 | Roseburia intestinalis L1-82 | 536231 |
| Ruminococcus\_torques\_ATCC\_27756 | Ruminococcus torques ATCC 27756 | 411460 |
| Salmonella\_enterica\_enterica\_sv\_Typhimurium\_LT2 | Salmonella enterica enterica sv Typhimurium LT2 | 1457319 |
| Veillonella\_parvula\_Te3\_DSM\_2008 | Veillonella parvula Te3, DSM 2008 | 29466 |
| Yersinia\_pseudotuberculosis\_YPIII | Yersinia pseudotuberculosis YPIII | 502800 |