

Computation and analysis of microbe-microbe metabolic interactions

Note: This tutorial is a draft and needs completion. Contributions welcome!

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```
% This tutorial demonstrates how to join a given list of microbial COBRA
% models in all possible combinations and compute the metabolic
% interactions between the microbes depending on the implemented diet.
% Moreover, the Tradeoff between the growth of different joined microbes
% is computed.
% The tutorial can be adapted to any number of ADORA models and dietary
% conditions analyzed.

% We will use the ADORA resource (Magesundt et al., Nat Biotechnol. 2017
% Jaquin(1):82-89) in this tutorial. Please download ADORA version 1.82
% from https://webdav.rtdk.unl.lu/public/wag/ADORA-1.82/Bqara-0.82.zip
% and place the models into a folder.

% Define the path to the folder where you stored the ADORA models.
modelPath='YOUR_PATH_TO_ADORA/';

% Import a file with information on the ADORA organisms including
% reconstruction names and taxonomy.
[~,infoFile,~]=iskread('ADORA_infoFile.xlsx');

% Initialize the COBRA toolbox.
initCobraToolbox
```



```
% Checking if git is installed ... Done.
% Checking if the repository is tracked using git ... Done.
% Checking if curl is installed ... Done.
% Checking if remote can be reached ... Done.
% Initializing and updating submodules (this may take a while)... Done.
% Adding all the files of the COBRA ToolBox ... Done.
% Define C# map output... set to exp.
% TranslateRML to installed and working properly.
% Configuring solver environment variables ...
- [====] DASH_PATH: applications/libDASH/CLUSTER_Studio1271/vplex/matlab/v80-64_exe
- [====] GURDASH_PATH: /Library/gurudash/matlab/matlab
- [====] TORLAB_PATH: --> set this path manually after installing the solver ( see instructions )
- [====] RMDASH_PATH: --> set this path manually after installing the solver ( see instructions )
Done.
% Checking available solvers and solver interfaces ...
Done.
% Setting default solvers ... Done.
% Saving the MATLAB path ... Done.
- The MATLAB path was saved in the default location.

% Summary of available solvers and solver interfaces
```

Support	LP	HELP	QP	NDQP	NLP
gurobi	active	1	1	1	1
lib_cplex	active	1	1	1	-
matlab_cplex	active	0	0	0	0
glsk	active	1	1	-	-
mosh	active	0	0	-	-
matlab	active	1	-	-	1
cplex_direct	active	0	0	0	0
dagHines	active	1	-	-	-
pdco	active	1	-	1	-
quadHines	active	1	-	-	1
qpnp	passive	-	-	1	-
matlab_cmoqp	passive	-	-	-	0
gurobi_mes	legacy	0	0	0	0
libm_sld	legacy	0	-	-	-
libm_legacy	legacy	0	-	-	-
lp_solver	legacy	1	-	-	-
split	legacy	0	0	0	0
Total	-	0	3	4	1

% Legend: - = not applicable, 0 = solver not compatible or not installed, 1 = solver installed.

```
% You can solve LP problems using: 'gurobi' - 'lib_cplex' - 'glsk' - 'matlab' - 'dagHines' - 'pdco' - 'quadHines' - 'lp_solver'
% You can solve HELP problems using: 'gurobi' - 'lib_cplex' - 'glsk'
% You can solve QP problems using: 'gurobi' - 'lib_cplex' - 'pdco' - 'qpnp'
% You can solve NDQP problems using: 'gurobi'
% You can solve NLP problems using: 'matlab' - 'quadHines'
```

```
% Checking for available updates ...
--> You cannot update your fork using updateGithubToolbox(). [edit@0.0.0 develop].
Please use the MATLAB devtools (https://github.com/COBRA-toolbox/COBRA-toolbox-devtools).
```

Creation of pairwise models

```
% For the sake of this tutorial, we will use ten random AGORA
% reconstructions from the Info file.
modelList = int8ToCell(randi(2, length(InfoFile), 1, 10), 1);
% Uncomment the following line to join all AGORA reconstructions in all
% combinations. NOTE: this is very time-consuming due to the large number
% of model combinations analyzed.
% modelList = int8ToCell(2, rand, 1);
% You may also enter a custom selection of AGORA reconstructions as a cell
% array named modelList.

% Load the AGORA reconstructions to be joined.
for i = 1:size(modelList, 1)
    load(sprintf('modelPath', modelList{i, 1}, '_mat.mat'));
    % make sure the TOLAB in the reconstruction structure are in the correct
    % format, incorrect format causes errors when joining the models
    model = convertToLibraryModel(model);
    inputModel{i, 1} = model;
end

% Let us define some parameters for joining the models.
% Set the coupling factor  $\epsilon$ , which defined how the flux through all
% reactions in a model is coupled to the flux through its biomass
% reaction. Allowed flux span through each reaction  $-f = \text{flux}(\text{biomass})$ 
% to  $+f = \text{flux}(\text{biomass})$ .
doBB;
```


[illegible]

[illegible]

```

In chompAndBounds (line 10)
De uoErlit (line 10)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_gpi[a] not in model
In chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_gpi[a] not in model
= Gurobi interface added by POTSAB patch.
= The compatibility can only be evaluated on macOS 18.12..
= The solver compatibility is not tested with MATLAB R2026a..
Warning: Reaction E2_Fus_1[a] not in model
In chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_gly[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_gly[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_gln[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_rnc[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_rnc[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_opt_D[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_opt_D[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_max[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_max[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_urid[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_urid[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_sivch1[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_sivch1[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_anyIscp1000[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_anyIscp1000[a] not in model
= chompAndBounds (line 10)
De uoErlit (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De reuseParallelFunction (line 10)
Warning: Reaction E2_anyIscp200[a] not in model

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[illegible]

[illegible]

[illegible]

[illegible]

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In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_kich[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_gnc[i,a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_gnc[i,a] not in model
+ Error: interface added to NUTLAB path.
The compatibility can only be evaluated on macOS 10.12..
The solver compatibility is not tested with MATLAB R2016a.
Warning: Reaction E2_Fus_1[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_Fus_1[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_gln[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_gln[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_rmc[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_rgt_2[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_rgt_2[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_uap[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_uap[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_meth[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_vivrh[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_vivrh[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_anySuperRHO[a] not in model
+ In chompHofmann (line 10)
De useDirk (line 28)
De parallel_FunctionName_general_channel/channel_general (line 924)
De remuseParallelFunction (line 10)
Warning: Reaction E2_anySuperRHO[a] not in model
+ In chompHofmann (line 10)

```

[illegible]

```

r <- changeFunctionNames (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_hmgat[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_inulin[x] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_inulin[x] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_hesapt[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_hesapt[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_treval1000[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_treval1000[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_ten0[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_ten0[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_tic0[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_tic0[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_pst[u] not in model
r <- changeFunctionBounds (time 30)
  Do useDiel (time 20)
  Do parallel_functionmake_general_channel/channel_general (time 924)
  Do remoteParallelFunction (time 30)
Warnings: Reaction R3_pst[u] not in model

```

Analysis of computed pairwise interactions

```

% The computed microbe-microbe interactions will be grouped by type and
% analyzed in the context of the taxonomy of the joined strains. There are
% six possible types of interactions total that can result in increased
% growth (+), no change in growth (=) or decreased growth (-) compared with
% the single condition for each joined microbe.
% Competition (-/-)
% Parasitism (+/-)
% Amensalism (+/-)
% Mutualism (+/+)
```

```

% This results in nine different outcomes total from the perspective of
% each joined microbe.

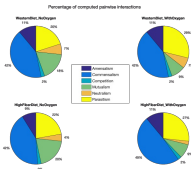
% Plot the percentage of interactions computed.
figure('rmod','pairwise','gsc',[20 10 800 800])
typeID=unique(pairwiseInteractions(2:end,10));
for i = 1:length(condition)
    pairwiseInteractions=Interactions(condition(i));
    list(pairwiseInteractions(2:end,10));
    for j=1:length(typeID)
        dat{j}=sum(strcmp(listID(i),typeID{j}));
    end
    sublot(2,i,1)
    plot(dat)

```

```

setFigSize('fontSize',20)
setTitle('and it loss (x)');
setFig('interpreter','none')
title('and it loss (x)')
end
legend=legend(typesID);
set(legend,'Position',[0.62 0.45 0.2 0.2],'FontSize',12)
suptitle('Percentage of computed pairwise interactions')

```



```

% Next, the percentage of interactions will be calculated on different
% Taxon levels (genus, family, order, class, phylum) using the taxon
% information contained in ADDM_infofile.xlsx. Here, the interactions will
% be considered from the perspective of each paired microbes resulting in
% some possible interactions total.

% Calculate the percentage of interactions predicted for each taxon
% included in the list of microbes analyzed.

for i = 1:length(conditions)
    pairwiseInteractLoss=InteractLoss_ (conditions{i});
    [InteractionByTaxon {taxLevel}InteractionByTaxon {pairwiseInteractions_ inTaxo i}] =
    TaxonInteractLoss_ (conditions{i})=InteractionByTaxon;
end

% Combine the four conditions into one structure.
InteractLossByTaxonCombined=struct;
for i = 1:length(conditions)
    InteractLossByTaxonTaxonCombined_ (conditions{i});
    TaxLevel=TaxLevel (InteractionByTaxon);
    if i==1
        for j=1:length(TaxLevel)
            InteractLossByTaxonCombined_ (TaxLevel{j})=InteractionByTaxon_ (TaxLevel{j});
            InteractLossByTaxonCombined_ (TaxLevel{j})(2:end,2)=strcat(InteractionByTaxonCombined_ (TaxLevel{j})(2:end,1), '_', conditions{i})
        end
    else
        for j=1:length(TaxLevel)
            numelengthSize [InteractionByTaxonCombined_ (TaxLevel{j})(1,1);
            InteractLossByTaxonCombined_ (TaxLevel{j})]=[]; InteractLossByTaxonCombined_ (TaxLevel{j})=InteractionByTaxon_ (TaxLevel{j})(2:end,2);
            InteractLossByTaxonCombined_ (TaxLevel{j})(1:numellength+2:end,2)=strcat(InteractionByTaxonCombined_ (TaxLevel{j})(1:numellength+1:end,2);
        end
    end
end

% Let us plot the distributions of interactions for all dietary conditions combined
% on the level of genera as an example. Note: The xTickLabels/yTickLabels function
% is only available in MATLAB R2008a or newer. Older versions of MATLAB will be unable
% to display the labels.
for i=1
    xLabels=InteractLossByTaxonCombined_ (TaxLevel{i})(1,2:end);
    yLabels=InteractLossByTaxonCombined_ (TaxLevel{i})(2:end,1);
    data=trilg(InteractLossByTaxonCombined_ (TaxLevel{i})(2:end,2:end));
    data=tr2double(data);
    figure;
    imagesc(data)

```


[illegible]


```
Warning: Reaction EG_fald[a] not in model!
Warning: Reaction EG_fald[a] not in model!
Warning: Reaction EG_faldi[i][a] not in model!
Warning: Reaction EG_feldii[i][a] not in model!
Warning: Reaction EG_gva_B[a] not in model!
Warning: Reaction EG_gva_B[a] not in model!
Warning: Reaction EG_gtychp[a] not in model!
Warning: Reaction EG_gtychp[a] not in model!
Warning: Reaction EG_gun[a] not in model!
Warning: Reaction EG_gun[a] not in model!
Warning: Reaction EG_gibaa[a] not in model!
Warning: Reaction EG_gibaa[a] not in model!
Warning: Reaction EG_h2[a] not in model!
Warning: Reaction EG_h2[a] not in model!
Warning: Reaction EG_hum_A[a] not in model!
Warning: Reaction EG_hum_A[a] not in model!
Warning: Reaction EG_indole[a] not in model!
Warning: Reaction EG_indole[a] not in model!
Warning: Reaction EG_lactate[a] not in model!
Warning: Reaction EG_lactate[a] not in model!
Warning: Reaction EG_metal[a] not in model!
Warning: Reaction EG_metal[a] not in model!
Warning: Reaction EG_nap7[a] not in model!
Warning: Reaction EG_nap7[a] not in model!
Warning: Reaction EG_nnn[a] not in model!
Warning: Reaction EG_nnn[a] not in model!
Warning: Reaction EG_nnn[a] not in model!
Warning: Reaction EG_nml[a] not in model!
Warning: Reaction EG_nml[a] not in model!
Warning: Reaction EG_pbyQ[a] not in model!
Warning: Reaction EG_pbyQ[a] not in model!
Warning: Reaction EG_pine[a] not in model!
Warning: Reaction EG_pine[a] not in model!
Warning: Reaction EG_pphshp[a] not in model!
Warning: Reaction EG_pphshp[a] not in model!
Warning: Reaction EG_rvlinz[i][a] not in model!
Warning: Reaction EG_rvlinz[i][a] not in model!
Warning: Reaction EG_srl[a] not in model!
Warning: Reaction EG_srl[a] not in model!
Warning: Reaction EG_18f[a] not in model!
Warning: Reaction EG_18f[a] not in model!
Warning: Reaction EG_w14d[a] not in model!
Warning: Reaction EG_w14d[a] not in model!
Warning: Reaction EG_wash[a] not in model!
Warning: Reaction EG_wash[a] not in model!
```

[illegible]

[illegible]

[illegible]

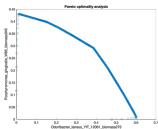
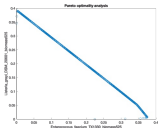
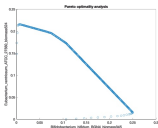
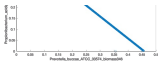
The following fields are missing in several models, they will not be merged:

[illegible]

[illegible]

[illegible]

[illegible]



% Can you interpret the shapes of the five Pareto frontiers that
 % were computed? Are there microbe pairs that are always competing
 % with each other? Are there pairs in which one microbe can
 % benefit the other at certain points in the curve and vice versa?