Anexos

PARTE I

Exercise 1

Tabela 1_Valores de fluxo de produção nativos do L-lactato e D-lactato. Os valores foram obtidos através da simulação FBA recorrendo ao Mewpy package.

```
L-Lactate wild type flux:  0.0
--------------***--------------
D-Lactate wild type flux:  0.0
```

Exercise 2

Tabela 2_Valores máximos e mínimos de fluxo FVA para o L-Lactato e D-Lactato. Valores obtidos utilizando o Mewpy package.

```
------------------***-----------------------
Implementação no Mewpy:
------------------***-----------------------
   Reaction ID  Minimum    Maximum
0  EX_lac__L_e      0.0   1.114182
1  EX_lac__D_e      0.0  12.256000
------------------***-----------------------
```

PARTE II

Exercise 1

<u>Alínea a</u>

Tabela 3_Virulence factor of the Mycobacterium tuberculosis complex.

Category	Gen Name	Rv number	Description	Attenuation evidences			References
				Model	Result	Complementation	
Lipids and Fatty Acid Metabolism	*kasB*	Rv2246	3-oxoacyl-[acyl-carrier protein] synthase 2 kasb	C57BL/6 mice (lda)	Reduced CFUs in organs and lung pathology Increased animal survival	Yes	25
Mycolic acid synthesis	*mmaA4*	Rv0642c	Methoxy mycolic acid synthase 4	C57BL/6 mice (lda/iv)	Reduced CFUs in organs	Yes	26
				C57BL/6 mice (iv)[†]	Failed to persist in the spleens	ND	
	pcaA	Rv0470c	Mycolic acid synthase (cyclopropane synthase)	C57BL/6 mice (iv)	Failed to persist in organs Increased animal survival	Yes	27
				C57BL/6 mice (lda)	Reduced CFUs in lung	Yes	28
	mymA operon	Rv3083 to Rv3089	Propable Monooxygenase (Hydroxylase)	Activated J774 macrophages and guinea pigs (sc)	Reduced CFUs	ND	32
	-	Rv2869c	Membrane bound metalloprotease	C57BL/6 mice (lda)	Reduced CFUs in lung	Yes	33
	treS	Rv0126	Trehalose synthase	C57BL/6 mice (iv)	Reduced CFUs in lung Increased animal survival	ND	34
Synthesis of complex lipids				C57BL/6J mice (in) and MAM MH-S	Reduced CFUs	ND	36
PDIM	*pks15* *pks1*	Rv2946c Rv2947c	Probable polyketide synthases	B6D2 F1 mice (lda)	Increased animal survival	ND	37
				Rabbits (intracisternally)	Reduced CFUs in cerebrospinal fluid and organs	ND	38

*The mutant used was made in *M. bovis*; [†]in *M. bovis* BCG or [‡]in both *M. tuberculosis* and *M. bovis* BCG. Route of infection: lda, low dosis aerosol; a, aerosol; it: intratracheal; iv, intravenous; ip, intraperitoneal, im, intramuscular; sc, subcutaneous. Complementation: ND, a complemented strain was not reported by the authors; No, a complemented strait was done but the phenotype was not restored; Yes, a complemented strait was done and the phenotype restored; Yes[a], the phenotype was restored by the insertion of the entire operon; Partial, a complemented strait was done and the phenotype partially restored; NU: a complemented strait was done but not used by the authors. Abbreviations: CHP, conserved hypothetical proteins; MBMDM, murine bone marrow-derived macrophages; MAM, murine alveolar macrophage; HBMDM, human blood monocyte-derived macrophages.

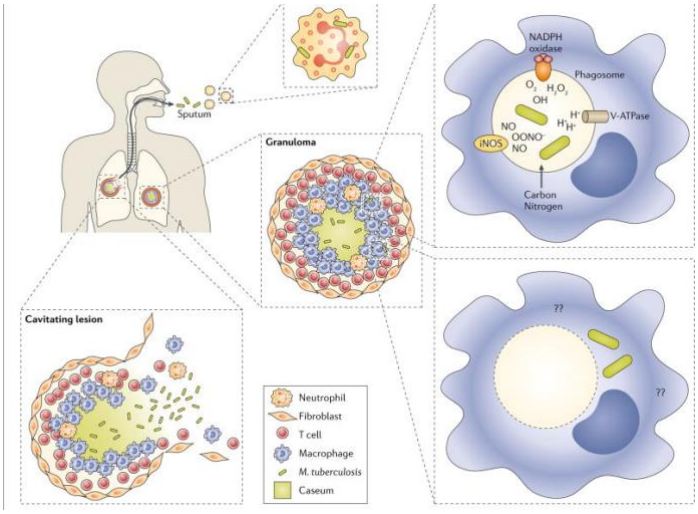

Figura 1_Ciclo de vida de Mycobacterium tuberculosis. (Ehrt et al., 2018)

Alínea b

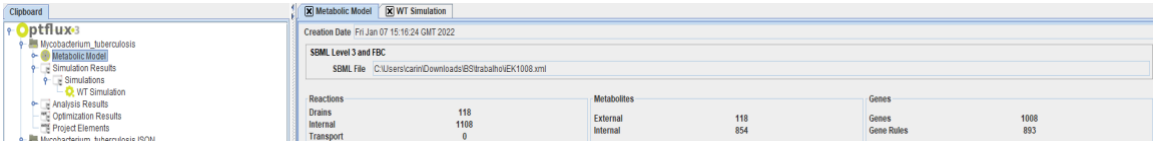

Figura 2_Criação do modelo metabólico importando os dados do ficheiro SBML.

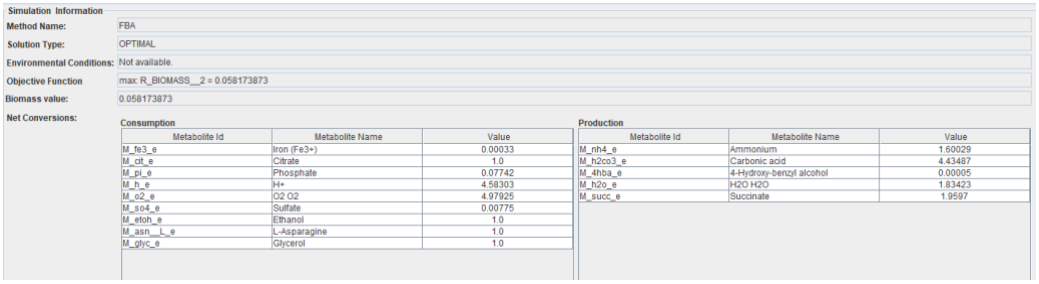

Figura 3_Simulação wild-type maximizando a biomassa, com metabolitos produzidos e consumidos, com respetivos fluxos.

```
from cobra.io import read_sbml_model
model = read_sbml_model('C:/users/maryg/biologia de sistemas/trabalho/iNJ661.xml.gz')
```

```
from mewpy.simulation import get_simulator
simul = get_simulator(model)
```

Figura 4_Model e simulador in python.

```
Method:SimulationMethod.FBA

[7]: # FBA com alteração
     result = simul.simulate()

     result

[7]: objective: 0.30596969829082155
     Status: OPTIMAL
     Constraints: OrderedDict([('EX_glc__D_e', (-1000.0, 1000.0)), ('EX_o2_e', (-1000, -1000))])
     Method:SimulationMethod.FBA
```

Figura 5_FBA com otimização de biomassa.

Alínea c

Tabela 4_Excerto de lista de reações críticas identificadas.

Critical Reactions Id	Critical Reactions Name
R_GLUPRT	Glutamine phosphoribosyldiphosphate amidotransferase
R_4HBt	MNXR68734
R_MI3P3	1D-myo-inositol-3-phosphate lyase (isomerizing)
R_PRAMPC	Phosphoribosyl-AMP cyclohydrolase
R_G16MTM7	G16MTM7
R_G16MTM8	G16MTM8
R_G16MTM9	G16MTM9
R_FAStm2602	FAStm2602
R_TDM54	TDM54
R_FAStm2601	FAStm2601
R_DXPS	1-deoxy-D-xylulose 5-phosphate synthase
R_PSD160	Phosphatidylserine decarboxylase (n-C16:0)
R_TDM52	TDM52
R_TDM51	TDM51
R_TAT9	TAT9
R_QULNS	Quinolinate synthase
R_TMHAS2	TMHAS2
R_TMHAS3	TMHAS3
R_G16MTM1	Glycolipid 1,6-alpha-D-mannosyltransferase
R_TMHAS1	TMHAS1
R_ASPK	Aspartate kinase
R_G16MTM4	G16MTM4
R_G16MTM5	G16MTM5
R_G16MTM6	G16MTM6
R_G1PTT	Glucose-1-phosphate thymidylyltransferase
R_DASYN160190	CDP-diacylglycerol synthetase (C16:0, C19:0)
R_AFTA	Arabinofuranosyl transferase (M.tb)
R_MYCOPP	MNXR90581
R_PPBNGS	Porphobilinogen synthase
R_SUCCt2r	Succinate transport via proton symport
R_PPA	Inorganic diphosphatase
R_IPPS	2-isopropylmalate synthase
R_GLNS	Glutamine synthetase
R_PPCOAC	Propionyl-CoA carboxylase
R_SULabc	Sulfate transport via ABC system
R_KARA1	Ketol-acid reductoisomerase (2,3-dihydroxy-3-methylbutanoate)
R_BIOMASS__2	
R_FAStm2801	FAStm2801
R_FAStm2802	FAStm2802
R_AACPS11	AACPS11
R_METS	Methionine synthase
R_AACPS10	AACPS10

Tabela 5_Excerto de lista de genes críticos identificados.

Critical Genes
G_Rv1338
G_Rv3602c
G_Rv3627c
G_Rv0013
G_Rv2973
G_Rv0367c
G_Rv0486
G_Rv3754
G_Rv753c
G_Rv0482
G_Rv1315
G_Rv1308
G_Rv1309
G_Rv1306
G_Rv1307
G_Rv1304
G_Rv3051c
G_Rv1305
G_Rv1302
G_Rv3606c
G_Rv1311
G_Rv1310
G_Rv2400c
G_Rv0423c
G_Rv1380
G_Rv1381
G_Rv1389
G_Rv3565
G_Rv1384
G_Rv1385
G_Rv1383
G_Rv0422c
G_Rv3790
G_Rv0225
G_Rv3795
G_Rv3794
G_Rv3793
G_Rv3792
G_Rv3791
G_Rv2539c
G_Rv2995c
G_Rv1599
Critical Genes

Genes and reactions essentiality

Gene and reaction essentiality tests identify, respectively, the list of genes and reactions whose deletion would prevent the organism to grow.

```
[29]: simul.essential_reactions()
      ...
[30]: simul.essential_genes()
      100%|██████████| 661/661 [00:36<00:00, 18.06it/s]
```

Figura 6_Reações e Genes essenciais (código python).

Alínea d

Tabela 6_Inibidor da Reação

top print hide Go to Inhibitor Search

INHIBITOR	ORGANISM	UNIPROT	COMMENTARY	LITERATURE	IMAGE
D-fructose 2,6-diphosphate	Mycobacterium tuberculosis	I6Y2G3	noncompetitive inhibitor	728738	