Genome scale network reconstruction of the pathogen *Enterococcus faecalis*

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Introduction

E. faecalis is a gram-positive bacterium that is receiving more attention due to its "two-face" behavior. This natural inhabitant of the mammalian gastrointestinal tract is also an opportunistic pathogen responsible for urinary tract infections, nosocomial infections, bacteremia and infective endocarditis. Its intrinsic physiological properties such as inherent antibiotic resistance and exceptional ability to adapt to harsh conditions provide this organism with an enormous advantage during the infection process.

Objectives

We propose to reconstruct the genome scale metabolic network of *E. faecalis.* The reconstruction is based on the genome sequencing information available, as well as online databases and literature evidence. Later, the model will be tested and validated in the laboratory, by performing experiments based on the simulations. The model will support the interpretation and better understanding of the metabolomic and proteomic data generated in our labs.

Methods

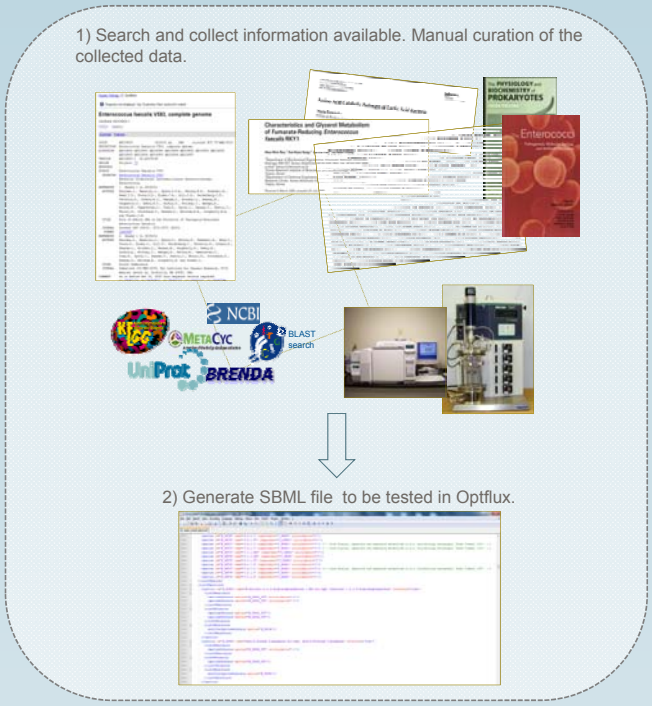

Figure 1: Data integration of different levels of information.

Results

It is currently known that *E.faecalis* is able to synthesize all amino acids using minimal medium (data not shown) although it grows poorly. The current data available in the literature and annotated genome do not contemplate information about biosynthesis of all amino acids and therefore it was necessary to lump some of the reactions required for the biosynthesis in order for the model to work and produce the missing amino acids.

The simulations were performed using the software: Optflux (***www.optflux.org)***

Table 1: Definition of the drains reactions considering that *E.faecalis* is able to grow in minimal medium and to synthesize all amino acids

LB	Organic acids	UP
0	MALA	+∞
0	LCTT	+∞
0	PYR	0
0	FORM	+∞
0	ACET	+∞

LB	Aroma compounds	UP
0	AALD	+∞
0	DTYL	+∞
0	GLCN	+∞
0	ACTN	+∞

LB	Sugars/Sugar alcohols	UB
0	SALC	0
0	ARBT	0
-10	GLUC	0
0	MANN	0
0	TRHL	0
0	FRUT	0
0	SUCR	0
0	MNTL	0
0	CELB	0
0	MALT	0
0	GLYC	0
0	ETOH	0
0	RIB	0
0	SORT	0
0	MurNAc	0

LB	Amino acids	UB
0	ARG	0
0	ALA	0
0	LEU	0
-∞	ILE	0
0	GLU	0
0	GLN	0
0	GLY	0
0	ASN	0
0	ASP	0
0	SER	0
0	THR	0
-∞	MET	0
0	CYS	0
0	HIS	0
0	TYR	0
0	TRP	0
0	VAL	0
0	PHE	0
0	PRO	0
0	LYS	0

LB	Others	UB
	GLUM	
	ADE	
	NICO	
	GABA	
	SLF	
	AGM	
	BET	
0	ORN	0
	PUTR	
	ASC	
0	GALM	0
0	GALMAc	0

LB	Gases/Io	UB
	H+	
	Na+	
	K+	
0	CO2	1000
0	O2	0
	Pi	
	Pii	
	H2O	
	NH3	

Organic acids and aroma compounds: MALA-malate, LCTT-lactate, PYR-pyruvate, FORM-formate, ACET-acetate, AALD-acetaldehyde, DTYL-diacetyl, GLCN-D-gluconate, ACTN-acetoin;
Sugars and Sugar alcohols: SALC-salicin, ARBT-arbutin, GLUC-glucose, MANN-mannose, TRHL-trehalose, FRUT-fructose, SUCR-sucrose, MNTL-manitol, CELB-cellobiose, MALT-maltose, GLYC-glycerol, ETOH-ethanol, RIB-ribose, SORT-sorbitol, MurNAc-acetylmuramic acid;
Aminoacids: ARG-arginine, ALA-alanine, LEU-leucine, ILE-isoleucine, GLU-glutamate, GLN-glutamine, GLY-glycine, ASN-asparagine, ASP-aspartate, SER-serine, THR-threonine, MET-methionine, CYS-systeine, HIS-histidine, TYR-tyrosine, TRP-tryptophan, VAL-valine, PHE-phenilalanine, PRO-proline, LYS-lysine;
Others: GLUM-glucosamine, ADE-adenine, NICO-nicotinamide, GABA, SLF-sulfate, AGM-agmantine, BET-betaine, ORN-ornitine, PUTR-putrescine, ASC-ascorbate, GALM-galactosamine, GALMAc-acetyl-D-galactosamine;
Gases/ions: Pi-phosphate, Pii-diphosphate
LB – Lower Bound, UP – Upper Bound

OptFlux outcome:

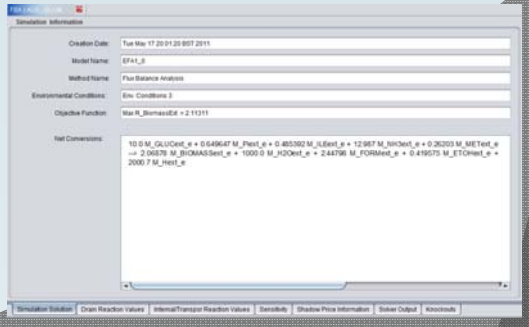

Figure 2: Snapshot of OptFlux screen displaying the overall reaction of biomass production

Conclusions and future directions

The metabolic model generated for this bacterium allows the correlation of flux distributions with different environmental conditions. It is also possible to determine the minimal medium required for growth.

As an example, figure 2 shows the overall conversion of a defined minimal medium (shown in table 1) in bi-products and biomass1.

Additionally, metabolomic data generated by our group allowed the identification of key compounds/reactions in the bacterium metabolism that were beforehand unclear, namely the evidence that *E. faecalis* is able to synthesize all amino acids, adding valuable information to the model. The next step will be the validation of the results based on published data and also in laboratory. As it has been previously shown for other organisms, the genome scale network reconstruction may serve as a valuable tool to predict the phenotypic behaviour under various genetic and environmental conditions, as well as to perform metabolic engineering simulations (e.g. gene deletion experiments) and drug target identification.

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