Metabolic modeling of the nitrogen fixation and biopolymers production by *Azotobacter vinelandii* DJ

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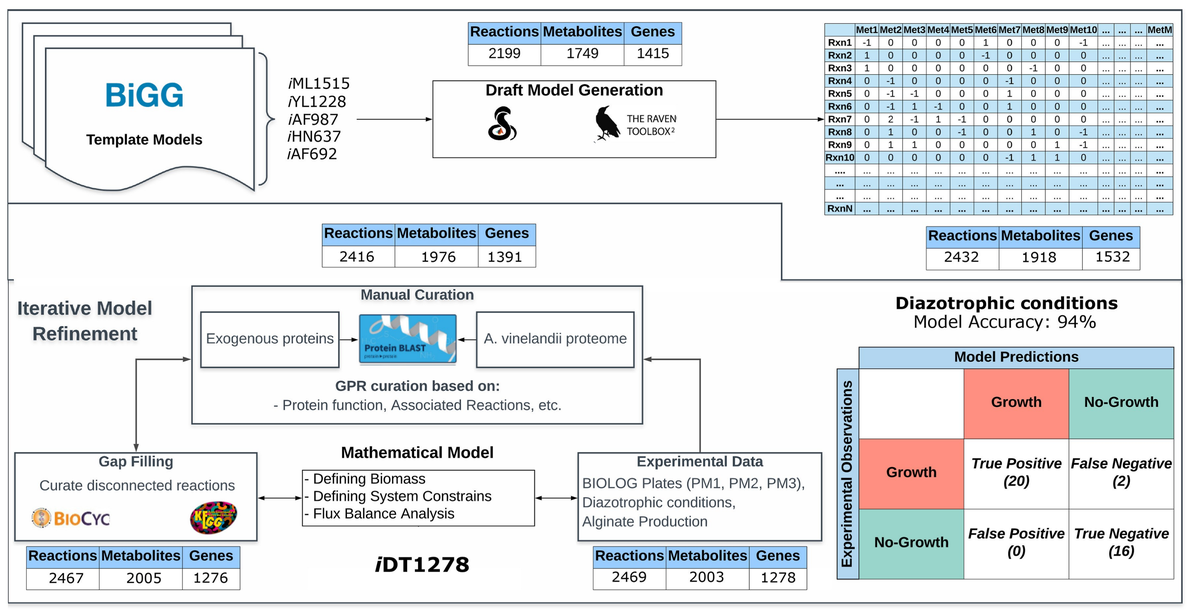
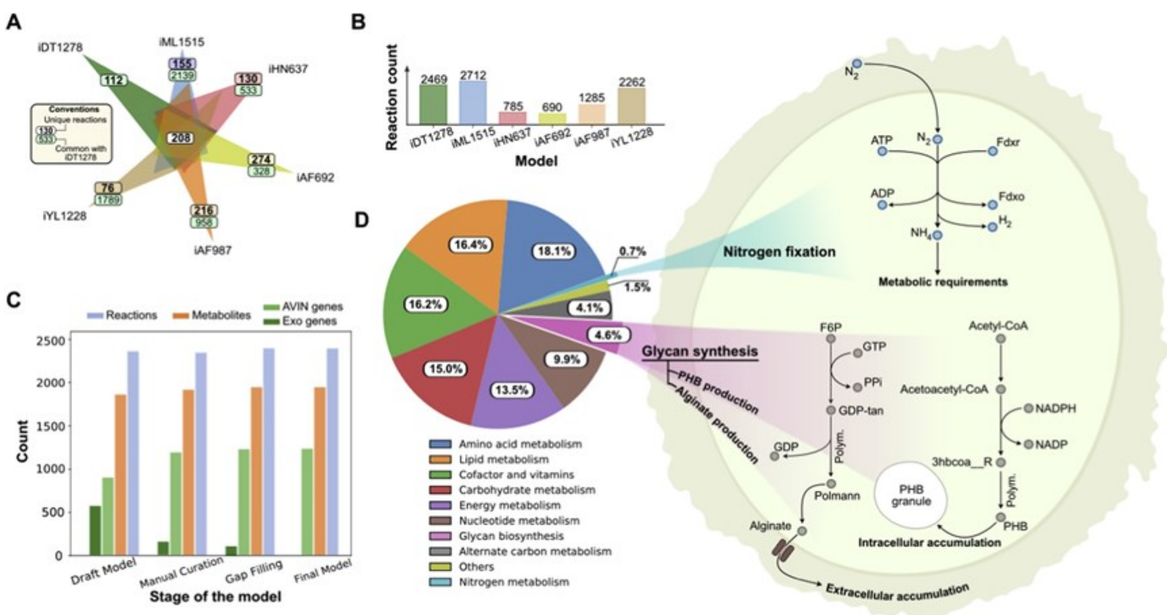
BACKGROUND

Nitrogen fixation is an important metabolic process carried out by different microorganisms, which converts molecular nitrogen into inorganic nitrogenous compounds such as ammonia (NH_3) (Gyurján et al., 1995). These nitrogenous compounds play important roles for biogeochemical cycles on earth and for the synthesis of essential biomolecules at industrial level. *Azotobacter vinelandii* is a well-known gram-negative soil bacterium capable of converting atmospheric nitrogen gas (N_2) into soluble ammonia (NH_3) as well as into other important nitrogenous soluble compounds. A. vinelandii can also produce the biopolymers alginate and polyhydroxybutyrate (PHB) depending on nutritional requirements. Alginate is biodegradable exopolysaccharide (Remminghorst & Rehm, 2006) produced to reduce internal oxygen concentration necessary to fix nitrogen. PHB is synthetized by this microorganism under high carbon/nitrogen ratios as a carbon and energy reserve in the form of cysts (Zúñiga et al., 2011). We developed the metabolic model (M-model) of Azotobacter vinelandii DJ to contextualize metabolic processes associated with nitrogen fixation, ammonium assimilation, and production of organic nitrogen on genome-scale. Our model was successfully validated using high-throughput phenotypic data and physiological data.

METHODS



RESULTS



The final *Azotobacter vinelandii* DJ metabolic model (*i*DT1278) consists of 2,003 metabolites, 2,469 reactions and 1,278. Specific metabolic capabilities of *A. vinelandii* DJ such as nitrogen fixation, PHB and alginate production represent around 3% of the metabolic reactions. *i*DT1278 includes a BOF was determined from the first reaction to predict the alginate production since *A. vinelandii* DJ (Noar et al., 2015). *i*DT1278 represents, to our knowledge, the most comprehensive M-model of the diazotroph *A. vinelandii* available to date. The model was validated under a wide range of different growth conditions (diazotrophic and non-diazotrophic growth), using high-throughput phenotypic data as well as literature information. Initially, iDT1278 was tested under six different experimental conditions, specifically, carbohydrates under diazotrophic and non-diazotrophic conditions.

Subsequently, FBA (Orth et al., 2010) was performed for a group of 38 carbon sources in diazotrophic and H_2-consuming conditions (Wong & Maier, 1985). Statistical results show for the subset of 38 carbon sources an accuracy of 95%, with 20 true positive predictions (100% positive predicted) and 16 true negative predicted results (89% negative predicted). Additional experimental validation was performed using Biolog plates for a set of carbon (PM1 and PM2) and nitrogen (PM3) sources to determine the growth rate values of *A. vinelandii* DJ. Out of 190 carbon sources from the Biolog plates, 123 compounds were identified in the model; the simulations were performed under two specific conditions: diazotrophic and non-diazotrophic simulation conditions. The same procedure used in PM1 and PM2 experiments was followed to estimate the growth rates with 75 different nitrogen sources. For this case, simulations were performed using pyruvate as the carbon source. Table. 1 shows the complete analysis of the experimental and predicted data for all carbon and nitrogen sources; statistical parameters (accuracy, sensitivity, specificity, positive predicted, negative predicted and Matthews correlation coefficient) were calculated for non-diazotrophic conditions. Ultimately, biopolymers production (alginate and PHB) was validated through experimental data retrieved from the literature. We evaluated the model accuracy to growth and alginate and PHB production using four carbon sources under diazotrophic and non-diazotrophic conditions. Simulations were confirmed to accurately predict (true positive predictions) alginate production rates

Table 1. Statistics of the predictions under carbon and nitrogen sources: true positive (TP), true negative (TN), false positive (FP), false negative, and MCC. Statistical analysis of the estimations for 38 carbon sources under nitrogen fixation and H_2 consumption conditions

Statistics	Carbon (PM1 and PM2)	Nitrogen (PM3)	Carbon+H2 (Wong and Maier, 1985)
TP	58	6	20
TN	50	62	16
FP	3	6	0
FN	10	1	2
Accuracy	0.89	0.91	0.95
Sensitivity	0.85	0.86	0.91
Specificity	0.95	0.91	1
Positive predicted	0.95	0.5	1
Negative predicted	0.84	0.98	0.89
MCC	0.28	0.62	0.67

CONCLUSION

Here we have created the most comprehensive genome-scale metabolic model for *A. vinelandii* DJ (*i*DT1278) deploying in great detail nitrogen assimilation, nitrogen fixation, as well as on alginate and PHB production. The model consists of 1,278 genes involved in 2,469 reactions. *i*DT1278 predicted accurately the growth ratio and production values of alginate and PHB production under diazotrophic and non-diazotrophic conditions. To our knowledge, this is the first M-model at genome-scale capable to simulate several carbon and nitrogen conditions (close to 250 conditions) with a high precision for growth values and polymer production (PHB and alginate) even when comparing internal metabolic fluxes.

REFERENCES

Gyurján, I., Korányi, P., Preininger, É., Varga, S. S., & Paless, G. (1995). Artificial Plant-Azotobacter Symbiosis for Atmospheric Nitrogen Fixation. In *Azospirillum VI and Related Microorganisms* (pp. 401–413). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-79906-8_46

Noar, J., Loveless, T., Navarro-Herrero, J. L., Olson, J. W., & Bruno-Bárcena, J. M. (2015). Aerobic Hydrogen Production via Nitrogenase in Azotobacter vinelandii CA6. Applied and Environmental Microbiology, 81(13), 4507–4516. https://doi.org/10.1128/AEM.00679-15

Remminghorst, U., & Rehm, B. H. A. (2006). Bacterial alginates: from biosynthesis to applications. Biotechnology Letters, 28(21), 1701–1712. https://doi.org/10.1007/s10529-006-9156-x

Zúñiga, C., Morales, M., Le Borgne, S., & Revah, S. (2011). Production of poly-β-hydroxybutyrate (PHB) by Methylobacterium organophilum isolated from a methanotrophic consortium in a two-phase partition bioreactor. Journal of Hazardous Materials, 190(1–3), 876–882. https://doi.org/10.1016/j.jhazmat.2011.04.011

Wong, T. Y., & Maier, R. J. (1985). H2-dependent mixotrophic growth of N2-fixing Azotobacter vinelandii. Journal of Bacteriology, 163(2), 528–533.