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Research Article

Nutrient Availability Impacts Intracellular Metabolic Profiles in Digital Organisms

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Abstract

The ability of organisms to utilize environmental chemicals as nutrients and adapt to changes in nutrient availability is a hallmark of life. Yet despite different environments, the concentration and osmolarity of intracellular metabolites are relatively constant across different organism. Although adaptation experiments can be performed, they are usually labour intensive and must be carried out in stepwise or gradual manner. On the other hand, digital organisms or computer-simulated organisms can be used to study adaptations to extreme conditions. Here, we examine the effects of nutrient levels on the metabolic profiles of organisms. Our results show that nutrient availability results in significantly different average intracellular metabolite amounts ($F = 5166$, p-value < 1E-200) at 1500^{th} generation despite the range within one order but there is significant decline of the impact of nutrient availability on the amounts of intracellular metabolites with increasing generations ($r = -0.995$, $F = 385$, p-value = 3.98E-05). However, mean intracellular amounts of specific metabolites are significantly different across all 12 nutrient availabilities ($14 \leq F \leq 1927$, 4.1E-304 ≤ p-value ≤ 1.6E-22). This suggests that the impact of nutrient availability is beyond the overall intercellular metabolite amounts but at the level of individual metabolites.

Keywords: Organisms; Metabolism; Nutrients

Introduction

Metabolism can be defined as the process by which an organism uses chemicals in its environment to synthesize required metabolites and biomolecules for growth. The similarity of metabolic pathways across organisms in different environments [1] suggests that organism must adapt to survive a specific environment, known as ecological niche [2]. Although such adaptation may involve spontaneous mutations [3-5] as a result of imperfect DNA replication [6], stress as a result of different environments can provide pressure for selection [7-11].

Despite adapting to different ecological niches, concentrations of intracellular metabolites are similar. An early study by Schmidt-Nielsen [12] reported that intracellular potassium in most vertebrate or invertebrate cells to be about 100 to 150 milliosmolarity. Subsequently, Fagerbakke., *et al*. [13] found that the concentrations of several intracellular metabolites to be comparable in several aquatic bacteria. This is supported by Park., *et al*. [14] whom reported comparable ($0.64 \leq r \leq 0.88$) intracellular metabolite concentrations between a mammalian cell line, yeast, and *Escherichia coli*. Taking the similarity of metabolic pathways [1] and intracellular metabolite concentrations [14] together, this suggests a significant role genetic optimization within the organism as a result of adaptation to ensure comparable intracellular metabolite concentrations despite varying environmental metabolite availability.

Although such laboratory experiments had been carried out [7-11], they are usually time-consuming and labour-intensive [15]. This gives rise to studies on improving efficiency [16]. Moreover, experimental adaptations are often carried out in stepwise or gradual manner [11] to maintain stress to be below sub-lethal level. Digital organisms (DOs), which are computer-simulated organisms [17,18] and had been used to explore various evolutionary scenarios [19-25] with alternative metabolisms [26], is considered as instances of life rather than simulations of life [27] as DOs can be considered alive [28]. Furthermore, DOs can be used to examine adaptations to extreme conditions [29] which are not suitable for biological organisms.

Using digital organism simulations, this study aims to examine the effects of the amount of environmental chemicals (acting as nutrients) on the metabolic profiles of organisms. Our results show that nutrient availability has an impact on intracellular metabolite amounts but this impact reduces with increasing generations. However, mean intracellular amounts of specific metabolites are significantly different across all 12 nutrient availabilities.

Materials and Methods

Simulation system

Digital Organism Simulation Environment (DOSE) [30,31] with D2 genomic interpreter was used as the simulation platform. D2 was modified from Dennis Interpreter [29], which was based on DOSE's native genome interpreter, Ragaraja [32]. The modifications were as follow (Appendices A and B): Firstly, the same 24 intracellular metabolites (Metabolite A to Y) and the first 35 reactions (R1 to R35) from Dennis Interpreter [29] were used. However, reactants were used in the production of products in R1 to R35, which were identical to biological enzymatic reactions. Secondly, environmental chemical conditions were defined as a list of 24 element, corresponding to the 24 intracellular metabolites defined. Thirdly, instead of only 2 active transporters [33], R36 and R37 to import extracellular metabolites C and O respectively, 13 active importers were defined (R36 to R48) for extracellular metabolites A, C, E, G, I, K, M, O, Q, S, U, W, and Y, respectively. In addition, proportion of extracellular metabolite(s), default at 1E-9 to represent the relative size between one DO and that of an ecological cell. Fourthly, 12 exporters were defined (R49 to R60) to export 50% of intracellular metabolites A, B, E, F, I, J, M, N, Q, R, U, and V respectively to their respective environmental chemical conditions. Lastly, 10 additional reactions (R61 to R70) were added to ensure that all metabolites can be produced and used in at least 1 reaction. In Dennis Interpreter [29], 3 metabolites (A, E, and N) had no usage reactions and 5 metabolites (C, I, O, Q, and V) had no production interactions. The average number of usage and production reactions per metabolite were 3.18 (standard deviation of 1.332) and 3.50 (standard deviation of 2.236) respectively. After these modifications, the average number of usage and production reactions per metabolite in D2 Interpreter were 3.60 (standard deviation of 1.225) and 3.60 (standard deviation of 2.893) respectively.

Reaction Type	Definition	Remarks
Enzymatic Reactions (n = 45)	R1: B + Y → E + W	Reactants and products for reaction was defined in Dennis Interpreter [29]
	R2: C + Q → D + R	
	R3: C + D → G + N	
	R4: C + W → L + S	
	R5: C + U → G + M	
	R6: C + V → E + P	
	R7: B + C → M + X	
	R8: F + P → B + E	
	R9: G + M → K + N	
	R10: J + S → E + M	
	R11: D + K → L + W	
	R12: F + K → G + M	
	R13: K + L → E + W	
	R14: L + M → E + N	
	R15: H + L → J + N	
	R16: M + S → H + T	
	R17: I + M → A + W	
	R18: O + R → F + W	
	R19: I + O → J + M	
	R20: C + O → X + Y	
	R21: H + Q → J + M	
	R22: R + X → E + Y	
	R23: S + X → B + K	
	R24: F + T → A + X	
	R25: T + U → E + K	
	R26: G + U → B + J	
	R27: F + U → M + R	
	R28: M + S → E + R	
	R29: K + V → F + S	
	R30: V + X → E + F	
	R31: R + W → J + S	
	R32: B + W → U + Y	
	R33: Q + Y → A + P	
	R34: P + Y → B + N	
	R35: F + Y → B + R	
	R61: A + N → O + V	New definition in D2 genomic interpreter
	R62: D + E → I + Q	
	R63: N + T → C + H	
	R64: E + N → C + T	
	R65: A + W → D + Q	
	R66: E + R → V + Y	
	R67: B + E → C + U	
	R68: M + N → O + U	
	R69: J + W → C + I	
	R70: G + J → D + T	

Importers (n = 13)	R36: Extracellular A (eA) → A + 1E-9 x eA	Redefined in D2 genomic interpreter
	R37: Extracellular C (eC) → C + 1E-9 x eC	
	R38: Extracellular E (eE) → E + 1E-9 x eE	New definition in D2 genomic interpreter
	R39: Extracellular G (eG) → G + 1E-9 x eG	
	R40: Extracellular I (eI) → I + 1E-9 x eI	
	R41: Extracellular K (eK) → K + 1E-9 x eK	
	R42: Extracellular M (eM) → M + 1E-9 x eM	
	R43: Extracellular O (eO) → O + 1E-9 x eO	
	R44: Extracellular Q (eQ) → Q + 1E-9 x eQ	
	R45: Extracellular S (eS) → S + 1E-9 x eS	
	R46: Extracellular U (eU) → U + 1E-9 x eU	
	R47: Extracellular W (eW) → W + 1E-9 x eW	
	R48: Extracellular Y (eY) → Y + 1E-9 x eY	
Exporters (n = 12)	R49: A → 50% of A, 50% of A add to eA[1]	New definition in D2 genomic interpreter[1] eA refers to extracellular A
	R50: B → 50% of B, 50% of B add to eB	
	R51: E → 50% of E, 50% of E add to eE	
	R52: F → 50% of F, 50% of F add to eF	
	R53: I → 50% of I, 50% of I add to eI	
	R54: J → 50% of J, 50% of J add to eJ	
	R55: M → 50% of M, 50% of M add to eM	
	R56: N → 50% of N, 50% of N add to eN	
	R57: Q → 50% of Q, 50% of Q add to eQ	
	R58: R → 50% of R, 50% of R add to eR	
	R59: U → 50% of U, 50% of U add to eU	
	R60: V → 50% of V, 50% of V add to eV	

Metabolite	Production Reactions	Usage Reactions	Importer	Exporter
A	R17, R24, R33	R61, R65	R36	R49
B	R8, R23, R26, R34, R35	R1, R7, R32, R67		R50
C	R63, R64, R65, R67, R69	R2, R3, R4, R5, R6, R7, R20	R37	
D	R2, R70	R3, R11, 62		
E	R1, R6, R8, R10, R13, R14, R22, R25, R28, R10	R62, R64, R66, R67	R38	R51
F	R18, R29, R30	R8, R12, R24, R27, R35		R52
G	R3, R5, R12	R9, R26, R70	R39	
H	R16, R63	R15, R21		
I	R62, R69	R17, R19	R40	R53
J	R15, R19, R21, R26, R31	R10, R69, R70		R54
K	R9, R23, R25	R11, R12, R13, R29	R41	
L	R4, R11	R13, R14, R15		
M	R5, R7, R10, R12, R19, R21, R27	R9, R14, R16, R17, R28, R68	R42	R55
N	R3, R9, R14, R15, R34	R61, R63, R64, R68		R56
O	R61, R68	R18, R19, R20	R43	
P	R6, R33	R8, R34		
Q	R62, R65	R2, R21, R22	R44	R57
R	R2, R27, R28, R35	R18, R22, R31, R66		R58
S	R4, R29, R31	R10, R16, R23, R28	R45	
T	R16, R64, R70	R24, R25, R63		
U	R32, R67, R68	R5, R25, R26, R27	R46	R59
V	R61, R66	R6, R29, R30		R60
W	R1, R11, R13, R17, R18	R4, R31, R32, R65, R69	R47	

Appendix A: Definition of Reactions.

X	R7, R20, R24	R22, R23, R30		
Y	R20, R22, R32, R66	R1, R33, R34, R35	R48	

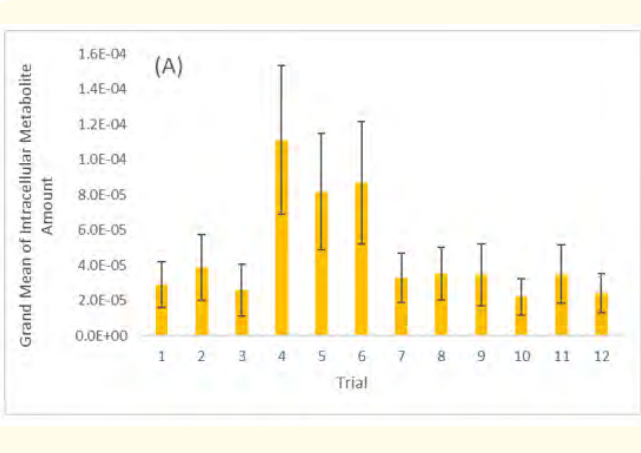
Appendix B: Metabolite Usage and Production.

General simulation setup

Each simulation replicate had 100 DOs, simulated for 1500 generations. The genome of each DO was initiated with 700 genes, 10 repeats from Gene 1 (corresponding to R1) to Gene 70 (corresponding to R70). Mutation rate was set at 1% (about 7 gene mutations per DO per generation). The selection process from one generation to the next is based on previous studies [29,34-36]. Briefly, the lowest decile of the organisms by fitness were removed per generation but in event where more than 50% of the population could be removed, a random selection of 10 organisms were removed instead. After removal, a random selection of remaining DOs after removal were replicated to top up the population to 100 DOs for the next generation. Each DO is given an empty cytoplasm (all intracellular metabolites were set to zero) before executing the metabolism coded by its genome.

Simulation trials

Two control trials (high and low) and 12 experimental trials were set up (Figure 1), with 30 simulation replicates per trial. The high and low control trials consisted of 1000 units and 10 units for each of the importable extracellular metabolites (A, C, E, G, I, K, M, O, Q, S, U, W, and Y) respectively. The non-importable extracellular metabolites (B, D, F, H, J, L, N, P, R, T, V, and X) were set at 500 units each. Experimental trial 1 (decreasing metabolites) consisted of 1000, 692, 479, 332, 230, 159, 110, 76, 53, 36, 25, 17, and 12 units for extracellular metabolites A, C, E, G, I, K, M, O, Q, S, U, W, and Y; respectively. Experimental trial 2 (increasing metabolites) consisted of 12, 17, 25, 36, 53, 76, 110, 159, 230, 332, 479, 692, and 1000 units for extracellular metabolites A, C, E, G, I, K, M, O, Q, S, U, W, and Y; respectively. Experimental trial 3 (fluctuation A) consisted of 1000 units each for extracellular metabolites A, E, I, M, Q, U, and Y; with 10 units each for extracellular metabolites C, G, K, O, S, and W. Experimental trial 4 (fluctuation B) was the opposite of trial 3, and consisted of 10 units each for extracellular metabolites A, E, I, M, Q, U, and Y; with 1000 units each for extracellular metabolites C, G, K, O, S, and W. Experimental trial 5 (middle-high) consisted of 500 units each for extracellular metabolites A, C, E, G, I, K, and M; with 1000 units each for extracellular metabolites O, Q, S, U, W, and Y. Experimental trial 6 (high-middle) was the opposite of trial 5, consisted of 1000 units each for extracellular metabolites A, C, E, G, I, K, and M; with 500 units each for extracellular metabolites O, Q, S, U, W, and Y. Experimental trial 7 (low-middle) was the reduced level of trial 5, consisted of 10 units each for extracellular metabolites A, C, E, G, I, K, and M; with 500 units each for extracellular metabolites O, Q, S, U, W, and Y. Experimental trial 8 (middle-low) was the reduced level of trial 6 and opposite of trial 7, consisted of 500 units each for extracellular metabolites A, C, E, G, I, K, and M; with 10 units each for extracellular metabolites O, Q, S, U, W, and Y. Experimental trial 9 (low and increasing) consisted of 5 units each for extracellular metabolites A, C, E, G, I, K, and M; with 64, 111, 193, 334, 578, and 1000 units for extracellular metabolites O, Q, S, U, W, and Y, respectively. Experimental trial 10 (decreasing to low) was the opposite of trial 9, and consisted of 1000, 578, 334, 193, 111 and 64 units for extracellular metabolites A, C, E, G, I, and K, respectively; and 5 units each for extracellular metabolites M, O, Q, S, U, W, and Y. Experimental trial 11 (U-shape) consisted of 1000, 410, 168, 69, 28, 12, 5, 12, 28, 69, 168, 410, and 1000 units for extracellular metabolites A, C, E, G, I, K, M, O, Q, S, U, W, and Y; respectively. Experimental trial 12 (inverse V) was the opposite of trial 11, and consisted of 5, 12, 28, 69, 168, 410, 1000, 410, 168, 69, 28, 12, and 5 units for extracellular metabolites A, C, E, G, I, K, M, O, Q, S, U, W, and Y; respectively.



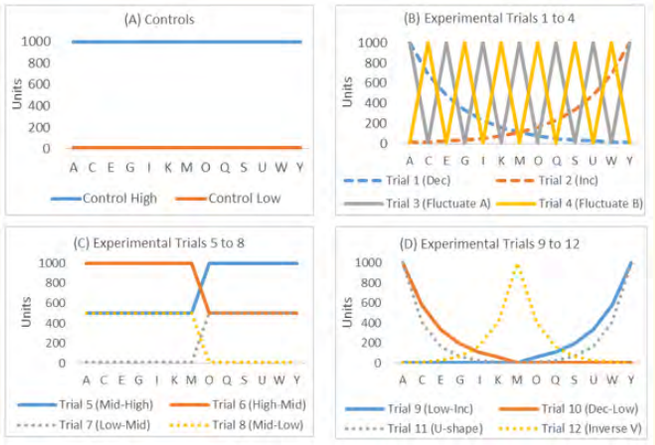


Figure 1: Extracellular metabolite scenarios in controls and experimental trials. The graphs illustrate the relative units (on vertical axis) for each extracellular metabolite (on horizontal axis). Panel A shows high and low controls. Panel B shows Trials 1 to 4 - Trial 1 is decreasing quantities while Trial 2 is increasing quantities from metabolite A to Y. Trials 3 and 4 are fluctuating between high and low quantities in opposite directions. Panel C shows Trials 5 to 8 - Trials 5 and 6 are opposite of each other while Trials 7 and 8 are opposite of each other. Trial 5 have medium quantities of metabolites A to M with high quantities of metabolites O to Y while Trial 6 have high quantities of metabolites A to M with medium quantities of metabolites O to Y. Trial 7 have low quantities of metabolites A to M with medium quantities of metabolites O to Y while Trial 8 have medium quantities of metabolites A to M with low quantities of metabolites O to Y. Panel D shows Trials 9 to 12 - Trial 9 and 10 are opposite of each other while Trials 11 and 12 are opposite of each other. Trial 9 has very low quantities of metabolites A to M with increasing quantities of metabolites O to Y while Trial 10 has decreasing quantities of metabolites A to M with very low quantities of metabolites O to Y. Trial 11 has decreasing quantities of metabolites A to M with increasing quantities of metabolites O to Y while Trial 11 has increasing quantities of metabolites A to M with decreasing quantities of metabolites O to Y.

Results and Discussion

Nutrient Availability Impacts on Intracellular Metabolite Amount. Our simulation results show that the nutrient availability results in significantly different average intracellular metabolite amounts (Figure 2A; F = 5166, p-value < 1E-200) at 1500th generation despite the range within one order, from 2.21E-05 (Trial 10) to 1.11E-04 (Trial 4). This suggests that nutritional availability may have an impact on the amounts of intracellular metabolites. If we consider the constant osmolarity within the cell [12], different amounts of intracellular metabolites may be observed as different cell sizes. This is supported by Pulina., *et al.* [37] reporting correlation between reduced nutrient availability and small phytoplankton cell sizes.

Comparing the Pearson's correlations between the grand mean of intracellular metabolite amounts and total importable metabolite amounts of 250th generation and 1500th generation using 12 trials and both high and low controls, there is steady decrease in correlations from 0.825 at 250th generation and 0.836 at 1500th generation but not significant (Figure 3A; Z = 0.973, n = 14, p-value = 0.165) between these 2 time points. The decline of correlations from 250th generation to 1500th generation is significantly correlated (Figure 3B; r = -0.995, F = 385, p-value = 3.98E-05), which suggests that the impact of nutrient availability decreases as the number of generations increases. This is consistent with various studies showing that bacteria rapidly adapts genetically to different nutritional availability [38,39]. Taken together, our results suggests that although nutrient availability impacts on the amounts of intracellular metabolites, the impact is diminished with increasing generations.

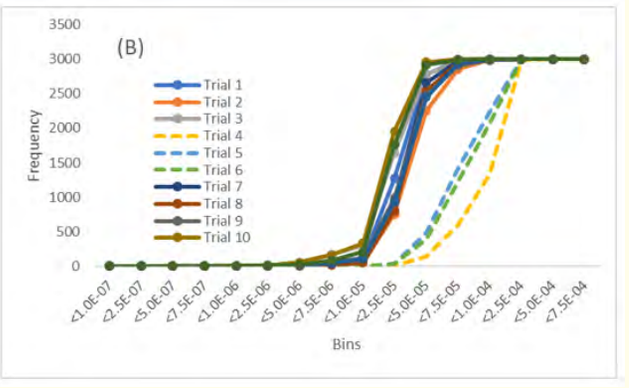


Figure 2: Intracellular Metabolite Amounts in Different Trials at 1500th Generation. Panel A shows grand mean of intracellular metabolite amounts across the 12 trials. Error bar denotes standard error. Panel B shows the distribution of intracellular metabolite amounts per trial where each trial consists of 100 digital organisms.

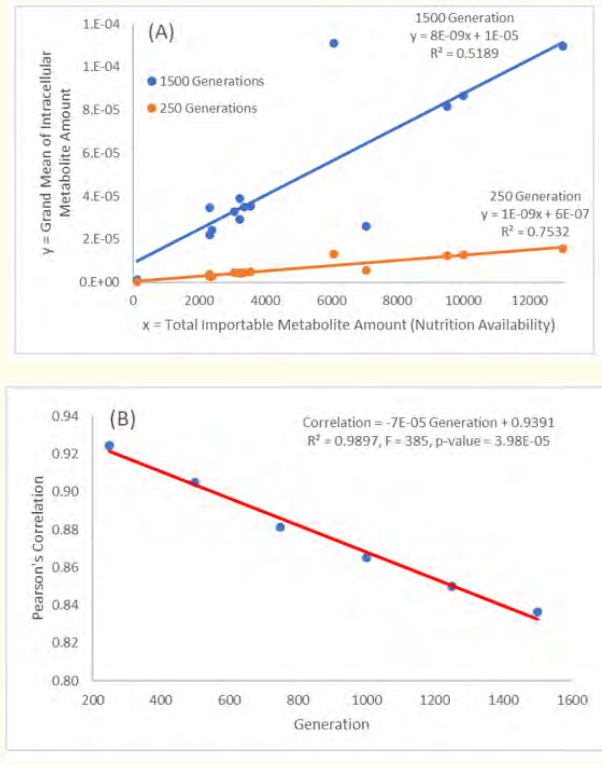


Figure 3: Effects of Nutrient Availability on Intracellular Metabolite Amounts. Panel A shows the regression at 250th and 1500th generation. Panel B shows the regression between the correlation of nutrient availability on intracellular metabolite amounts and generation count.

Substantial variations of relative intracellular metabolite amounts in different environments

Comparing the means of metabolite amounts across the 12 trials, our results (Table 1) suggests that the grand means of metabolites are significantly different across the 12 trials ($F \geq 14$, p-value $\leq$ 1.6E-22). The most significantly and least significantly metabolites are metabolites F ($F = 14$, p-value = 1.6E-22) and metabolite K ($F = 1927$, p-value = 4.1E-304) respectively (Figure 4). This suggests that the relative nutrient availability has an impact on the relative amounts of intracellular metabolites. This is consistent with a study by Huang., *et al*. [40] showing that the metabolic profile of human gastric cancer cell line SGC7901 cultured in RPMI1640 was distinctly different from that cultured in DMEM despite similar morphology and proliferation rates. Sampaio., *et al*. [41] also found that environmental nutrient levels has an impact on the metabolome of plants. Collectively, this suggests that the impact of nutrient availability is beyond the overall intercellular metabolite amounts but at the level of individual metabolite levels.

Metabolite	F	p-value	Metabolite	F	p-value
A	258	3.9E-160	N	71	2.64E-82
B	95	8.6E-98	O	1072	8.9E-261
C	1637	4.8E-292	P	270	3.9E-163
D	190	1.4E-139	Q	173	8.0E-134
E	223	2.8E-150	R	20	3.4E-31
F	14	1.6E-22	S	1686	3.2E-294
G	1925	4.8E-304	T	637	5.0E-223
H	598	1.8E-218	U	212	6.7E-147
I	228	1.1E-151	V	22	1.7E-33
J	124	6.0E-113	W	1230	6.3E-271
K	1927	4.1E-304	X	843	3.0E-243
L	155	1.1E-126	Y	1307	2.1E-275
M	258	6.3E-160			

Table 1: One-way ANOVA Analysis of Grand Means of Intracellular Metabolite Amount. The mean amounts of metabolites from each of the 100 DOs per replicate were calculated, resulting in 30 mean amounts of metabolites for each trial. The null hypothesis is the grand mean (mean of means) of metabolite amount is equal across all 12 trials.

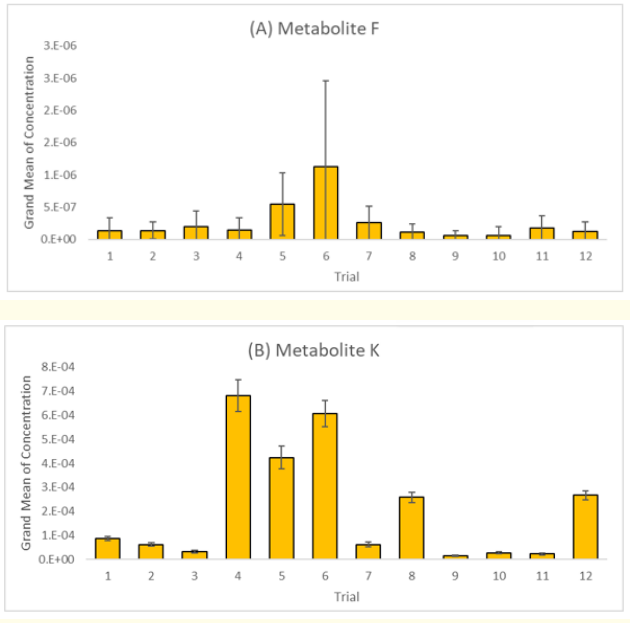


Figure 4: Grand Means of Metabolite F and Metabolite K Across 12 Trials. Panel A shows the grand means for metabolite F (largest p-value of 1.6E-22). Panel B shows the grand means for metabolite K (smallest p-value of 4.1E-304). The error bars denote standard error.

Conclusion

Using digital organisms, we show that nutrient availability has a significant impact on the overall level of intracellular metabolites by possibility effecting the levels of individual metabolites.

Supplementary Materials

The supplementary figures of this study can be downloaded from https://bit.ly/EnvCellSupplement, and the supplementary materials of this study can be downloaded from https://bit.ly/EnvCellData.

Note

Katheresan S Sooriya Kannan and Tanmay Patil are joint first authors.

Conflict of Interest

The authors declare no conflict of interest.

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