Conversion of *Escherichia coli* to Generate All Biomass Carbon from CO_2

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Presenter: Trayee Dhar **Time & location:** 15:00-15:40

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Background: Nature is distinctively rich mostly with two kinds of organisms- autotrophs and heterotrophs. Autotrophs can generate their food by metabolizing inorganic substances (CO_2) or light. On the other hand, heterotrophs are dependent on other organisms or organic molecules for formation of their cell biomass. In the modern age of synthetic biology, scientists will to introduce autotrophic nutritional mode into heterotrophic bacteria which may lead us to sustainable environment , food and energy production by using heterotrophic bacterial model *E. coli*. In this study authors successfully converted *E. coli* to autotrophy by engineering its metabolic pathway by introducing CO_2 fixation pathway (Calvin cycle) with the help of heterologous expression of several metabolically useful genes. Their approach focuses on adaptive laboratory evolution to achieve full autotrophic growth of *E. coli* by cultivating engineered parental strain in a chemostat with formate and with sparged 10% CO_2 facilitating constant organic sugar (xylose) starvation. In evolved autotrophic *E. coli*, all the biomass carbon were observed to be introduced from inorganic CO_2 and formate was used to serve as electron donor for CO_2 fixation cycle.

Objective: To develop autotrophic *E. coli* bacteria by introducing a CO_2 fixation cycle with metabolic rewiring and laboratory evolution based strategy.

Result: A parental strain for adaptive evolution was engineered in a way to introduce Rubisco driven carboxylation which harbors pfkAB and zwf genes knockout and overexpression of Rubisco, Prk, CA, and FDH. These modifications enabled the strain to assimilate CO2 for xylose (an organic sugar) catabolism via the Rubisco-Prk shunt and the strain is unable to grow in autotrophic condition. they constantly introduced organic sugar starvation in a Xylose- limited chemostat to produce organic sugar independent *E. coli* cells. The chemostat was filled with formate containing medium and it was continuously sparged with CO_2 enriched air (10%) which provided *E. coli* cells with suitable condition to propagate and also implying tremendous selection pressure to utilize CO_2 as the only CO_2 source while energy production was led by formate oxidation by FDH. After the inoculation of the ancestral strain into the chemostat, dependency on sugar started to decrease from day 120 with decreasing D- xylose in medium . Interestingly, samples taken from the chemostat from day 203 (150 chemostat generations) of the experiment and onward, were found to grow on M9 minimal media supplemented with only formate and elevated CO_2. Evolved isolated clones could grow with the given condition with a doubling time of 18± 4 hours. For validation of autotrphy evolved cells were grown in media containing comprehensively ^{13}C- labeled formate and $^{13}CO_2$ for 10 generations (until isotopic steady state). After analyzing the ^{13}C labeling patterns of different metabolites using LC/MS they observed 98% of carbon atoms of biomass building blocks across the central metabolism to be ^{13}C labeled generated solely from CO_2 and formate. While elucidating the genetic basis of trophic mode conversion they found small number of mutations in evolved autotrophic strains , which could be acquired from adaptive evolution.

Conclusion: This outstanding study showed the possibility of converting trophic mode of bacteria which could be a stepping stone for studying evolutionary transition and could advance synthetic biology toward sustainable bioproduction.

Reference: Antonovsky N et. al. Sugar synthesis from CO2 in *Escherichia coli*. Cell, 2016, pp-115-25.