Is a sugar of intermediate difficulty required for microbes to evolve the ability to process hard-to metabolize sugars?

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INTRODUCTION:

The question I am asking is about the optimal conditions for microbes to evolve the ability to metabolize difficult sugars, and if there is a relationship between the sugars on the Petri dish on which an organism's ancestors started their evolutionary path, and its evolutionary progress. The hypothesis is that having an intermediate sugar will assist microbes in evolving the capacity for feeding on high difficulty sugars. If this is correct, the microbes that live in a dish with sugars of intermediate difficulty are likely to more evolve into organisms that metabolize complex sugars than those started without the intermediate. Those that occupy a dish with no sugar should then have few or no adaptations to eat any sugars, and those that are in a dish lacking intermediate sugars will not evolve into ones that can eat the hard. In other words, is evolution a gradual process that takes small steps or does it allow the large changes in an organism's make up or habits to proliferate?

If the presence of intermediate sugars does not help microbes evolve the ability to metabolize harder ones, then evolution of this ability will take place in all of my test dishes or in none. Another possibility is that it may occur randomly and have no relationship to the conditions in which the microbes live.

It appears that I am not alone in controlling the food intake of microbes. Steven E. Finkel and Roberto Kolter have deprived *Escherichia coli* of nutrients so that it would remain in a dormant state. They have found that even when in this dormant state, mutation takes place in a random manner and the microbes with advantageous traits are in fact selected for even while in this latent state[1], suggesting that even my sugar-deprived bacteria may come to form a fit and dynamic population, and there is a chance that they may randomly evolve features whose development I am hoping is not random al all, but is in fact the result of evolutionary "steps."

Richard Lenski's work with *Escherichia coli* bacterium provides me with further evidence that microbial evolution can be random and does not always lead to the best possible outcome, *E. coli* do not normally metabolize citrate, however Richard Lenski's 20,000 generations of this bacteria did just that. This scientist and his colleges are not sure what exactly caused the change, but because microbes reproduce rapidly and are easy to store, they were able to freeze each generation and are not looking to find what caused this mutation 10,000 generations earlier[2].

Recent studies have shown evolution can act on a single gene, and as a result, the ability metabolize sugar may also be lost as well as evolved. "Indiomarine Ioihensis, a marine bacterium that lives near sulphurus hydrothermal vents underwent significant gene changes over millions of years[3]" that helped it to obtain carbon from amino acids rather than from sugars. They are now trying to map the family of the gene and the relationship of one species to another to try to determine the gene's "signature" and its normal rate of evolution.

METHODS:

To test my hypothesis, I set up 4 dishes. In the first dish, there were no additional sugars. The second contained a combination of Notose (an easy sugar) and Orose (a hard sugar). The third had Andose (Moderate) sugars and Orose, and the fourth Notose and Andose. I will run each simulation three times in a 30 by 30 dish with a constant mutation rate of 3 for 3000 steps and average the results to determine which group was most successful in evolving the ability to metabolize Orose or Andose in the very least.

RESULTS:

This study has concluded that a sugar of intermediate difficulty is not required to for microbes to evolve the ability to process sugars that are more difficult to metabolize, however, having a sugar of intermediate difficulty improves the overall fitness of the organisms and may help to retain the trait to metabolize more difficult sugars within the group over extended periods of time. None of the groups of microbes developed the ability to metabolize the hard sugar (Oro) or even the medium (And). The odd thing, is that in the final tally, 0.582% of the microbe population with only hard (Oro) and easy (Not) sugars on their dish had the ability to digest another easy sugar (Nanose) that was not present on the dish after 3000 steps, where as around 3% had were able to do this at the same time interval in an environment where an intermediate sugar was present.

54% of the microbes in the Not/ And dish evolved the ability to metabolize Not sugar, as compared to 50% of Not/ Oro group, which is a not such significant difference. I would be inclined to conclude that the evolution to process sugars other than Not was random and it was by luck that the simulation had been stopped at a point where it was present. At 3000 updates, the Not/Oro group had the same ability to metabolize Orn (which was not present in any of the dishes) as the Not/ And group, where Oro was absent (this trait was present in 0.366% of both populations), suggesting that this ability was a random mutation that would not be retained in the population as time went on. To further break this down; While the results may look as it the trait had been retained over the course of 3000 steps, it is fact 2 individuals raised in Not/ Orn (around 0.2% of the total population) that had this ability in only one of the three tests at 2000 steps, and then 1 individual had it at 3000 steps in only one of the experiments.

A close examination of the “no sugar” group proves particularly illuminating in regards to the general pattern of the evolution of the ability to metabolize sugars. In experiment 2 with no sugar, at 141 steps, 22 organisms could process Not and 34 Nan (out of a total population of 500!) but by step 353, they completely lost the ability to process Nan, then at 496 they were no longer able to process Not. Then this mutation would come back at random intervals peak at 8 organisms the fall back down to zero again. The same kind of pattern persisted in all groups, where a mutation had occurred but since there was no pay off or reward for having that ability, it was screened out by natural selection. However, this brings up the question of the Not/Orn group, where such ability would have clearly been advantageous. I think that the group that was able to digest Not became so dominant that the mutation that could do Orn was not able to establish itself in larger numbers since the Not group was proliferating. To test out this idea, I ran a further series of tests where all three of the sugars were present, three times. It turned out not to be the case. After 13,000 steps, there was no significant difference between the populations of the Not/And groups at 3000 steps and that at 12,000 steps. The microbes simply would not evolve the ability to digest anything other than the two easiest sugars. There could be several reasons for this: perhaps the space was too small and the group that thrived initially continued to dominate the space as there was simply no room for groups with other abilities, or they were so good at surviving in this environment that there was no need to evolve the ability process difficult sugars since this required more energy and effort. Maybe selection pressures were lacking and the bacteria survived very well on easy sugars; why evolve the ability to metabolize the harder ones when they were clearly outcompeting the population that had done this? There seems not to be enough evolutionary advantage to the mutation of being able to process medium or hard sugars for it to selected for. Having said this, at 12, 000 the population of Nonose eating microbes became stable at an average of 24 individuals (out of a total of 900), give or take a few. Perhaps an investigation of the optimal size of the environment for evolution of this type to take place is needed before any valid conclusions can be drawn as to whether or not intermediate sugars are needed to evolve the ability to process harder ones.

To conclude, this experiment has shown that there has to be a clear benefit in retaining a trait in the population, which was lacking in this experiment, and perhaps even more importantly, it is not enough to simply evolve a trait. In order for it to be relevant to the evolutionary process, it has to persist, and proliferate so that the majority of the population goes on to acquire it by it being passed down to the next generation of organisms. .

ENDNOTES

1. Finkel, E. Stephen. "Evolution of Microbal Diversity During Prolonged Starvation *Proceedings of The National Academy of Sciences of the United States* 107. 12 (2010), 1, http://www.pnas.org/content/96/7/4023.full. (accessed March 24, 2010).
2. Homes, Bob. "Bacteria Make Major Evolutionary Shift in the lab *New Scientist* (2008), 1-3, http://www.newscientist.com/article/dn14094-bacteria-make-major-evolutionary-shift-in-the-lab.html. (accessed March 24, 2010).
3. "Microbial Evolution *eLab* (2010), http://www.scientistlive.com/European-Science-News/Biotechnology/Microbial_evolution/20049/. (accessed March 24, 2010).

APPENDIX

The total population in every dish at 1000, 2000 and 3000 updates is 900 organisms.

AFTER 1000 updates

Sugars on plate	Average no. that can digest Not	Average no. that can digest Nan	Average no. that can digest Orn	Average fitness
None	0	0	0	0.246
Easy (Not)/ Hard (Oro)	283.33	14.33	0	0.296
Moderate (And)/ Hard (Oro)	0.33	2.6	0	0.236
Easy (Not)/ Moderate (And)	333.33	10.66	0.33	0.313

AFTER 2000 UPDATES

Sugars on plate	Average no. that can digest Not	Average no. that can digest Nan	Average no. that can digest Orn	Average fitness
None	0.66	2.66	0.66	0.316
Easy (Not)/ Hard (Oro)	435.66	7.33	0.66	0.363
Moderate (And)/ Hard (Oro)	0	0.33	0	0.28
Easy (Not)/ Moderate (And)	465	7	0.33	0.366

AFTER 3000 UPDATES

Sugars on plate	Average no. that can digest Not	Average no. that can digest Nan	Average no. that can digest Orn	Average fitness
None	0	0	0	0.356
Easy (Not)/ Hard (Oro)	456.66	2.66	0.33	0.403
Moderate (And)/ Hard (Oro)	0	0	0	0.293
Easy (Not)/ Moderate (And)	488	15	0.33	0.466