Laboratory studies on the viability of life in \( \text{H}_2 \)-dominated exoplanet atmospheres

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Theory and observation for the search for life on exoplanets via atmospheric ‘biosignature gases’ is accelerating, motivated by the capabilities of the next generation of space- and ground-based telescopes. The most observationally accessible rocky planet atmospheres are those dominated by molecular hydrogen gas, because the low density of \( \text{H}_2 \) gas leads to an expansive atmosphere. The capability of life to withstand such exotic environments, however, has not been tested in this context. We demonstrate that single-celled microorganisms (Escherichia coli and yeast) that normally do not inhabit \( \text{H}_2 \)-dominated environments can survive and grow in a 100% \( \text{H}_2 \) atmosphere. We also discuss the astonishing diversity of dozens of different gases produced by \( \text{E. coli} \), including many already proposed as potential biosignature gases (for example, nitrous oxide, ammonia, methanethiol, dimethylsulfide, carbonyl sulfide and isoprene). This work demonstrates the utility of laboratory experiments to better identify which kinds of alien environments can host some form of possibly detectable life.

There are several ideas of how rocky exoplanets (small planets with radii smaller than about 1.7 Earth radii) may have formed and maintained \( \text{H}_2 \)-dominated atmospheres. Rocky exoplanets release \( \text{H}_2 \) gas to their atmospheres as water reacts with metallic Fe in accreting materials during planet formation\(^1\). A planet that accreted from Fe-rich primitive material (for example, similar to EH chondritic meteorites) and water ice may have an \( \text{H}_2 \)-dominated atmosphere up to a few percent of the total planet mass, if all the iron and water reacted\(^1\). This extreme end-member scenario provides rationale that planets may have an \( \text{H}_2 \)-dominated atmosphere even after losing much of the envelope from stellar extreme-ultraviolet (EUV) radiation, or even if smaller than maximally possible amounts of \( \text{H}_2 \) outgassed during planet accretion. Furthermore, super–Earths more massive (with higher surface gravity) and colder than Earth may maintain an \( \text{H}_2 \) atmosphere against thermal escape\(^1\). A mechanism for replenishing an \( \text{H}_2 \) atmosphere against atmospheric escape may occur in super–Earths with predominantly icy interiors: the high pressures convert methane ice into ethane, butane or even elemental carbon, continually releasing \( \text{H}_2 \) (ref. 13).

Super–Earth exoplanets may have captured a \( \text{H}_2 \)-He atmosphere from the protoplanetary disk, in contrast to planets that formed an \( \text{H}_2 \) atmosphere from outgassing. Planets of a few Earth masses beyond about 2 au from their host star, away from destructive host star XUV radiation may maintain a primordial \( \text{H}_2 \)-He atmosphere of 1 to 100 bars, provided the planet has protective magnetic fields\(^1\). Note that at orbital separations of 2 au or greater, the planet will still be habitable as \( \text{H}_2 \) acts as a powerful greenhouse gas\(^4\), due to collision-induced infrared \( \text{H}_2 \) opacity. Slow preferential escape of \( \text{H}_2 \) in special cases may lead to a secondary He atmosphere after billions of years\(^1\). Although not yet observable, rogue rocky planets ejected from their planetary systems can also retain massive primordial \( \text{H}_2 \) atmospheres\(^1\). Despite very cold outer layers, the surface could be clement from interior radioactive heat trapped by the \( \text{H}_2 \) atmosphere greenhouse. Note that in this work we focus on atmospheres that are distinct from more massive gas envelopes found on Neptune- and sub-Neptune-sized exoplanets.

It is known that Earth had very small amounts of \( \text{H}_2 \) in its early atmosphere, up to about 0.1%\(^7\) of the Earth mass. The \( \text{H}_2 \) producing for hundreds of millions of years\(^1\) or possibly even for 2.5 billion years up to the Great Oxidation Event\(^7\). Today, the little \( \text{H}_2 \) produced on Earth is consumed by microorganisms, oxidized in the atmosphere or lost to space.

Initial attempts to observe rocky exoplanet atmospheres of any kind are limited by current telescope capabilities. The few observed with the most capable instrument, the Hubble Space Telescope WFC3 have not shown evidence for \( \text{H}_2 \)-dominated atmospheres (for example, Trappist-1 d, e, f, g\(^1\)) and LHS 1132b\(^1\), although hazy or cloud-covered \( \text{H}_2 \)-dominated atmospheres are not ruled out by the observations. Yet, exoplanet atmosphere studies via transmission spectroscopy\(^1\) and direct imaging\(^1\) are solidly established: dozens of hot giant exoplanet atmospheres have been successfully observed with detection of gases including H\(_2\), CO, H\(_2\)O, CH\(_4\), TIO and inferences of clouds or hazes\(^1,10\). Astronomers are therefore confident small exoplanet atmospheres for planets orbiting small red dwarf stars can be observed via transmission spectra with the NASA–European Space Agency (ESA) James Webb Space Telescope and via direct imaging with the extremely large ground-based telescopes now under construction. The goal in observing rocky planet atmospheres includes the identification of greenhouse gases to estimate exoplanet surface temperature, the search for water vapour, indicative of surface liquid water needed for all life as we know it, and the search for ‘biosignature’ gases that might be attributed to life.

Rocky exoplanets with \( \text{H}_2 \) atmospheres will be far easier to detect and study than those with atmospheres composed of higher mean molecular weight gases such as CO\(_2\) and N\(_2\). Atmospheric pressure (and density) falls off exponentially with increasing altitude from the planetary surface, with an e-folding factor of ‘scale height’, \( H = kT / \mu m_g \). Here \( k \) is Boltzmann’s constant, \( T \) is temperature, \( g \) is surface gravity and \( \mu \) is the mean molecular weight, and \( m_g \) is the atomic mass unit (roughly the mass of a hydrogen atom). For atmospheres observed in transmission or reflection (but not
A few other groups of microorganisms have been studied known to be toxic to life in either small or large quantities (negative health effects for animals are associated only with asphyxiation through displacement of oxygen in the lungs), microorganisms have not been shown to grow in pure 100% H₂ atmospheres before.

We are building on limited past work. Only a small set of simple microorganisms that are normally dependent on H₂ to survive (and are thus accommodated to H₂) have been studied in high-H₂-concentration environments. For example, methanogens (and acetogens) are routinely grown in 80% H₂ and 20% CO₂. Very few other groups of microorganisms have been studied in high H₂ gas concentrations (for example, sulfate-reducing bacteria, and hydrogen-oxidizing bacteria, such as knallgas bacteria). Eukaryotes have not been studied in high-concentration H₂ environments (except in a couple of isolated cases in passing) and no studies exist of yeast in high-H₂ environments. Although H₂ is not known to be toxic to life in either small or large quantities (negative health effects for animals are associated only with asphyxiation through displacement of oxygen in the lungs), microorganisms have not been shown to grow in pure 100% H₂ atmospheres before.

Approach

We conducted growth experiments (Fig. 1 and Supplementary Fig. 1) on two species of microorganisms Escherichia coli strain K-12 and yeast Saccharomyces cerevisiae strain S288C in our custom-built bioreactor system. The system consists of small borosilicate bottles with 30 ml of culture media (standard media for E. coli and yeast cell culture growth media) and 126 ml of headspace. The headspace in each bottle was flushed with appropriate gases via a needle injected through a sterile rubber stopper. The anaerobic experiments (100% H₂, 100% He, 20% CO₂ and 80% N₂) were started in serum bottles that had been previously flushed with oxygen-scrubbed N₂ before flushing with the target gas. Four bottles (air, 100% H₂, 100% He, 20% CO₂ and 80% N₂) were placed in an incubator shaker at 28 °C. We ensured the gas concentration remained stable and the cultures remained anoxic over the duration of the experiment with micromolar O₂ levels via continuous measurements using our new, precise and accurate O₂ sensors (Supplementary Figs. 2 and 3). The bottles were continuously monitored for a quick assessment of cell culture turbidity by a custom camera. We sampled the culture periodically to assess growth of the culture (E. coli by optical density measurements (OD600) and yeast by cell counting with a haemocytometer). See the Supplementary Information for more details on the approach and methods.

Because of equilibrium gas exchange, the liquid medium will be saturated with H₂. The solubility of H₂ in water is lower than N₂ and O₂ (but of the same order of magnitude), with O₂ being the most soluble of the three gases (approximately 1.6 times more soluble than H₂)23. We monitor O₂ partial pressure with custom sensors to show that the O₂ stays at trace levels for the duration of the experiments. The gas-phase partial pressures of O₂ are less than 60 µbar for the E. coli experiment and down to a few microbars for the yeast experiment (Supplementary Information), translating into about 70 and 2 nM of dissolved O₂ concentration in water, respectively. For context, these O₂ gas concentration values are close to the upper limits of those found on Archean Earth21, before Earth’s atmosphere was oxygenated.

Results

We consider a pure 100% H₂ atmosphere as a control; if life can survive in a 100% H₂ atmosphere then it can also survive in an H₂-dominated atmosphere. We show that a 100% H₂ atmosphere has no detrimental effects on microorganisms that do not normally
inhabit H2-rich conditions. We chose E. coli and yeast because they are the standard model organisms used in biology. E. coli as a representative of the domain Bacteria and yeast for Eukarya. We show that both E. coli, a simple single-celled prokaryote, as well as yeast, a more complex single-celled eukaryote, can survive and reproduce in liquid cultures surrounded by a 100% H2 gas environment.

The growth curves (Figs. 2 and 3, for E. coli and yeast, respectively) demonstrate that the organisms are reproducing normally. For E. coli grown in a 100% H2 atmosphere, the maximal cell concentration (number of cells per unit volume) is just slightly over two times less than in the control of E. coli grown in air. If the availability of O2 is low, E. coli switches from aerobic respiration to the less efficient energy metabolism (smaller amount of energy produced per unit of catabolized organic material) based on either anaerobic respiration or fermentation, but the experimental data show near-identical maximal growth rates. We note that E. coli (and yeast) derives energy from materials in the liquid culture medium. Other controls of a pure He gas environment show similar E. coli growth curves as compared to the H2 gas environment, for the same reasons. The control containing a 20% CO2/80% N2 gas mixture shows demonstrably slower growth compared with the H2 and He gas environments, likely because dissolved CO2 makes the medium more acidic, slowing growth, although this does not seem to have an effect on maximum cell concentration.

Yeast has a substantially lower maximal cell concentration in the pure H2 environment (and in other anaerobic cases), two and a half orders of magnitude lower than for the control experiment of yeast growing in air, accompanied by a roughly three times longer generation time. Yeast growth results in the controls of He and 20% CO2/80% N2 are within uncertainties identical to those of the pure H2 environment, with no distinction for the 20% CO2/80% N2. Yeast is not affected by the acidification of the medium as it is known to grow in moderately acidic media with pH as low as 4 (ref. 24).

We explain the disproportionately low growth rates and maximal cell densities of yeast as due to the absence of biochemically relevant O2, which is unrelated to energy metabolism. Independent of aerobic respiration25, O2 is a crucial substrate in the biosynthesis of many biochemicals essential to different eukaryotes, for example, unsaturated fatty acids, quinones, porphyrin rings of hemes and chlorophylls, and sterols. For yeast specifically, it is well known that O2 is required as a biosynthetic substrate for several critical metabolites (for example heme and sterols, such as ergosterol). Under anaerobic conditions, yeast cells start to rely exclusively on an import of exogenous sterols from the environment24. Thus, the lack of availability of sterols in the culture medium is likely the main limiting growth factor in the very low O2 environment of our experimental conditions. The exceptionally slow growth rates for yeast should not apply to all eukaryotes. While yeasts are obligatorily dependent on sterols for growth, other eukaryocytes (which exclusively inhabit O2-free environments) substitute sterols with other (sterol-like) molecules that do not require O2 as a substrate for synthesis25.

Discussion

Pockets of high H2 concentration (from, for example, Ca decay, serpentinization) exist on modern Earth, including microniches in mines with up to 30% to 88% H2 by volume26. These H2-rich environments are also populated by microbes (such as sulfur-reducing bacteria and some archaea). Life on Earth uses H2 for chemosynthesis, for anaerobic respiration. It is unknown if more complex eukaryotic microorganisms are inhabiting such high H2 niches, but our experiments support the idea that in principle they could be.

An atmosphere with H2 is likely to be accompanied by methane, CH4. CH4 will form in the atmosphere at moderate temperatures if four times as much hydrogen as carbon is present27. Also, along with H2, CH4 may outgas gradually from an initial large interior H2O reservoir to replenish volatiles in the atmosphere28. CH4 gas is non-toxic in the sense that CH4 does not readily chemically react in the detrimental fashion with the biochemicals of the cell. The negative biological effects of CH4, as for H2 and N2, would be associated only with the displacement of O2 and creating too low O2 partial pressures for aerobic organisms29.

Fig. 2 | Growth curves of E. coli. The vertical axis shows the cell concentration in the culture in units based on the optical density at 600 nm (OD600) and the horizontal axis shows time in hours. Error bars are reported at 2σ according to the machine measurement uncertainty. τ is the doubling time of cells in the culture. The growth curves are colour coded according to the legend, showing that high concentrations of H2 (and other gases) does not impair survival and cell division of E. coli.

Fig. 3 | Growth curves of yeast. The vertical axis shows the number of yeast cells per unit volume in the culture and the horizontal axis shows time in hours. Error bars are uncertainties in counting and reported at 2σ (see the Supplementary Information for details). τ is the doubling time of cells in the culture. The growth curves are colour coded according to the legend, showing that high concentrations of H2 (and other gases) does not impair survival and cell division of yeast.
An H₂-dominated atmosphere with its trace amounts of reduced gases is conducive to the origin of life, not detrimental, because reduced precursor molecules are presumed to be needed for life’s origin (for example, see ref. 5). For example, important organic precursor molecules (such as nitriles or carbonyls including aldehydes, ketones and amides, and so on) that could eventually participate in the formation of biologically important molecules such as nucleotides (for example in RNA and DNA) or amino acids (for example in proteins) can form much more easily if reduced gases are present than if they are not. This is especially relevant if life originated on the surface of the planet (for example, see ref. 17) and not at deep sea vents.

As an example of biosignature gases that could be present in an H₂-dominated atmosphere, we can consider gases produced by E. coli. E. coli synthesizes an impressive number of volatile molecules (45) with a wide variety of functional groups (10; Supplementary Tables 1 and 2). Several gases produced by E. coli have already been studied as promising exoplanet biosignature gases (including ammonia, methanethiol, dimethylsulfide, carbonyl sulfide, carbonyl disulfide, nitrous oxide, isoprene, and possibly produced by E. coli methane and phosphate; Supplementary Table 1) and have relatively distinctive spectral features from each other (Fig. 4). Yeast produces even more gases than E. coli (75 versus 45), but the gases fall into fewer functional group categories (Supplementary Table 2). Many of the gases produced by E. coli or yeast have yet to be evaluated as biosignatures, including a large number of carbonyls and alcohols that are challenging to distinguish spectrally.

That such a simple organism as E. coli—and a single species at that—has a diverse enough metabolic machinery capable of producing a range of gases with useful spectral features is very promising for biosignature gas detection on exoplanets. While most of the gases are produced in small quantities on Earth there are exoplanet environments where the gases if produced in larger quantities could build up.

We showed previously that any biosignature gases produced can, under reasonable stellar EUV environments, survive photochemically in H₂-dominated atmospheres32. To emphasize that an H₂-rich atmosphere (that is, with H radicals) can support the accumulation of E. coli- and yeast-produced potential biosignature gases as compared to anoxic or oxidized atmosphere (with OH or O radicals) we compare radical reaction rates (from the National Institute of Standards and Technology (NIST)33). We find that for all but one or two volatiles produced by E. coli or yeast, with reaction rates available at or close to room temperature, the H radical reaction rates are lower than the O-bearing radical (OH and O) reaction rates (Supplementary Tables 3a–c). Therefore, if a gas can accumulate in an anoxic or oxidized atmosphere it should also, in principle, be able to accumulate in an H₂-rich atmosphere.

We note that we chose a 100% H₂ gas environment as a control. Actual atmospheres dominated by H₂ will always have other gas components that are products of planetary geology or atmospheric photochemistry. Furthermore, rocky planets will have to be colder than Earth, have a more massive surface gravity than Earth and/or a replenishment mechanism to maintain an H₂-dominated atmosphere. We used microorganisms that do not normally inhabit H₂-rich conditions, and at first glance might not be well adapted to H₂-rich atmospheres. Although E. coli is known to live in anaerobic conditions such as in the gut of many animals and has previously been studied in a pure N₂ environment34, and yeast is commonly used in the fermentation industry for beer, both microorganisms are capable of surviving and reproducing in a 100% H₂ environment. The fact that both a simple cellular architecture such as E. coli (a prokaryote) and a much more complex single-cellular microorganism such as yeast (a eukaryote) can thrive in a pure H₂ gas environment, and produce a variety of byproduct gases, opens the possibility for a much broader spectrum of habitats for life on diverse habitable worlds.

Data availability
We supplied the source data for Figs. 2 and 3, which you can find as supplementary files as well as at https://dspace.mit.edu/handle/1721.1/123824. The other data that support the plots within this paper and other findings of this study are available from the authors on request.

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References
1. Ringwood, A. E. Origin of the Earth and Moon (Springer, 1979).
2. Elkins-Tanton, L. T. & Seager, S. Ranges of atmospheric mass and composition of super–Earth exoplanets. Astrophys. J. 685, 1237–1246 (2008).
3. Rogers, L. A., Bodenheimer, P., Lissauer, J. J. & Seager, S. Formation and structure of low-density exo-Neptunes. Astrophys. J. 738, 59 (2011).
4. Walker, J. C. G. Evolution of the Atmosphere (Macmillan, 1977).
5. Levi, A., Kenyon, S. J., Podolak, M. & Prilipnik, D. H-Atmospheres of icy super-Earths formed in situ in the outer solar system: an application to a possible planet nine. Astrophys. J. 839, 111 (2017).
6. Pierrehumbert, R. & Gaidos, E. Hydrogen greenhouse planets beyond the habitable zone. Astrophys. J. Lett. 734, L13 (2011).
7. Hu, R., Seager, S. & Yung, Y. L. Helium atmospheres on warm Neptune- and sub-Neptune-sized exoplanets and applications to GJ 436b. *Astrophys. J.* 807, 8 (2015).

8. Stevenson, D. J. Life-sustaining planets in interstellar space? *Nature* 400, 32 (1999).

9. Kasting, J. F. in *Treatise on Geochemistry* 2nd edn, Vol. 6, 157–175 (Elsevier, 2013).

10. Zahnle, K., Gacesa, M. & Catling, D. C. Strange messenger: a new history of hydrogen on Earth, as told by xenon. *Geochim. Cosmochim. Acta* 244, 56–85 (2019).

11. De Wit, J. et al. Atmospheric reconnaissance of the habitable-zone Earth-sized planets orbiting TRAPPIST-1. *Nat. Astron.* 2, 214–219 (2018).

12. Diamond-Lowe, H., Berta-Thompson, Z., Charbonneau, D. & Kempton, E. M.-R. Ground-based optical transmission spectroscopy of the small, rocky exoplanet GJ 1132b. *Astron. J.* 156, 42 (2018).

13. Seager, S. & Sasselov, D. D. Theoretical transmission spectra during extrasolar giant planet transits. *Astrophys. J.* 537, 916–921 (2000).

14. Marois, C. et al. Direct imaging of multiple planets orbiting the star HR 8799. *Science* 322, 1348–1352 (2008).

15. Seager, S. & Deming, D. Exoplanet atmospheres. *Annu. Rev. Astron. Astrophys.* 48, 631–672 (2010).

16. Madhusudhan, N., Knutson, H., Fortney, J. J. & Barman, T. in *Protopstars and Planets VI* (eds Beuther, H. et al.) 739–762 (University of Arizona Press, 2014).

17. Balch, W. E., Fox, G. E., Magrum, L. J.,Woese, C. R. & Wolfe, R. S. Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* 43, 260–296 (1979).

18. Peters, V., Janssen, P. H. & Conrad, R. Efficiency of hydrogen utilization during uniphilic and mixotrophic growth of *Acetobacterium woodii* on hydrogen and lactate in the chemostat. *FEMS Microbiol. Ecol.* 26, 317–324 (1998).

19. Pajusalu, M., Borlina, C. S., Seager, S., Ono, S. & Boask, T. Open-source sensor for measuring oxygen partial pressures below 100 microbars. *PLoS ONE* 13, e020667 (2018).

20. Kaye, G. W. C. & Laby, T. H. *Tables of Physical and Chemical Constants* (Longman, 1986).

21. Pajusalu, M., Borlina, C. S., Seager, S., Ono, S. & Boask, T. Open-source sensor for measuring oxygen partial pressures below 100 microbars. *PLoS ONE* 13, e020667 (2018).

22. Buzas, Z. S., Dallmann, K. & Szajani, B. Influence of pH on the growth and ethanol production of free and immobilized *Saccharomyces cerevisiae* cells. *Biotechnol. Bioeng.* 34, 882–884 (1989).

23. Waldbauer, J. R., Newman, D. K. & Summons, R. E. Microaerobic steroid metabolism and the molecular fossil record of Archean life. *Proc. Natl Acad. Sci. USA* 108, 13409–13414 (2011).

24. Davies, B. S. J. & Rine, J. A role for sterol levels in oxygen sensing in *Saccharomyces cerevisiae*. *Genetics* 174, 191–201 (2006).

25. Takishita, K. et al. Lateral transfer of tetrahymanol-synthesizing genes has allowed multiple diverse eukaryote lineages to independently adapt to environments without oxygen. *Biol. Direct* 7, 5 (2012).

26. Gregory, S. P., Barnett, M. J., Field, L. P. & Milodowski, A. E. Subsurface microbial hydrogen cycling: natural occurrence and implications for industry. *Microorganisms* 7, 53 (2019).

27. Schaefer, L. & Degley, B. Jr Chemistry of atmospheres formed during accretion of the Earth and other terrestrial planets. *Icarus* 208, 438–448 (2010).

28. Levi, A., Sasselov, D. & Podolak, M. Structure and dynamics of cold water super-Earths: the case of occluded CH4 and its outgassing. *Astrophys. J.* 792, 125 (2014).

29. Jo, J. Y. et al. Acute respiratory distress due to methane inhalation. *Tuber. Respir. Dis.* 74, 120–123 (2013).

30. Shaprio, R. *Origins: A Skeptic’s Guide to the Creation of Life on Earth* (Bantam Dell Pub. Group, 1987).

31. Benner, S. A. et al. When did life likely emerge on Earth in an RNA-first process? *ChemSystemsChem* 2, e1900035 (2020).

32. Seager, S., Bains, W. & Hu, R. Biosignature gases in H2-dominated atmospheres on rocky exoplanets. *Astrophys. J.* 777, 95 (2013).

33. Linstrom, P. J. & Mallard, W. G. The NIST Chemistry Webbook: a chemical data resource on the Internet. *J. Chem. Eng. Data* 46, 1059–1063 (2001).

34. Cox, C. S. The survival of *Escherichia coli* in nitrogen atmospheres under changing conditions of relative humidity. *Microbiology* 45, 283–288 (1966).

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Author contributions

S.S. conceived the original idea and wrote the paper with the help of J.J.P. and M.P. M.P. designed and implemented the experimental set-up. S.S. and M.P. planned the experiments with the help of J.J.P. J.H. and M.P. performed the experiments with the help of J.J.P. All authors analysed the data.

Competing interests

The authors declare no competing interests.

Additional information

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