

# 1 *Thermotoga neapolitana*

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8 Like other Thermotogales, *Thermotoga neapolitana* produces a large quantity of hydrogen using proton  
9 as the final electron acceptor. They can utilize a wide range of simple and complex sugars, proteins,  
10 peptides, as well as agricultural and food wastes as the carbon and nitrogen sources. Their thermostable  
11 enzymes have been used in many industrial settings, such as food processing and consumer products.  
12 Biohydrogen gas can power fuel cells, releasing water as the only waste, an extremely appealing prospect  
13 in urban transportation. In addition, *T. neapolitana* possesses a unique pathway called capnophilic lactic  
14 fermentation (CLF, capnophilic means “requiring CO<sub>2</sub>”), which captures CO<sub>2</sub> from the environment and  
15 combines it with acetate to form L-lactate, without harming the yield of hydrogen. L-lactate monomers  
16 can be polymerized into polylactic acid (PLA), a highly demanded feedstock material for biodegradable  
17 plastics and biocompatible medical devices.

18

19 The CLF pathway enables a non-competitive NADH-dependent synthesis of L-lactic acid and hydrogen.  
20 The fermentation process is activated by CO<sub>2</sub> and includes both a catabolic branch and an anabolic branch.  
21 The former produces acetate through acetyl-CoA from glycolysis, and the latter converts acetyl-CoA and  
22 CO<sub>2</sub> to pyruvate by pyruvate:ferredoxin oxidoreductase (PFOR) and then synthesizes lactate by lactate  
23 dehydrogenase (LDH). In addition to an energetic flow derived from glycolysis, flavin-based oxido-  
24 reductase enzymes, such as NAD-ferredoxin oxidoreductase (RNF) and NADH-dependent reduced  
25 ferredoxin:NADP oxidoreductase (NFN), supply reduced ferredoxin and NADH to support concomitant

1 synthesis of lactic acid and hydrogen. Heterologous expression of a thermostable acetyl-CoA synthetase,  
2 which catalyzes the irreversible acetate assimilation, enhances lactic acid synthesis and CO<sub>2</sub> recovery.

3

4 **TAXONOMY AND CLASSIFICATION:**

5 **DOMAIN:** Bacteria

6 **PHYLUM:** Thermotogae

7 **CLASS:** Thermotogae

8 **ORDER:** Thermotogales

9 **FAMILY:** Thermotogaceae

10 **GENUS:** *Thermotoga*

11 **SPECIES:** *neapolitana*

12 Gram-negative, strict anaerobe, optimal growth near 80°C, obligate chemoheterotroph, rod-shaped,  
13 surrounded by a sheath-like outer membrane called “toga”, does not form spores.

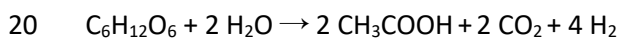
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15 **KEY FACTS:**

16 *T. neapolitana* was first isolated in 1986 from submarine hot springs in the Bay of Naples, Italy.

17

18 *T. neapolitana* can convert carbohydrate-rich biomasses into H<sub>2</sub> by dark fermentation (DF) with yields  
19 close to the Thauer limit of 4 moles of hydrogen per mole of glucose, according to the following reaction:



21

22 Production of hydrogen gas by hyperthermophilic bacteria such as *T. neapolitana* allows a better  
23 resistance to high hydrogen partial pressure and low risk of contamination during fermentation.

24

1 After sparging with CO<sub>2</sub>, *T. neapolitana* activates an anaplerotic pathway named capnophilic lactic  
2 fermentation (CLF) which leads to 97.6% pure L-lactic acid without changing the H<sub>2</sub> yield compared to DF.

3

4 The CLF pathway was first discovered in *T. neapolitana* but missing in *T. maritima* and other  
5 Thermotogales and Pseudothermotogales.

6

7 The genomes of *T. neapolitana* and *T. sp.* strain RQ7 share an average nucleotide identity of 98.49%,  
8 suggesting that RQ7 is a strain of *T. neapolitana*.

9

10 RQ7 is naturally competent and is a useful tool for genetic manipulation of this group of important  
11 thermophiles.

12

13 The discovery of a bifunctional aldehyde/alcohol dehydrogenase clarifies the pathway of ethanol  
14 production in *T. neapolitana*.

15

16 System biology tools have been developed to model *T. neapolitana* metabolic profiles, such as the kinetic  
17 modeling of H<sub>2</sub> production under CLF conditions and the genome-scale metabolic modeling of RQ7.

18

19 Expression of a *T. neapolitana* endoglucanase in transgenic hybrid poplar trees resulted in more digestible  
20 lignocellulosic biomass, offering a potential way to reduce pretreatment costs for the bioenergy industry.

21

## 22 **Acknowledgments**

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24

1 **Declaration of interest**

2 No interests are declared.

3

4 **Literature**

- 5 1. Belkin, S. *et al.* (1986) A new sulfur-reducing, extremely thermophilic eubacterium from a  
6 submarine thermal vent. *Appl. Environ. Microbiol.* 51, 1180-1185
- 7 2. Dipasquale, L. *et al.* (2014) Capnophilic lactic fermentation and hydrogen synthesis by  
8 *Thermotoga neapolitana*: An unexpected deviation from the dark fermentation model. *Int J*  
9 *Hydrogen Energy.* 39, 4857-4862
- 10 3. Han, D. *et al.* (2014) Natural transformation of *Thermotoga* sp. strain RQ7. *BMC Biotechnology*  
11 14, 39
- 12 4. Wang, Q. *et al.* (2021) A novel bifunctional aldehyde/alcohol dehydrogenase catalyzing  
13 reduction of acetyl-CoA to ethanol at temperatures up to 95 °C. *Sci. Rep.* 11, 1050-7
- 14 5. Pradhan, N. *et al.* (2021) Kinetic modeling of hydrogen and L-lactic acid production by  
15 *Thermotoga neapolitana* via capnophilic lactic fermentation of starch. *Bioresour. Technol.* 332,  
16 125127
- 17 6. Gautam, J. and Xu, Z. (2021) Construction and Validation of a Genome-Scale Metabolic Network  
18 of *Thermotoga* sp. Strain RQ7. *Appl. Biochem. Biotechnol.* 193, 896-911
- 19 7. Xiao, Y. *et al.* (2018) Expression of a hyperthermophilic endoglucanase in hybrid poplar modifies  
20 the plant cell wall and enhances digestibility. *Biotechnol. Biofuels* 11, 225-7.
- 21 8. d'Ippolito, G. *et al.* (2014) Recycling of Carbon Dioxide and Acetate as Lactic Acid by the  
22 Hydrogen-Producing Bacterium *Thermotoga neapolitana*. *ChemSusChem* 7, 2678-2683
- 23 9. d'Ippolito, G. *et al.* (2020) CO<sub>2</sub>-Induced Transcriptional Reorganization: Molecular Basis of  
24 Capnophilic Lactic Fermentation in *Thermotoga neapolitana*. *Frontiers in Microbiology* 11, 171

- 1 10. Esercizio, N. *et al.* (2021) Improvement of CO<sub>2</sub> and Acetate Coupling into Lactic Acid by Genetic
- 2 Manipulation of the Hyperthermophilic Bacterium *Thermotoga neapolitana*. *Microorganisms* 9,
- 3 8, 1688
- 4