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Genomic investigation of the filamentous foam forming *Candidatus* 'Microthrix parvicella' isolated from activated sludge

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Introduction

Candidatus 'Microthrix parvicella' (henceforth referred to as 'Microthrix') is a Gram positive, filamentous member of the *Actinobacteria* (Fig. 1). It is observed in activated sludge wastewater treatment plants where it is often associated with the poor sludge separation problems known as 'bulking' and 'foaming' (Fig. 2).

Despite the importance of 'Microthrix' little is known about its physiology and why it thrives in these treatment plants; an improved understanding is likely to provide the key to improving future control strategies.

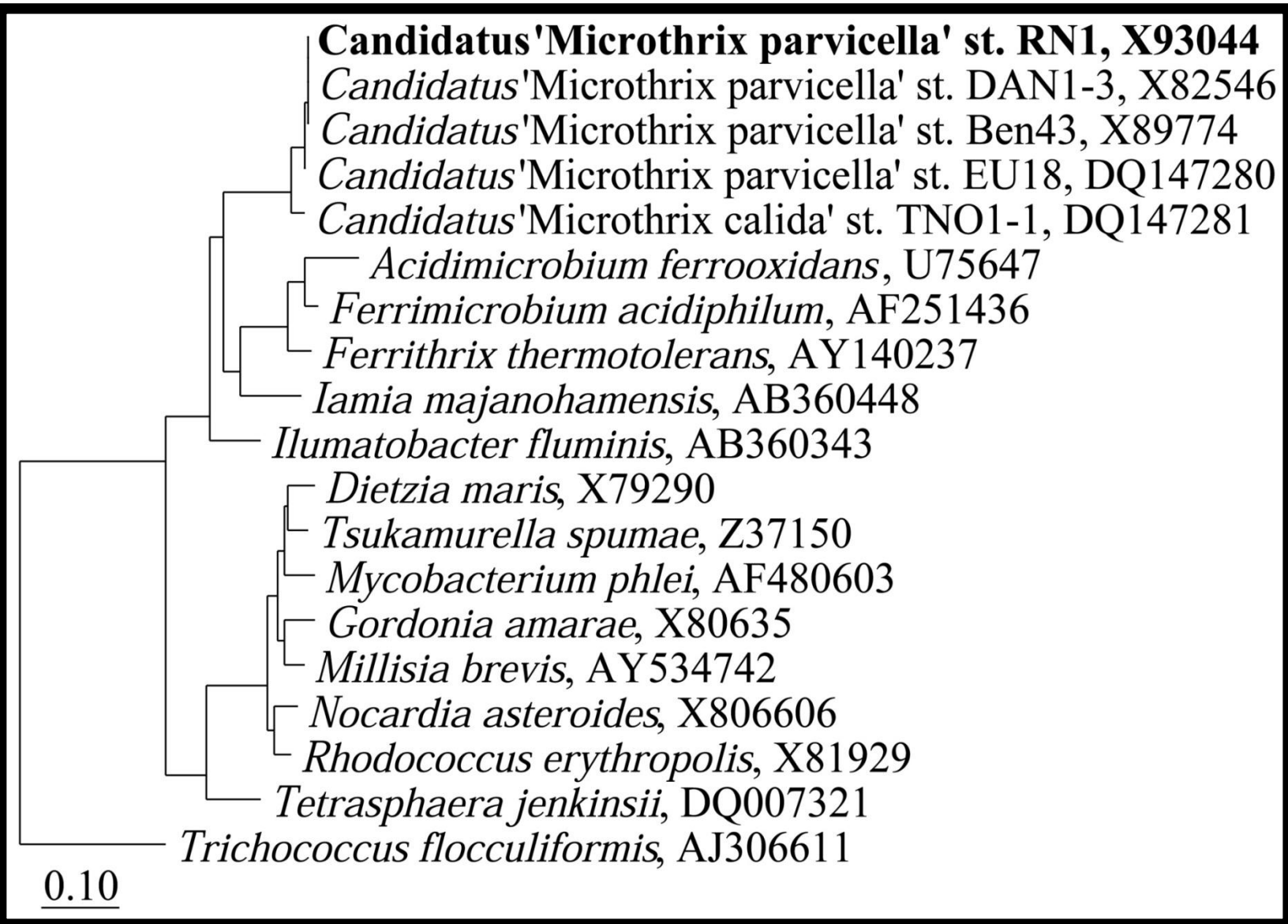


Fig 1. 16S rRNA gene ML phylogenetic tree of 'Microthrix' isolates and selected related sequences.

Aim

To develop a putative metabolic model to explain the success of 'Microthrix' under the dynamic conditions of activated sludge treatment (Fig. 3).

Approach

- An almost complete genome Of 'Microthrix' strain RN1 (Fig. 1) was obtained using the 454 sequencing method.
- Annotations were made with the MaGe platform (Genoscope) for the key metabolic pathways, entailing the carbon, nitrogen and phosphorus metabolism.

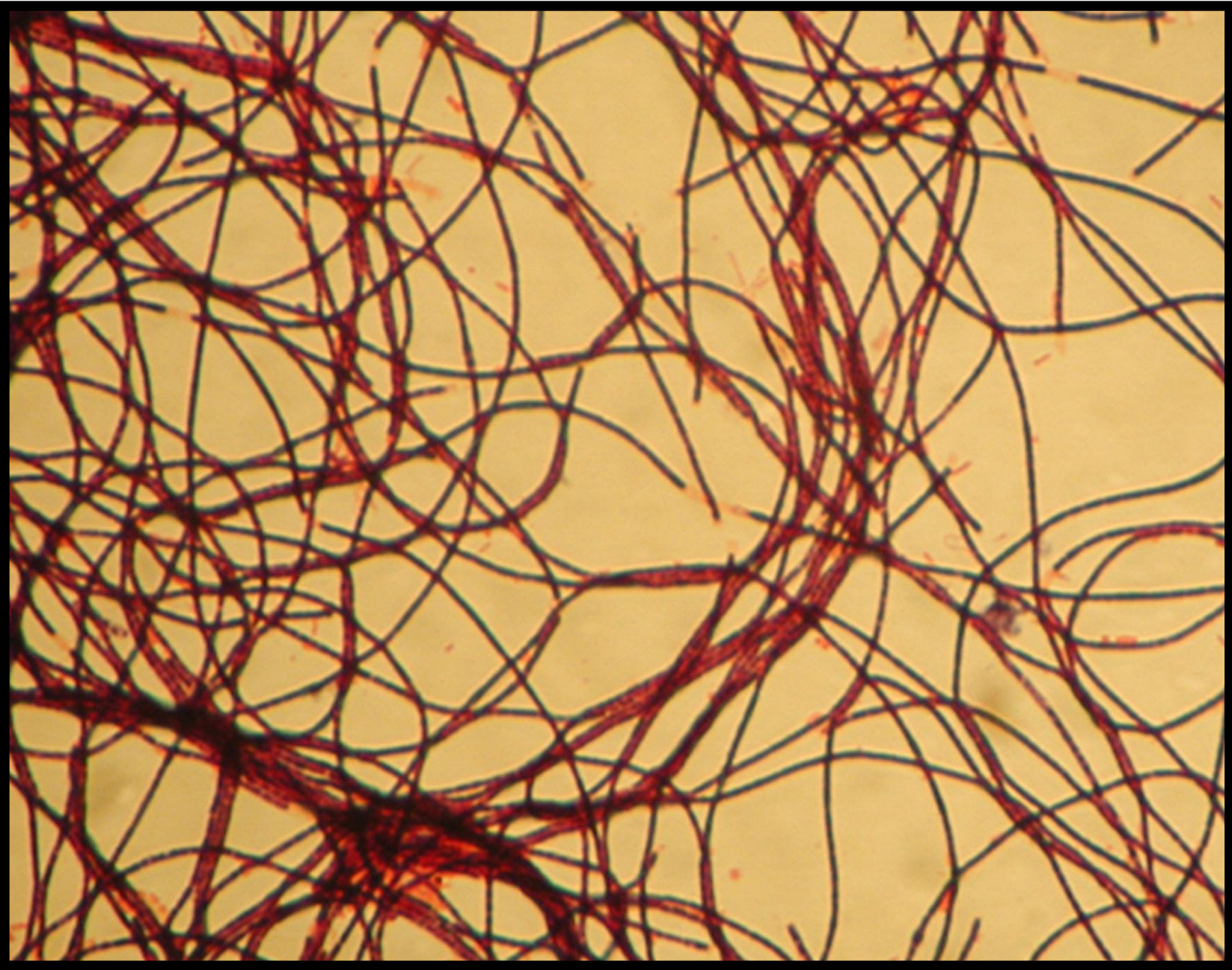


Fig 2. Gram stain micrograph of the biomass from an activated sludge plant dominated with 'Microthrix'.

Summary of key pathways

Pathway	Present in genome?
Central carbon metabolism	
Oxidative TCA cycle	Yes
Reductive TCA cycle	No
Glyoxylate shunt	No
Ethylmalonyl-CoA	Yes
Glycolysis EM	Yes
Glycolysis ED	No
Gluconeogenesis	Yes
Fermentation	No
Storage polymers	
Polyhydroxyalkanoate synthesis	No
Glycogen synthesis	No
Polyphosphate synthesis	Yes
Triacylglycerol synthesis	Yes
Denitrification	
Nitrate (NO ₃ ⁻) → Nitrite (NO ₂ ⁻)	Yes
Nitrite (NO ₂ ⁻) → Nitric oxide (NO)	Yes
Nitric oxide (NO) → Nitrogen (N ₂)	No

Table 1. Summary of selected pathways in the 'Microthrix' genome.

Summary of metabolic model

Based on the annotation of key pathways the main features of the proposed metabolic model for 'Microthrix' in activated sludge is summarised as follows (Fig. 3).

Anaerobic conditions:

- Uptake and storage of lipids as intracellular triacylglycerols (TAGs).
- Hydrolysis of polyphosphate provides energy for carbon uptake and storage.

Aerobic/anoxic conditions:

- Stored TAGs are utilised via the β -oxidation pathway, ethylmalonyl-CoA pathway and TCA cycle providing energy and carbon for growth and cell maintenance.
- Phosphate is taken up and stored as polyphosphate.

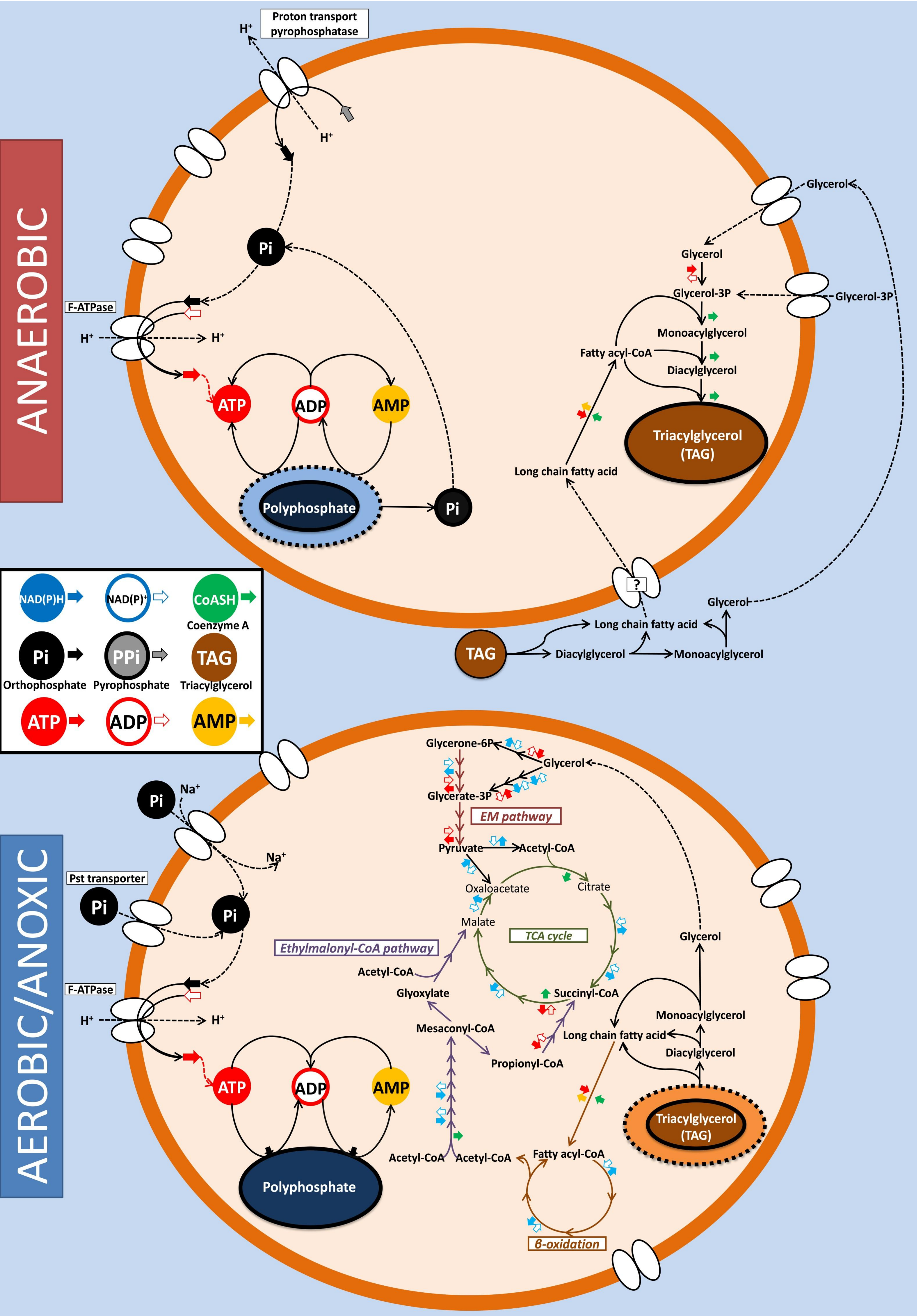


Fig 3. Diagrammatic summary of the energy and carbon metabolism of 'Microthrix' in activated sludge wastewater treatment systems.

Closing remarks

- As indicated by *in situ* data the 'Microthrix' genome indicates that it is a lipid specialist.
- The annotated genome provides the foundation for -omic based methods such as transcriptomic and proteomic studies that will validate this model and provide further insight into its *in situ* physiology.
- The annotation analysis of the 'Microthrix' genome is an ongoing project.