¹ Chain elongators, friends and foes

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9 **HIGHLIGHTS:**

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- Chain elongation microbiomes are surprisingly diverse and contain many unclassified microorganisms.
 - Microbial interactions between chain elongators and other microbes mostly result in decreased bioprocess efficiency.
 - Adequate process conditions can minimise parasitic interactions.

BACKGROUND: The utilization of wastes as new raw materials in the 16 manufacturing of goods and commodities is a central challenge of the 17 circular economy. Among the various routes and concepts put forward, the 18 production of medium-chain carboxylic acids (MCCA; e.g. caproic acid, 19 caprylic acid, etc.) by microbial chain elongation has recently garnered 20 much interest due to the market value of the target molecules (> $\leq 2 \cdot kg^{-1}$) 21 and wide range of potential applications (e.g. antimicrobial, anticorrosion 22 agent, precursor or plasticisers, etc.). Furthermore, their hydrophobicity 23 facilitates product recovery from fermentation broths compared to short-24 chain carboxylic acids (SCCA)¹. To date, most reports – as well as ongoing 25 commercial endeavours - producing MCCA have relied on the use of mixed 26 cultures for the transformation of (waste-derived) feedstocks into MCCA. 27 While a mixed culture approach is suitable for waste-fed processes, it 28 hinders the understanding of the microbiology and microbial ecology in 29 these systems. This aspect is of crucial importance when aiming to use such 30 microbiomes for bioproduction, which requires both process controllability 31 and reliability. Here we dissect the knowledge and information available in 32 literature (both from pure and mixed culture studies) to identify the most 33 common chain elongation culprits and their satellite communities, discuss 34 their most relevant metabolic and physiological features and how such 35 interactions can impact an MCCA-bioproduction process. 36

RESULTS & DISCUSSION: To the best of the authors' knowledge, there 37 are to date 15 reported caproic acid producers, of which 8 were reported 38 since 2010, and 4 in 2020 alone (3 part of not yet peer-reviewed studies). 39 Out of these 15, only 7 have been confirmed to utilize the reverse beta-40 oxidation pathway. All caproic acid producing organisms belong to the 41 phylum Firmicutes. If one analyses the microbial community data (based 42 on 16S rRNA bacterial gene sequencing data; V4 region) available for chain 43 elongation microbiomes one realizes that: i) most studies report rather 44 diverse communities (even those fed with synthetic media), seldom 45 containing a dominating Operational Taxonomic Unit (OTU), and rich in 46

unclassified microorganisms (see Figure 1A for an example on ethanol chain
elongation)²⁻⁶; ii) most dominant genera do not seem to be linked to
characterized chain elongators, some of them even belonging to other phyla
such as Proteobacteria or Bacteroidota; and iii) microbiomes from different
laboratories are clearly distinct (see Figure 1B for an example on a BrayCurtis dissimilarity PCoA from ethanol chain elongation samples).



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Figure 1. Reported chain elongating microbiomes fed with synthetic ethanol media. (A) Relative abundance of top 12 genera as OTUs. (B) Bray-Curtis dissimilarity PCoA.

Given the fact that chain elongation technologies aim to use wastes for 57 bioproduction, it is essential to understand the chain elongating microbial 58 communities and the trophic interactions between the different quilds to 59 design efficient bioprocesses. While chain elongators seem to have little 60 friends (with the exception of lactic acid bacteria producing lactic acid 61 and/or ethanol as potential electron donors), many microbial groups 62 compete for substrates (e.g. acetoclastic methanogens, sulfate reducing 63 bacteria (SRB), propionic acid bacteria, etc.) or decrease the process 64 hydrogenotrophic efficiency by consuming the products (e.g. 65 methanogens, homoacetogens, etc.). Operational conditions such as pH or 66 product extraction shape the microbiome and affect these interactions. 67

CONCLUSION: The application of chain elongation technologies is moving far ahead of the current understanding of the microbiology and microbial ecology of these systems. There is a clear need for the identification and characterization of unknown organisms, as well as moving from identity into function. Additionally, knowledge is lacking on the interactions between functional guilds and how to control them. Dedicating research to these knowledge gaps will be key to design efficient bioprocesses.

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