Bioresource Technology 259 (2018) 128–135

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Co-culture-based biological carbon monoxide conversion by *Citrobacter amalonaticus* Y19 and *Sporomusa ovata* via a reducing-equivalent transfer mediator

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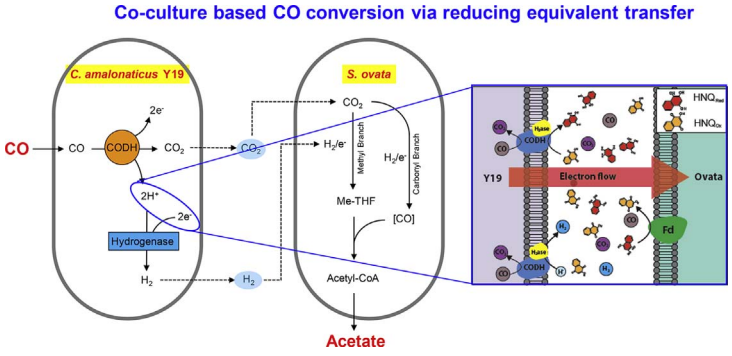
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Carbon monoxide
Co-culture
Reducing equivalent
Citrobacter amalonaticus Y19
Sporomusa ovata

ABSTRACT

The biological conversion of carbon monoxide (CO) has been highlighted for the development of a C1 gas biorefinery process. Despite this, the toxicity and low reducing equivalent of CO uptake make biological conversion difficult. The use of synthetic co-cultures is an alternative way of enhancing the performance of CO bioconversion. This study evaluated a synthetic co-culture consisting of *Citrobacter amalonaticus* Y19 and *Sporomusa ovata* for acetate production from CO. In this consortium, the CO_2 and H_2 produced by the water-gas shift reaction of *C. amalonaticus* Y19, were utilized further by *S. ovata*. Higher acetate production was achieved in the co-culture system compared to the monoculture counterparts. Furthermore, syntrophic cooperation via various reducing equivalent carriers provided new insights into the synergistic metabolic benefits with a toxic and refractory substrate, such as CO. This study also suggests an appropriate model for examining the syntrophic interaction between microbial species in a mixed community.

1. Introduction

Synthesis gas (syngas), consisting of CO, H_2, and CO_2, is a promising feedstock for the bio-based production of organic chemicals and fuels (Dürre and Eikmanns, 2015; Munasinghe and Khanal, 2010). CO, which accounts for 50–70% of the flue gas produced from the basic oxygen furnace in steel mill processes, is also a major component of the off-gas in many industrial processes (e.g., the steel industry and biomass

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https://doi.org/10.1016/j.biortech.2018.02.129
Received 26 December 2017; Received in revised form 27 February 2018; Accepted 28 February 2018
Available online 02 March 2018
0960-8524/

gasification). Although CO is a well-known toxic ingredient to most organisms, acting as a respiratory inhibitor, several microorganisms from various taxonomic groups can tolerate and even convert CO to value-added chemicals, such as acetate and ethanol, via a microbial CO conversion process, called gas fermentation (Chang et al., 1999; Geelhoed et al., 2016; Sipma et al., 2006). Pure culture-based CO conversion was examined using *Citrobacter* sp., *Clostridium* sp., *Rhodospirillum* sp., and *Eubacterium limosum* KIST612 (Diender et al., 2015; Jung et al., 1999a, 2002; Park et al., 2017; Younesi et al., 2005). Genetic modification was attempted to improve the low conversion rate and yield of wild type strains. In particular, the genetic systems and metabolic engineering of *Clostridium* strains have been studied extensively for the production of acetate, butanol, ethanol, and isobutanol (Cho et al., 2015; Valgepea et al., 2017).

The heavy recombination work of a single strain frequently causes a metabolic burden, making it vulnerable to environmental changes that may be more severe in the presence of CO (Wu et al., 2016a). A previous study suggested that multiple species coexist and carry out complementary activities for the energy and carbon metabolism, developing ecological networks that cannot be achieved in a single species environment (Faust and Raes, 2012). Research on the distribution of the CO dehydrogenase gene in sub-seafloor sediment samples shows that anaerobic microbial ecosystems are also capable of utilizing CO (Hoshino and Inagaki, 2017). Therefore, a study of the synthetic microbial community has attracted interest to better understand the natural microbial interactions, dynamics, and ecology as well as to improve the performance of practical applications, such as the CO biorefinery.

Recently, synthetic microbial communities with a defined co-culture have been reported to perform complicated biological functions that may be difficult to achieve with individual strains or species (Hill et al., 2017; Kim et al., 2016; Weiss et al., 2017; Xiao et al., 2016; Zhang et al., 2015). Members of the microbial consortium interact with each other. This syntrophic cooperation allows a “division of labor”, suggesting that the overall bioproduction/bioconversion relies on the combination of each constituent strain (Lindemann et al., 2016). Zhang and Wang reported that modular co-culture engineering overcomes the limit of the metabolic capacity of a single strain as an alternative platform in a bioprocess for the production of valuable chemicals and energy (Zhang and Wang, 2016). Medium-chain fatty acids and higher alcohols have also been produced by a synthetic co-culture (Diender et al., 2016; Wu et al., 2016b). A thermophilic microbial co-culture, consisting of *C. hydrogenoformans* and *M. thermoautotrophicus*, could efficiently uptake CO or mixtures of $H_2/CO/CO_2$ to produce methane efficiently in CSTR-bioreactors at high conversion rates (Diender et al., 2018).

The division of labor approach also can be a biological tool to produce acetate from CO because the process is composed of two consecutive steps. Initially, microbial oxidation of CO is catalyzed by carbon monoxide dehydrogenase (CODH), $CO + H_2O \rightarrow CO_2 + 2H^+ + 2e^-$ ($E^{0\prime} = -0.52$ V), via the biological water-gas shift reaction (WGSR). CO_2 is then fixed into a methyl-group and converted to acetyl-CoA and biomass via the Wood-Ljungdahl pathway (WLP). Although bacterial communities possessing both reactions have been reported in the natural environment (Esquivel-Elizondo et al., 2017), mixed consortia make it difficult to examine the synthrophic association and its mechanism of CO conversion in a natural CO-rich community.

This study evaluated a synthetic microbial consortium using a defined co-culture of *Citrobacter amalonaticus* Y19 and *Sporomusa ovata* to convert CO to acetate (Fig. 1). Y19 possesses WGSR, simultaneously producing CO_2 and hydrogen from CO, and *S. ovata* is a well-known acetogen that converts CO_2 and hydrogen to acetate. The co-culture system was compared with a monoculture for CO conversion and acetate formation. The change in the population of each bacterium in the co-culture was analyzed by quantitative real-time PCR (q-PCR), and the advantages of cell growth on the syntrophic association between the two species were examined. The effects of various electron mediators, such as methyl viologen (MV), neutral red (NR), 2-hydroxy-1,4-naphthoquinone (HNQ), and hydroquinone (HQ), on reducing equivalent transfer in the co-culture system were also examined.

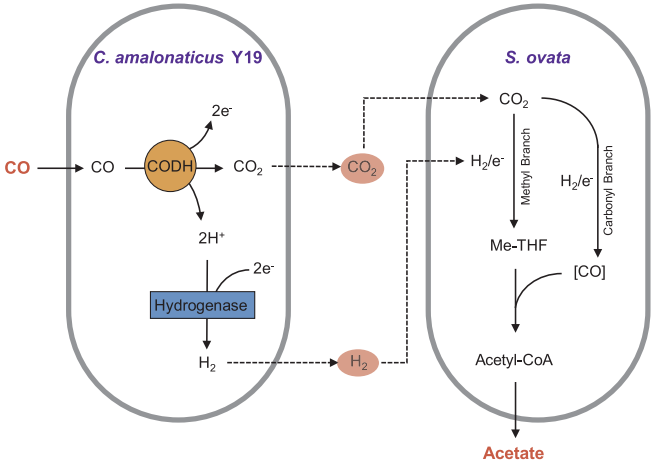

Fig. 1. Schematic representation of the co-culture of *C. amalonaticus* Y19 and *S. ovata*.

2. Materials and methods

2.1. Strains and culture condition

S. ovata was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Germany) and grown in modified DSMZ-recommended growth medium (DSMZ 311). *C. amalonaticus* Y19 (hereinafter referred to as Y19) was isolated and kindly provided by Prof. Park (Jung et al., 1999b). Both strains were stored at −80 °C before the experiment. The defined media were composed of the following components (per liter of distilled water): 0.5 g of NH_3Cl, 0.5 g of $MgSO_4{\cdot}7H_2O$, 0.25 g of $CaCl_2$, 2.25 g of NaCl, 2.00 mg of $FeSO_4{\cdot}7H_2O$, 0.3 g of L-Cysteine-HCl·H_2O, 0.3 g of $Na_2S{\cdot}9H_2O$, 0.35 g of K_2HPO_4, 0.23 g of KH_2PO_4, 4 g of $NaHCO_3$, 1 ml of a trace element solution, 1 ml of a selenite-tungstate solution, and 10 ml of a vitamin solution (Möller et al., 1984). Before starting the experiments, 50 ml of the defined media in 165 ml serum bottles were purged with CO_2/N_2 (20:80, v/v) and sterilized in 121 °C for 15 min. After autoclaving, the bottles were flushed with filtered Ar/CO (80:20, v/v) gas (20 ml/min) for 10 min.

2.2. Co-culture experiments

The co-culture experiments were conducted in a 165 ml serum bottle with 50 ml of the liquid phase. Y19 and *S. ovata* were harvested at the late exponential phase and washed twice with the defined media. Pre-cultures of Y19 were incubated in the media augmented with 0.5 g/L of yeast extract and 1 g/L of maltose in the presence of CO. The pre-culture of *S. ovata* were also grown in the media in the presence of CO_2 and H_2. After detecting growth in both pure cultures, Y19 and *S. ovata* were transferred to the defined media at an initial O.D of 0.01 and 0.5, respectively, initiating co-cultivation. The inoculated serum bottles were purged with CO_2/N_2 (20:80, v/v) to remove the oxygen, and then replenished with filtered Ar/CO (80:20, v/v) for 10 min (20 ml/min).

To examine the effects of the electron carrier mediators, 50 μM of methyl viologen (MV), neutral red (NR), 2-hydroxy-1,4-naphthoquinone (HNQ), and hydroquinone (HQ, Sigma Aldrich, USA) were added to the co-culture experiment to identify the electron transfer mechanism. All bottles were incubated in a shaking incubator (30 °C, 250 rpm).