XV WORLD FORESTRY CONGRESS
Building a Green, Healthy and Resilient Future with Forests
2–6 May 2022 | Coex, Seoul, Republic of Korea

Characterization of formate dehydrogenase from *Trametes versicor* for formate production from CO2 gas

Su-Yeon Lee, Soo-Min Lee, Mi-Jin Park, Seokyooun Jang, Soo-kyeong Jang

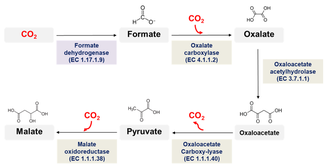
National Institute of Forest Science, Forest Products and Industry Department, Forest Industrial Materials Division

BACKGROUND

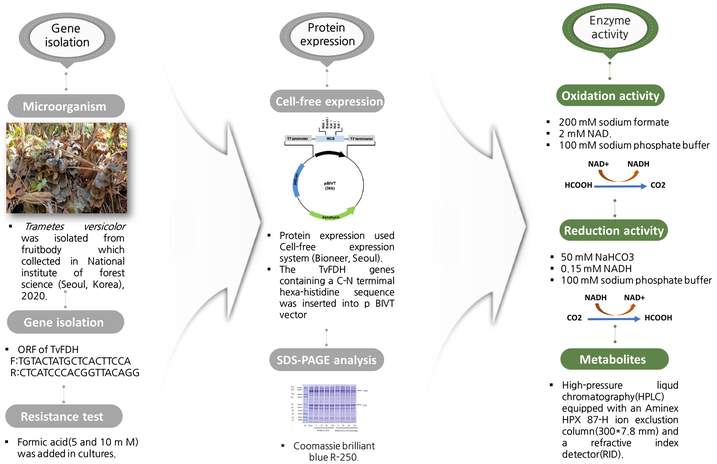
Carbon dioxide bio-conversion into useful chemicals(formic acid, methanol, ethylene, carbon monoxide etc.) has been studied as alternative and attractive production method, in recent years. Formate dehydrogenases (FDHs) are a heterogeneous group of enzymes that catalyze the oxidation of formic acid to carbon dioxide. Recently, several FDHs from bacteria, yeast, and plants has been shown to possess activity in the reverse reaction, the reduction carbon dioxide to formic acid under appropriate conditions.

Objective

Wood rot fungi belong to Basidiomycetes, they have especially acid-producing abilities, formic acid is known the major acidic metabolites. However, reduction activity of FDHs form wood-rotting fungi has not been characterized. In this study, we isolated and characterized the FDH(TvFDH) from wood rot fungus, *Trametes versicolor*.



Materials and methods



Results and discussion

Acid resistance test

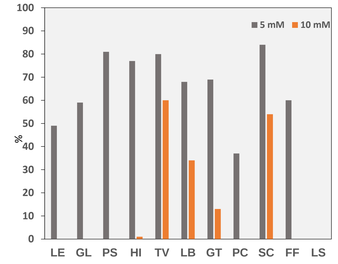


Fig1. Growth rate(%) of mycelium of wood rot fungi under the formic acid(5 and 10 mM)

LE: *Lentinula edodes*, GL: *Gymnopus luxurians*, PS: *Panellus stipticus*, HI: *Heterobasidion insulare*,TV: *Trametes versicolor*, LB: *Lenzites betulina*, GT: *Gloephyllum trabeum*, PC: *Pleurotus citrinipileatus*, SC: *Schizophyllum commune*, FF: *Flammulina filiformis*, LS: *Lepista sordida*

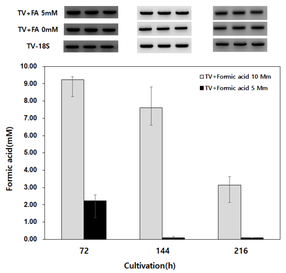


Fig2. formic acid consumption and gene expression(reverse-transcription PCR) during the cultivation days of *T. versicolor* with HPLC analysis.

Acid resistance of T. versicolor showed 80% and 60% at formic acid concentration(5 and 10 mM). T. versicolor grown in the presence of formic acid showed change the concentration, indicating that acid resistance is due to the oxidation activity from formic acid into CO2.

Protein expression

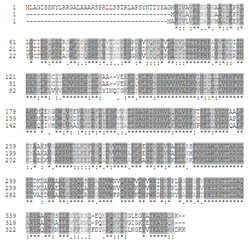


Fig3 sequence alignment of formate dehydrogenases.(TvFDH: *T. versicolor* FDH, CsFDH: *Ceriporiopsis subvermispora* FDH, CbFDH: *Candida boidinii*)

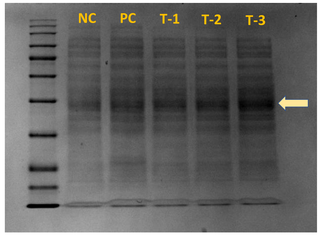


Fig 4. SDS-PAGE analysis of TvFDH, Lane 1: molecular mass of negative control(NC), lane 2: positive control(PC), lane 3: transformant 1, lane 4: transformant 2,lane 5: transformant 3

A protein band of the expected size(~42 kDa) was observed in T1~T3. Especially, a thick band of 42kDa was observed in the T3. These results suggest that the domain of TvFDH was folded into active forms in the cell-free system.

Enzyme activity

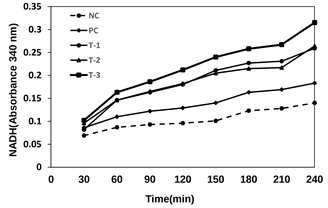


Fig 5. Oxidation activity of TvFDH through the NADH absorbance at 340 nm

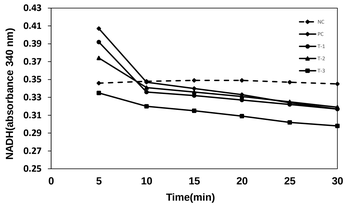
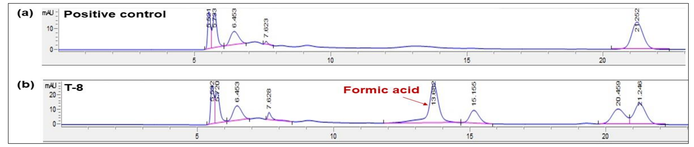


Fig 6. Reduction activity of TvFDH through the NADH absorbance at 340 nm

As shown in Fig 5 and 6, T-3 had the highest enzyme activity in the oxidation and reduction reaction. These results indicate that TvFDH possess a NADH-regenerating enzyme activity.

Metabolites



	N.C	P.C	T-1	T-2	T-3
Formic acid (mM)	-	-	0.09	0.12	0.17

The amount of formic acid produced through the enzymatic reaction is shown Figure 6. Cell -free extracts, containing TvFDH, produced 0.09 ~ 0.17 mM after 60 min, respectively

Conclusion

In this study, we have successfully cloned the full-length formate dehydrogenase from T. versicolor and expressed in cell-free protein expression system with a 6 His-linker tag fused to the N-terminus. We have also characterized the cell-free extract with NADH reduction and oxidation activity concentration variation at absorbance 340 nm. The TvFDH has considerable potential for industrial applications due to air stability, and the demonstration here that coupling with the NADH regenerating system significantly enhances CO2 reduction, even in the absence of product removal, is promising for the development of increasingly efficient biocatalytic processes for CO2 fixation.