Fermented milks and by-products
ألبان متخمرة و نواتج ثانوية (أ غ ذ 406)

- Lecture 5, for Food science program, level 4, 2019-2020

By:

Prof. Dr: Mohamed El-Alfy

- Professor Emeritus of dairy science, Dairy science department, Faculty of Agriculture, Benha University

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Propionic acid fermentation

Introduction

- Propionic acid (PA) is an important building block chemical and finds a variety of applications in organic synthesis, food, feeding stuffs, perfume, paint and pharmaceutical industries.
- Presently, PA is mainly produced by petrochemical route. With the continuous increase in oil prices, public concern about environmental pollution, and the consumers' desire for bio-based natural and green ingredients in foods and pharmaceuticals.
- Propionic acid (PA), a colorless liquid with a pungent odor and is an important C3–based building block chemical with a formula of CH3CH2COOH.

Table 1. Applications of propionic acid in various fields

Applications	Instructions
Feed and grain preservation	PA is inhibitory to *Aspergillus flavus*, aerobic *Bacillus*, *Salmonella* and yeast, and has been used as a mold inhibitor for animal feed, wet corn, silage and grain (Balamurugan *et al*., 1999).
Food preservatives	*Propionibacterium* have been granted a GRAS (generally recognized as safe) status by the United States Food and Drug Administration (FDA) (Salminen *et al*., 1998). PA can be used as preservatives in food industries to prevent the foods such as bread and cake from molding.
Herbicide synthesis	PA can be used for the synthesis of sodium 2, 2-dichloropropionate used as herbicide.
Perfume intermediates	PA is a precursor for the chemical synthesis of propionic ether and benzyl propionate, which can be used as additives in food and cosmetics (Kumar and Babu, 2006).
Pharmaceuticals intermediates	PA can be used for the synthesis of propionic andydride and chloropropionic acid as pharmaceutical intermediates (Kumar and Babu, 2006).
Synthesis of cellulose acetate propionate	PA can be used as the precursor for the synthesis of cellulose acetate propionate.
Other applications	PA can be used as an intermediate in the production of plastics, plasticizers, textile, and rubber auxiliaries, as well as dye intermediates.

Culture methods developed for microbial PA production

1-batch fermentation.

2- fed-batch fermentation.

3- cell-immobilized fermentation.

Microbial PA production

- **1-Strains used for PA production**

Typical strains for PA production are *Propionibacterium* spp., which are Gram-positive, non-motile, non-sporulating, rod-shaped, facultative anaerobes.

These strains include *P. thoenii*, *P. freuden- reichii*, *P. shermanii*, *P. acidipropionici*, and *P. beijingense*.

Table 2. Strains and corresponding details of microbial PA production

Strain	Culture mode	Substrates	PA production (g/L)	Productivity (g $L^{-1}h^{-1}$)	References
Propionibacterium acidipropionici	fed-batch	glycerol	44.62	0.20	Zhu *et al.*, 2010
	fibrous bed bioreactor	glycerol/ glucose/ lactate	~100	-	Zhang and Yang, 2009a, 2009b
	extractive fermentation	lactose	75	~1	Jin and Yang, 1998
	batch	lactate/sugarcane /molasses	15.06	0.26	Coral *et al.*, 2008
	fibrous bed bioreactor	glucose	71.8	-	Suwannakham *et al.*, 2006;
	batch	cheese whey	3.30	-	Morales *et al.*, 2006
	fed-batch	glucose/lactate	~30	-	Martinez-Campos. 2002
	immobilized cell fermentation	lactose	18.61	0.31	Coronado *et al.*, 2001
	cell recycle fermentation	xylose	-	2.7	Carrondo *et al.*, 1988
	batch/fed-batch/ extractive fermentation	lactose/glucose/ lactate	~15	-	Hsu and Yang, 1991; Lewis and Yang, 1992b, 1992c
	batch	glucose/ glycerol	~42	0.167	Barbirato *et al.*, 1997
Propionibacterium freudenreichii	multi-point fibrous-bed bioreactor (fed-batch)	glucose	67.05	0.14	Feng *et al.*, 2010a
	batch	wheat flour	20	-	Border *et al.*, 1987
Propionibacterium shermanii	batch	glucose	12.5	-	Quesada-Chanto *et al.*, 1998a
	batch	glucose/glycerol	~9	-	Himmi *et al.*, 2000
Propionibacterium microaerophilum	batch	glucose	~	-	Koussemon *et al.*, 2003
Propionibacterium beijingense	batch	glucose	11.32	-	He Y and Jin, 1990

2- Biosynthetic pathway of PA in Propionibacterium

PA synthesis includes two steps:

1) the formation of succinic acid by the condensation of two molecules of acetic acid.
2) 2) the formation of PA and CO_2 via the intermediate dissimilation of succinic acid. In

Propionibacterium PA is synthesized according to Eq. (1):

- $3CH_3CHOHCOOH \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$ (1)

- The biosynthesis of PA in *Propionibacterium* is related to the EMP pathway and dicarboxylic acid pathway. Theoretically, 2 moles of glucose can yield 3 moles of PA, 1 mole of acetic acid (AA), 1 mole of CO2, and 1 mole of H2O. Three moles of lactate can be converted to 2 moles of PA, 1 mole of AA, 1 mole of CO2, and 1 mole of H2O; and 1 mole of glycerol can generate 1 mole of PA and 1 mole of H2O. The reactions for three different carbon sources (glucose, lactate, and glycerol) are as follows :

- $1.5C_6H_{12}O_6 \rightarrow 2CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$ (2)
- $3CH_3CHOHCOOH \rightarrow CH_3CH_2COOH + CH_3COOH + CO_2 + H_2O$ (3)
- $CH_2OHCHOHCH_2OH \rightarrow CH_3CH_2COOH + H_2O$ (4)

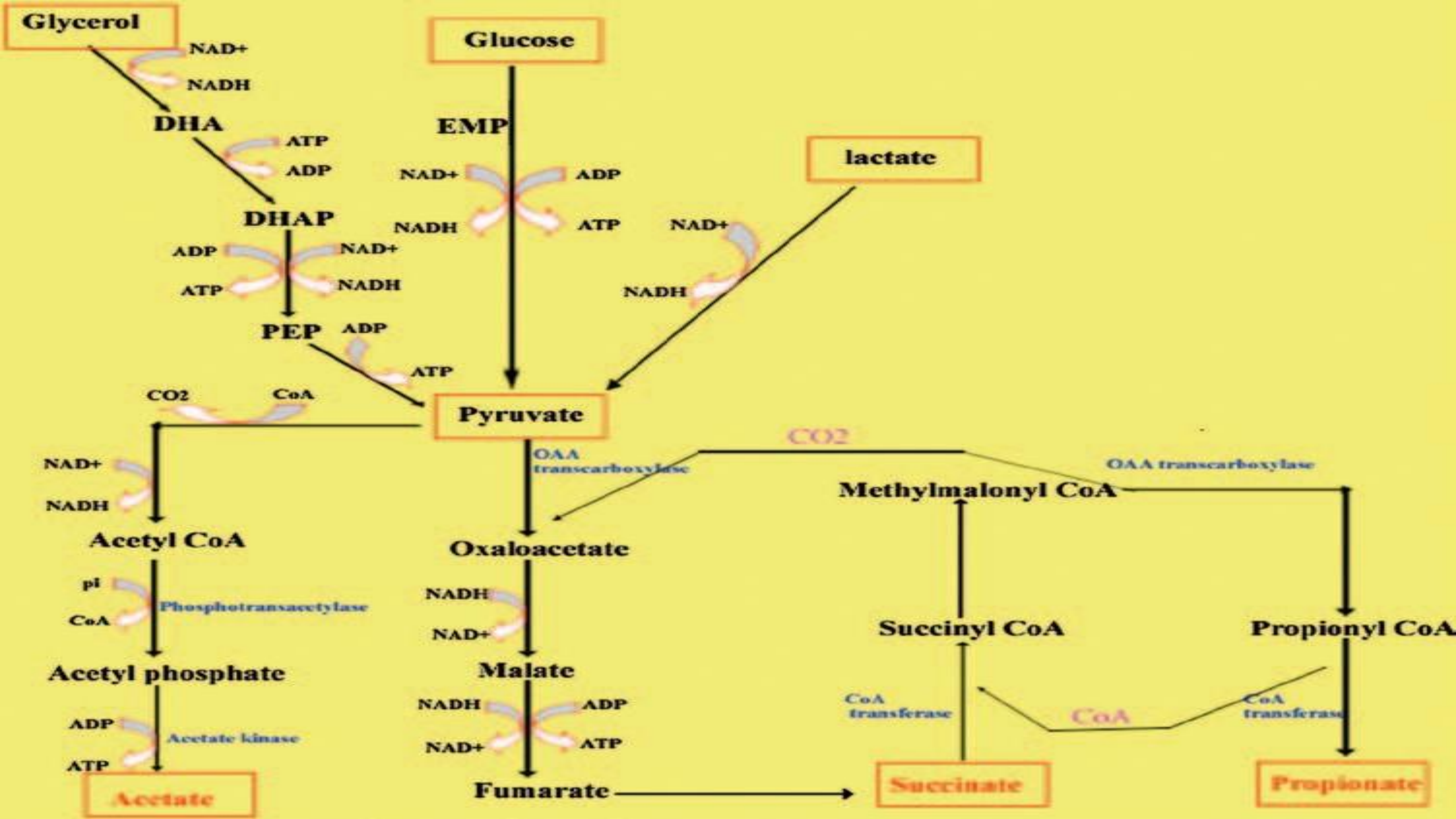
- The carbon sources (glycerol, glucose, and lactate) are metabolized into the same intermediate, pyruvate, which is a key metabolic node in the metabolic network of PA synthesis.
- A portion of the pyruvate is converted into acetate, and the rest is metabolized into malate and fumarate, which are then converted into succinate as a precursor of PA synthesis.
- There are three important cofactors involved in the regulation of PA synthesis, namely, ATP/ADP, NADH/NAD^+, and CoA/AcCoA. The regeneration rate of these cofactors determines the consumption rate of carbon sources and the synthetic rate of PA.
- Therefore, the regulation of the regeneration rate of these cofactors is a necessary part of metabolic engineering of *Propionibacterium* for enhanced PA production.

3- Carbon/nitrogen sources

- Several carbon sources such as glucose, fructose, maltose, sucrose, molasses, xylose, lactate, whey lactose hemicellulose and glycerol have been used for PA production.
- The oxidation state of the carbon source has a significant impact on the production of PA; the lower the oxidation state, the more favorable for PA synthesis due to the accelerated regeneration rate of NAD+, which is necessary for PA synthesis in *P. acidipropionici* .

4-Culture conditions

- The culture conditions, such as temperature and pH, also impact PA production.
- A temperature of 30°C is usually adopted for microbial PA production.
- A two-stage pH control strategy, involving a controlled pH of 6.5 for 48 h and then a pH of 6.0, was shown to enhance PA production.
- With this pH control strategy, the maximal PA concentration and glucose conversion efficiency achieved 19.21 g/L and 48.03%, respectively, and these parameters achieved 14.58 g/L and 36.45%, respectively, with a constant pH operation.


Glycerol
NAD+
NADH
DHA
ATP
ADP
DHAP
ADP
NAD+
ATP
NADH
PEP
ADP
ATP
Glucose
EMP
NAD+
ADP
NADH
ATP
lactate
NAD+
NADH
CO2
CoA
Pyruvate
NAD+
NADH
Acetyl CoA
pi
CoA
Phosphotransacetylase
Acetyl phosphate
ADP
ATP
Acetate kinase
Acetate
OAA transcarboxylase
CO2
OAA transcarboxylase
Methylmalonyl CoA
Oxaloacetate
NADH
NAD+
Malate
NADH
ADP
NAD+
ATP
Fumarate
Succinyl CoA
Propionyl CoA
CoA transferase
CoA
CoA transferase
Succinate
Propionate

References

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With my best wishes