

Microbial Life on Green Biomass and Their Use for Production of Platform Chemicals

Petra Schönicke, Robert Shahab, Rebekka Hamann, and Birgit Kamm

Contents

1	Introduction	22
2	Microorganisms on Green Plants	23
3	Microorganisms and Chemical Compounds in Silage	23
4	Organic Acid-Forming Bacteria	33
4.1	Acetic Acid (C2)	33
4.2	Propionic Acid (C3)	34
4.3	n-Butyric Acid (C4)	38
4.4	Isobutyric Acid (C4), Isovaleric Acid (C5), Isocaproic Acid (C6)	40
5	Sequence Chemical Products and Applications from Organic Acids	42
6	Future Perspectives	43
	References	44

Abstract This chapter describes the basics for the development of future biotechnology processes for the production of platform chemicals. Microbial life on green plants and harvested plants is very dynamic. Identified microorganisms on green plants and in silage as described in literature are listed in tables. But almost weekly new microorganisms are discovered, which constitute the site of a great variety of so far unknown metabolic pathways. Some microorganisms and their metabolic pathways to six organic acids used as platform chemicals and applications currently and in future are described.

P. Schönicke (✉) • R. Shahab • R. Hamann
Research Institute Bioactive Polymer Systems e.V., Teltow, Germany
e-mail: schoenicke@biopos.de

B. Kamm
Research Institute Bioactive Polymer Systems e.V., Teltow, Germany
Brandenburg Technical University, Senftenberg, Cottbus, Germany

Abbreviations

C	Carbon
MOs	Microorganisms

1 Introduction

All aerial parts of plants which perform photosynthesis and are usually in the growth phase can be called green biomass. Green biomass contains mainly carbohydrates, proteins, fibres, flavourings, colourings, vitamins, hormones, amino acids and enzymes, but less starch and lignin. The primary production of photosynthesis in green plants, such as C3 species in temperate climates, can yield up to 20 t dry matter and 4 t of proteins per ha per year, while C4 species in tropical climates can produce 80 t of dry matter and 6 t of proteins (Carlsson 1985). Economically, interesting are mainly alfalfa, clover and grass from permanent grassland and immature crops, but also green parts of plants, such as leaves as a by-product of the harvest of ripe crops (Kamm et al. 2006).

The area of green cropland cultivation in Europe (basis: 15 member states without new member states since a comprehensive European database on grassland areas is not available) amounts to 45 million ha and therewith to 35 % of the agricultural cropland. Based on an average yield of 10 t dry matter per hectare and year, 450 million tons of dry matter is produced annually by the 15 EU member states (FAO 2012). In Europe, the most important forage crop is alfalfa (Lucerne) due to its ability to absorb nitrogen from air and to enrich it in the soil. Alfalfa is cultivated on about 32 million hectares in the mentioned 15 EU member states. In the USA, intensive research in the field of biorefineries has been going on over the past 10 years. The Alfalfa New Products Initiative (ANPI), to which belong five of the states, aims at the intensification of the cultivation and use of Alfalfa. Thereby, known technologies, implemented at large scale only in France, like dehydration and fractionation are utilised. The high protein content and the favourable amino acid pattern make alfalfa exceptionally interesting for feedstuff production and research and development efforts on water-soluble proteins that are about 15 % of the average protein content (Lamsal 2004). The fraction of water-soluble carbohydrates form an important C-source among the nonstructural carbohydrates for microbial use by plant-associated bacteria (Seyfarth and Müller 1997). In harvested green biomass, one can find all microorganisms which already colonised the plant during growth and also those who ended up in the biomass during harvesting.

2 Microorganisms on Green Plants

The aerial parts of plants like leaves, caulis, buds, blossoms and fruits which are a habitat for microorganisms are called 'phyllosphere' (Whipps et al. 2008). Besides the soil, they offer a large habitat for bacteria, yeasts, fungi and protists, called 'epiphytic microorganisms', whereas the bacteria form the biggest group of these microorganisms (Lindow and Brandl 2003; Ruppel and Müller 2012). The phyllosphere is a very dynamic habitat because of strong fluctuating biotic and abiotic conditions of space and time (Kinkel 1997). A very high selective pressure exists because of the limited nutrient supply in many areas on the leaves, the UV-radiation during day time and often a prevalent dryness during the main growing period (in Central Europe) (Thompson et al. 1995; Andrews and Harris 2000). Additional population dynamics are generated during the ageing of the plants because of different survival strategies of the epiphytic microorganisms (Kinkel 1997). Bacteria like to live, for example, in cell agglomerations and are therefore more resistant against dryness and UV-radiation (Lindow and Brandl 2003).

In an analysis about quantity and biodiversity on leaves in a studied area at night, a higher individual number was determined, given that at night there is no UV-radiation and moisture is increasing because of dew production (Thompson et al. 1995). Also the point of time and the frequency of the harvest influence the composition of microorganism populations (Kinkel 1997). Late cut grass has a higher population concentration of heterotrophic bacteria as well as filamentous fungi, while the frequency of yeast and bacteria in the family of Micrococcaceae was always varying strongly (Behrendt et al. 2004).

Under lab conditions, only a few of the existing microorganism can be cultivated (Whipps et al. 2008; Müller and Ruppel 2014). With modern methods of the gene sequencing, which are culture independent, a significantly higher number of microorganism species can be determined, which are mostly unknown so far (Yang et al. 2001; Whipps et al. 2008). In Table 1, some of the identified microorganisms in the phyllosphere are listed alphabetically.

3 Microorganisms and Chemical Compounds in Silage

The production of silage for conservation of green fodder for the feeding of livestock during winter or biogas production has a long tradition in the agriculture of many countries. Extensive knowledge exists about techniques which contribute to a preferably optimal silage fermentation (Dogi et al. 2013). The requirements are a moisture content of the harvested plants of approximately 35 %, a sufficient content of fermentable sugars for lactic acid bacteria and solid compression for the reduction of trapped air (Driehuis and Oude Elferink 2000; Dunière et al. 2013). A good fodder quality can be obtained, if a strong pH-value reduction is achieved

Table 1 Some of the identified MOs of the phyllosphere are listed alphabetically

Microorganism	Plants, parts of plants	References
<i>Acidobacteria</i>	<i>Thlaspi geosingense</i>	Idris et al. (2004)
<i>Acinetobacter haemolyticus</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Acinetobacter sp.</i>	Citrus Valencia leaves	Yang et al. (2001)
<i>Acremonium</i>	Long-lived tropical leaves	Thompson et al. (1993), Inacio et al. (2002)
<i>Actinobacteria</i>	<i>Thlaspi geosingense</i> , <i>Campomanesia xanthocarpa</i> , <i>Capsicum annum</i> , <i>Solanum</i> <i>tuberosum</i> , <i>Crocus albiflorus</i>	Idris et al. (2004), Lambais et al. (2006), Rasche et al. (2006a, b), Reiter and Sessitsch (2006)
<i>Agrobacterium rubi</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Alternaria</i>	Long-lived tropical leaves	Thompson et al. (1993), Inacio et al. (2002)
<i>Alternaria</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dick- inson (1967, 1976), Wildman and Parkinson (1979)
<i>α-Proteobacteria</i>	<i>Thlaspi geosingense</i> , <i>Trichilia</i> <i>catigua</i> , <i>Trichilia clausenii</i> , <i>Campomanesia xanthocarpa</i> , <i>Zea mays</i> , <i>Capsicum annum</i> , <i>Solanum tuberosum</i> , <i>Crocus</i> <i>albiflorus</i>	Idris et al. (2004), Lambais et al. (2006), Kadivar and Stapleton (2003), Rasche et al. (2006a, b), Reiter and Sessitsch (2006)
<i>Arthrobacter atrocyaneus</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Arthrobacter globiformis</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Arthrobacter oxydans</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Arthrobacter protophormiae</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Aspergillus</i>	Long-lived tropical leaves	Thompson et al. (1993), Inacio et al. (2002)
<i>Aureobacterium saperdae</i>	Sugar beets, short-term dynamics	Thompson et al. (1995)
<i>Aureobasidium pullulans</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Aureobasidium</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dick- inson (1967, 1976), Wildman and Parkinson (1979)
<i>Aureobasidium pullulans</i>	<i>Acer platanoides</i> , <i>Hippophae</i> <i>rhamnoides</i>	Breeze and Dix (1981)
<i>Bacillus</i>	Heterotrophic	Wipat and Harwood (1999)
<i>Bacillus pumilus</i>	Valencia orange leaf, citrus Valencia leaf	Yang et al. (2001)
<i>Bacillus subtilis</i>		Kong et al. (1997)
<i>Bacillus thuringiensis</i>	Leaves, grass foliage	Hansen et al. (1998), Damgaard et al. (1998)

(continued)

Table 1 (continued)

Microorganism	Plants, parts of plants	References
<i>Bacterial groups</i>		Thompson et al. (1993), Inacio et al. (2002)
<i>Bacteroidetes</i>	<i>Thlapsi geosingense</i> , <i>Trichilia catigua</i> , <i>Trichilia clausenii</i> , <i>Campomanesia xanthocarpa</i> , <i>Zea mays</i> , <i>Solanum tuberosum</i>	Idris et al. (2004), Lambais et al. (2006), Kadivar and Stapleton (2003), Rasche et al. (2006a)
<i>Burkholderia cepacia</i>	Pathogen	Balandreau et al. (2001), Govan et al. (1996)
<i>Cladosporium</i>	Long-lived tropical leaves	Thompson et al. (1993), Inacio et al. (2002)
<i>Cladosporium</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dickinson (1967, 1976), Wildman and Parkinson (1979)
<i>Clostridia</i>	<i>Miscanthus sinensis</i>	Miyamoto et al. (2004)
<i>Clostridium bifermentans</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Cryptococcus</i>	Active coloniser, growth on healthy, intact, non-senescent leaves is relatively rare	Thompson et al. (1993), Inacio et al. (2002), Glushakova and Chernov (2004), Fokkema et al. (1979)
<i>Cyanobacteria</i>	<i>Campomanesia xanthocarpa</i> , <i>Crocus albiflorus</i>	Lambais et al. (2006), Reiter and Sessitsch (2006)
<i>Cyanobacteria Nostoc</i>	Autotrophic	Andrews and Harris (2000)
<i>Cytospora</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dickinson (1967, 1976), Wildman and Parkinson (1979)
<i>Dendrophoma</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dickinson (1967, 1976), Wildman and Parkinson (1979)
<i>Desulfurominas choroethenica</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Enterobacter agglomerans</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Enterobacter asburiae</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Enterococcus faecalis</i> <i>Ent. mundtii</i> <i>Ent. casseliflavus</i> <i>Ent. faecium</i> <i>Ent. sulfureus</i>	Bowel pathogen, bacteriocins on grass	Ott et al. (2001)
<i>Epicoccum</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dickinson (1967, 1976), Wildman and Parkinson (1979)
<i>Erwinia (Pantoea) ssp.</i>		Lindow and Brandl 2003
<i>Erwinia amylovora</i>	Flowers, causer of fire blight	Johnson and Stockwell (1998), Lindow et al. (1996)
<i>Erwinia amylovora</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Erwinia herbicola</i>	Sugar beets, short-term dynamics	Thompson et al. (1995)

(continued)

Table 1 (continued)

Microorganism	Plants, parts of plants	References
<i>Erwinia herbicola</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Erwinia rhapontici</i>	Sugar beets, short-term dynamics	Thompson et al. (1995)
<i>Erwinia rhapontici</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Escherichia coli</i>	Corn, beans, coriander	Brandl and Mandrell (2002), O'Brien and Lindow (1989)
<i>Firmicutes</i>	<i>Thlapsi geosingense</i> , <i>Campomanesia xanthocarpa</i> , <i>Zea mays</i> , <i>Capsicum annum</i> , <i>Solanum tuberosum</i> , <i>Crocus albiflorus</i>	Idris et al. (2004), Lambais et al. (2006), Kadivar and Stapleton (2003), Rasche et al. (2006a, b), Reiter and Sessitsch (2006)
<i>Fluorescent pseudomonads</i>	Heterotrophic	Andrews and Harris (2000)
<i>γ-Proteobacteria</i>	<i>Thlapsi geosingense</i> , <i>Trichilia catigua</i> , <i>Trichilia clausenii</i> , <i>Campomanesia xanthocarpa</i> , <i>Zea mays</i> , <i>Capsicum annum</i> , <i>Solanum tuberosum</i> , <i>Crocus albiflorus</i>	Idris et al. (2004), Lambais et al. (2006), Kadivar and Stapleton (2003), Rasche et al. (2006a, b), Reiter and Sessitsch (2006)
<i>Hydrogenophaga pseudoflora</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Lewia infectoria</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Marinobacter hydrocarbonoclasticus</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Methylobacterium mesophilicum</i> A47	<i>Lolium perenne</i>	Austin and Goodfellow (1979), Green and Bousfield (1983)
<i>Methylobacterium phyllosphaerae</i> CBMB27	<i>Oryza sativa</i> 'DongJin'	Madhaiyan et al. (2009)
<i>Methylobacterium platani</i> PMB02	<i>Platanus orientalis</i>	Kang et al. (2007)
<i>Methylobacterium</i> spp.	Cytokinins	Holland et al. (2002)
<i>Microbacterium lacticum</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Micrococcus kristinae</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Micrococcus roseus</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Microsphaeropsis</i>	Dormant spores, growth on healthy, intact, non-senescent leaves is relatively rare	Andrews et al. (1987), Dickinson (1967, 1976), Wildman and Parkinson (1979)
<i>Morchella esculenta</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Mucor</i>	Long-lived tropical leaves	Thompson et al. (1993), Inacio et al. (2002)
<i>Pantoea agglomerans</i>	<i>Gypsophila paniculata</i>	Manulis et al. (1998), Brandl and Mandrell (2002)

(continued)

Table 1 (continued)

Microorganism	Plants, parts of plants	References
<i>Penicillium</i>	<i>Hippophae rhamnoides</i> , long-lived tropical leaves	Thompson et al. (1993), Inacio et al. (2002)
<i>Pseudomonas aeruginosa</i>	Pathogen	Cho et al. (1975)
<i>Pseudomonas chlororaphis</i>	Cilantro (coriander)	Brandl and Mandrell (2002)
<i>Pseudomonas fluorescens</i> A, B, C, F, G	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Pseudomonas fluorescens</i>	Presence of a functional type III secretion pathway	Preston et al. (2001)
<i>Pseudomonas oleovorans</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Pseudomonas putida</i>	Sugar beet, presence of a functional type III secretion pathway	Preston et al. (2001), Thompson et al. (1995)
<i>Pseudomonas putida</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>P. syringae</i> pathovars <i>atrofaciens</i> , <i>glyciniae</i> , <i>lachrymans</i> , <i>morsprunorum</i> , <i>savastanoi</i> <i>fraxinus</i> , <i>savastanoi oleae</i> , <i>syringae</i> , <i>tabaci</i> , <i>targetes</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Pseudomonas</i> spp.	Surfactants (increase the wettability of leaf surfaces)	Bunster et al. (1989)
<i>Pseudomonas syringae</i>	Bean leaves, sugar beet leaves, alginate, ice activity	Brandl and Mandrell (2002), O'Brien and Lindow (1989), Kinkel (1997)
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	Snap bean, syringomycin (toxin, cell lysis)	Hutchison et al. (1995), Uppe et al. (2003), Quigley and Gross (1994)
<i>Pseudomonas tolaasii</i>	Tolaasin	Hutchison and Johnstone (1993)
<i>Rhodospiridium</i>	Active coloniser, growth on healthy, intact, non-senescent leaves is relatively rare	Fokkema et al. (1979)
<i>Rhodotorula</i>		Thompson et al. (1993), Inacio et al. (2002), Glushakova and Chernov (2004)
<i>Salmonella enterica</i>	Corn, beans, cilantro (coriander)	Brandl and Mandrell (2002), O'Brien and Lindow (1989)
<i>Salmonella enterica</i> serovars	Lettuce	Klerks et al. (2007)
<i>Serratia plymuthica</i>	Sugar beets, short-term dynamics	Thompson et al. (1995)
<i>Sphingomonas adhaesiva</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Sporobolomyces</i>		Thompson et al. (1993), Inacio et al. (2002), Glushakova and Chernov (2004)

(continued)

Table 1 (continued)

Microorganism	Plants, parts of plants	References
<i>Sporobolomyces</i>		Fokkema et al. (1979)
<i>Staphylococcus haemolyticus</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Staphylococcus simulans</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Unclassified organism</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Uncultured bacterium</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Uncultured delta proteobacterium</i>	Citrus Valencia leaf	Yang et al. (2001)
<i>Unidentified Cytophagales</i>	Valencia orange leaf, citrus Valencia leaf	Yang et al. (2001)
<i>Vibrio parahaemolyticus</i>	Polar flagellum, which is surface induced, acts as a sensory tactile device for the microbe	McCarter et al. (1992), McCarter and Silverman (1989)
<i>Xanthomonas campestris</i> pv. <i>campestris</i>	<i>Brassica campestris</i> leaves	Kuan et al. (1986)
<i>Xanthomonas campestris</i> pv. <i>undulosa</i>	Wheat leaves	Duveiller (1994)
<i>Xanthomonas maltophilia</i>	Sugar beet, short-term dynamics	Thompson et al. (1995)
<i>Xanthomonas phaseoli</i>	Bean leaves	Weller and Saettler (1980)
<i>Xanthomonas phaseoli fuscans</i>	Bean leaves	Weller and Saettler (1980)
<i>β-Proteobacteria</i>	<i>Thlpsi geosingense</i> , <i>Trichilia catigua</i> , <i>Trichilia clausenii</i> , <i>Campomanesia xanthocarpa</i> , <i>Zea mays</i> , <i>Capsicum annum</i> , <i>Solanum tuberosum</i> , <i>Crocus albiflorus</i>	Idris et al. (2004), Lambais et al. (2006), Kadivar and Stapleton (2003), Rasche et al. (2006a, b), Reiter and Sessitsch (2006)

through fast lactic acid fermentation during the first 2 days of the ensilage and if the silage also stays under complete air exclusion during further storing (Driehuis and Oude Elferink 2000; Shao et al. 2005). In case the silage is produced without inoculation, the ensiling is a natural fermentation process, in which competition takes place between epiphytic microorganisms (Li and Nishino 2013).

A compilation of identified microorganisms in silage in alphabetical order is included in Table 2.

The most famous microorganism groups in silage are lactic acid bacteria Enterobacteria, Clostridia and some *Bacillus* species (Hafner et al. 2013) (see also chapter [Microorganisms for Production of Lactic Acid and Organic Lactates](#)). Pre-ensiled crop is an excellent start material for the production of lysine (see chapter [Microorganisms for Biorefining of Green Biomass](#)).

Besides lactic acid in silages, other organic acids can be found, larger quantities of acetic acid but also propionic acid, butyric acid, isobutyric acid and isovaleric

Table 2 Identified MOs in silage are listed alphabetically

Microorganism	Products and impacts	References
<i>Absidia</i>		Driehuis and Oude Elferink (2000)
<i>Acetobacter</i>		Dunière et al. (2013)
<i>Acetobacter pasterianus</i>		Wang et al. (2014)
<i>Acinetobacter sp.</i>	Pre-ensiled crop	Li and Nishino (2013)
<i>Arthrinium</i>		Driehuis and Oude Elferink (2000)
<i>Aspergillus</i>		Driehuis and Oude Elferink (2000)
<i>Aspergillus fumigatus</i>	Mycotoxin	Driehuis and Oude Elferink (2000)
<i>Aspergillus ochraceus</i>	Ochratoxin A	Dunière et al. (2013)
<i>Aspergillus sp.</i>		Dunière et al. (2013)
<i>Bacillus cereus</i>	Can lead to food poisoning	Dunière et al. (2013), Driehuis and Oude Elferink (2000)
<i>Bacillus firmus</i>		Driehuis and Oude Elferink (2000)
<i>Bacillus lentus</i>		Driehuis and Oude Elferink (2000)
<i>Bacillus licheniformis</i>		Driehuis and Oude Elferink (2000)
<i>Bacillus polymyxa</i>		Driehuis and Oude Elferink (2000)
<i>Bacillus smithii</i>		Wang et al. (2014)
<i>Bacillus sphaericus</i>		Driehuis and Oude Elferink (2000)
<i>Byssoschlamys</i>		Driehuis and Oude Elferink (2000)
<i>Byssoschlamys nivea</i>	Mycotoxin	Driehuis and Oude Elferink (2000)
<i>Candida</i>		Driehuis and Oude Elferink (2000)
<i>Cladosporium sp.</i>		Dunière et al. (2013)
<i>Clostridium acidisoli</i>		Wang et al. (2014)
<i>Clostridium bifermentas</i>	Highly proteolytic	Driehuis and Oude Elferink (2000)
<i>Clostridium botulinum</i>	Pathogenic toxin	Dunière et al. (2013)
<i>Clostridium butyricum</i>	Weakly proteolytic	Dunière et al. (2013), Driehuis and Oude Elferink (2000)

(continued)

Table 2 (continued)

Microorganism	Products and impacts	References
<i>Clostridium sporogenes</i>	Highly proteolytic	Driehuis and Oude Elferink (2000)
<i>Clostridium tyrobutyricum</i>	Weakly proteolytic	Dunière et al. (2013), Driehuis and Oude Elferink (2000)
<i>E. coli</i> (STEC)	Shiga toxin	Dunière et al. (2013)
<i>E. coli</i> O157		Dunière et al. (2013)
<i>E. coli</i> O157:H7		Dunière et al. (2013)
<i>E. coli</i> O26		Dunière et al. (2013)
<i>Enterobacter aerogenes</i>		McGarvey et al. (2013)
<i>Enterobacter cloacae</i>		Wang et al. (2014)
<i>Enterobacter hormaechei</i>		McGarvey et al. (2013)
<i>Enterobacter ludwigii</i>		McGarvey et al. (2013)
<i>Enterobacter</i> sp.	2,3-butanediol, pre-ensiled crop	Li and Nishino (2013)
<i>Enterobacter</i> sp. FMB-1		McGarvey et al. (2013)
<i>Enterobacter</i> sp. J33		McGarvey et al. (2013)
<i>Enterobacter</i> sp. MPR16		McGarvey et al. (2013)
<i>Enterobacteria</i>	2,3-butanediol	Li and Nishino (2013)
<i>Enterococcus durans</i>		McGarvey et al. (2013)
<i>Epiphytic yeast</i>	CO ₂ ; alcohols	Dunière et al. (2013)
<i>Erwinia amylovora</i>		McGarvey et al. (2013)
<i>Erwinia herbicola</i>		Dunière et al. (2013)
<i>Erwinia persicina</i>		McGarvey et al. (2013)
<i>Fusarium</i>		Driehuis and Oude Elferink (2000)
<i>Fusarium</i> sp.	More than 20 mycotoxins, deoxynivalenol (DON), zearalenone (ZEN), fumonisin (FB)	Dunière et al. (2013)
<i>Geobacillus pallidus</i>		Wang et al. (2014)
<i>Geotrichum</i>		Driehuis and Oude Elferink (2000)
<i>Hafnia alvei</i>		Dunière et al. (2013)
<i>Hansenula</i>		Driehuis and Oude Elferink (2000)
<i>Klebsiella pneumoniae</i>		Dunière et al. (2013)

(continued)

Table 2 (continued)

Microorganism	Products and impacts	References
<i>Klebsiella sp.</i>	2,3-butanediol	Li and Nishino (2013)
<i>Kurthia sp.</i>		Wang et al. (2014)
<i>Lactobacillus acetotolerans</i>		Wang et al. (2014)
<i>Lactobacillus buchneri</i>		Wang et al. (2014), McGarvey et al. (2013)
<i>Lactobacillus diolivorans</i>		Wang et al. (2014)
<i>Lactobacillus diolivorans</i>		McGarvey et al. (2013)
<i>Lactobacillus lindneri</i>		McGarvey et al. (2013)
<i>Lactobacillus plantarum</i>		McGarvey et al. (2013)
<i>Lactobacillus sp. TS4</i>		McGarvey et al. (2013)
<i>Lactococcus garvieae</i>		McGarvey et al. (2013)
<i>Listeria innocua</i>	Cause animal disease	Dunière et al. (2013)
<i>Listeria ivanovii</i>	Cause animal disease	Dunière et al. (2013)
<i>Listeria monocytogenes</i>	Listeriosis	Dunière et al. (2013), Driehuis and Oude Elferink (2000)
<i>Listeria sp.</i>	Pathogenic	Dunière et al. (2013)
<i>Monascus</i>		Driehuis and Oude Elferink (2000)
<i>Morganella morganii</i>	2,3-butanediol	Li and Nishino (2013)
<i>Mucor</i>		Driehuis and Oude Elferink (2000)
<i>Mycobacterium bovis</i>	Causes bovine tuberculosis	Dunière et al. (2013)
<i>Mycobacterium tuberculosis</i>	Causes human tuberculosis	Dunière et al. (2013)
<i>Paenibacillus barengoltzii</i>		Wang et al. (2014)
<i>Pantoea brenneri</i>		McGarvey et al. (2013)
<i>Pantoea agglomerans</i>	Pre-ensiled crop	Li and Nishino (2013)
<i>Pantoea sp.</i>	2,3-butanediol, pre-ensiled crop	Li and Nishino (2013)
<i>Pediococcus pentosaceus</i>		McGarvey et al. (2013)
<i>Penicillium</i>		Driehuis and Oude Elferink (2000)

(continued)

Table 2 (continued)

Microorganism	Products and impacts	References
<i>Penicillium roqueforti</i>	Mycotoxin	Driehuis and Oude Elferink (2000)
<i>Penicillium verrucosum</i>	Ochratoxin A	Dunière et al. (2013)
<i>Penicillium sp.</i>		Dunière et al. (2013)
<i>Pseudomonas oleovorans</i>		McGarvey et al. (2013)
<i>Pseudomonas oryzihabitans</i>		McGarvey et al. (2013)
<i>Pseudomonas sp.</i>		Wang et al. (2014)
<i>Pseudomonas syringae</i>		Dunière et al. (2013)
<i>Rahnella aquatilis</i>		Dunière et al. (2013)
<i>Rahnella aquatilis</i>		Li and Nishino (2013)
<i>Saccharomyces</i>		Driehuis and Oude Elferink (2000)
<i>Scopulariopsis</i>		Driehuis and Oude Elferink (2000)
<i>Serratia fonticola</i>		Dunière et al. (2013)
<i>Torulopsis</i>		Driehuis and Oude Elferink (2000)
<i>Trichoderma</i>		Driehuis and Oude Elferink (2000)
<i>Trichosporon sp.</i>		Dunière et al. (2013)
Uncultured bacterium (band 1)	Pre-ensiled crop	Li and Nishino (2013)
Uncultured bacterium (band 3)	Pre-ensiled crop	Li and Nishino (2013)
Uncultured bacterium (band 31)		Li and Nishino (2013)
Uncultured bacterium (band 4)	Pre-ensiled crop	Li and Nishino (2013)
<i>Weissella kandleri</i>		McGarvey et al. (2013)
<i>Yersinia enterocolitica</i>	Causes yersiniosis	Dunière et al. (2013)

acid. Alcohols (mainly methanol and ethanol), ketones, ester and aldehydes can be determined as other volatile components in silages. While abiotic reactions can be responsible for the production of methanol and esters, the most important acids, alcohols and aldehydes, are caused by microbial activity (Hafner et al. 2013). Apart

Table 3 Identified chemical compounds in silage (Hafner et al. 2013)

Functional group	Chemicals
Alcohol	Methanol, ethanol, 1-propanol, 2-propanol, 2-propenol, 2-methyl-1-propanol, 1-butanol, 2-butanol, 3-methyl-1-butanol, 2-methyl-1-butanol, 1-pentanol, 1-hexanol, phenylmethanol, 2-phenylethanol
Ketone	Acetone, 2-butanone, 3-hydroxy-2-butanone
Aldehyde	Acetaldehyde, propionaldehyde, 2-methylpropanal, butyraldehyde, 2-methylbutanal, 3-methylbutanal, valeraldehyde, hexanal, heptanal

from lactic acid, 1,2-propanediol, propylene glycol and many esters, the following volatile organic compounds from silage were measured (Table 3).

The utilisation of green biomass for the production of platform chemicals in form of silage has two advantages. Firstly, green biomass as a feedstock would be available throughout the whole year, and secondly, by the acid impact, a soft pretreatment happens on the fibres. Simultaneously, the degradation of the proteins to amino acids continues.

For the application in biorefineries, a part of the so far undesired microorganisms from the food and forage production (e.g. silage) and their products can be newly evaluated. It is the case when undesirable by-products become desirable products, e.g. for the chemical industry. For that, the metabolic pathways are specifically utilised for these products and will be modified at time. A few examples of organic acids which could attain greater meaning and their microbial producers are described in the following section.

4 Organic Acid-Forming Bacteria

4.1 Acetic Acid (C2)

The worldwide production of acetic acid exceeds 7 million metric tons per year (Cheung et al. 2005) whereof approximately 2 million metric tons produced using biotechnological processes and renewable resources. There are two different big-scale production ways established. On the one hand, the chemical high-pressure Monsanto process with the catalytic conversion of methanol and carbon monoxide to acetic acid. On the other hand, a widely spread process is used which is known since ancient times. This process contains the biotechnological conversion of ethanol to acetic acid using aerobic acetic forming bacteria of the genus *Acetobacter*.

Under anaerobic conditions, e.g. *Clostridium aceticum* is able to use the homoacetic acid fermentation pathway to produce acetic acid at an optimum pH value of 8.3 and 30 °C. This pathway is divided into two parts. First, the digestible carbohydrates pass through the glycolysis and end up as pyruvate which is

Table 4 Characteristics of *Acetobacter aceti*

<i>Acetobacter aceti</i>		Gillis and de Ley (1980), Leisinger (1965)
Systematic classification	Kingdom	Bacteria
	Phylum	Proteobacteria
	Class	Alpha proteobacteria
	Order	Rhodospirillales
	Family	Acetobacteraceae
	Genus	Acetobacter
	Species	<i>A. aceti</i>
Synonyms	ATCC 15973, <i>Acetobacter aceti</i> subsp. <i>aceti</i>	
Source or first isolation	From alcohol turned to vinegar	
Characteristics	Gram-negative, peritrichously flagellated, anaerobic	
Morphology	Rod shaped	
Growth conditions	26 °C, medium 1: mannitol agar/broth, aerobic	
Pathogenicity	Class 1	
DNA GC content	55.9	

oxidatively decarboxylated. Acetyl-CoA is formed which will be further converted to acetate and secreted. The released carbon dioxide is converted within the Wood–Ljungdahl pathway (reductive-acetyl-CoA pathway) to acetate as well. This leads to a high overall yield of three molecules acetate per molecule glucose.

Because of the fact that green biomass is used as raw material, a complete conversion of the present carbohydrates should be intended. Especially, cellulose has to be converted to fermentable sugars within the process setup. In the future, it will be desirable to set up an economic process using acetogenic bacteria which are able to utilise a wide variety of carbohydrates including cellulose (Table 4).

Because of the low substrate costs compared to the fossil fuel-based methanol and the higher product yield compared to the ethanol oxidation, experts expect that this approach will be more successful and economic. The increment of the pH tolerance of the acetogenic bacteria will be an essential fact as well (Table 5).

4.2 Propionic Acid (C3)

Currently, propionic acid is produced by a petrochemical production way. The process is called oxo process. Ethylene reacts with synthesis gas (CO/H₂) to propionaldehyde which reacts with oxygen to propionic acid. Because of the raising oil price and the pursuit for oil independency and sustainable granting of industry appreciable chemicals, the public demand for biotechnological production of propionic acid is raised continuously for the last few years.

The worldwide annual production quantity in 2006 was estimated to 377,000 metric tons. BASF qualifies as the biggest manufacturer with 150,000 metric tons per annum.

Table 5 Characteristics of *Clostridium aceticum*

<i>Clostridium aceticum</i>		Wieringa (1936), Gottschalk and Braun (1981), Skerman et al. (1980), Braun and Gottschalk (1981), Karlsson et al. (1948)
Systematic classification	Kingdom	Bacteria
	Phylum	Firmicutes
	Class	Clostridia
	Order	Clostridiales
	Family	Clostridiaceae
	Genus	Clostridium
	Species	<i>C. aceticum</i>
Synonyms	ATCC 35044	
Source of first isolation	Mud	
Characteristics	Gram-positive, peritrichously flagellated, anaerobic	
Morphology	Rod shaped	
Growth conditions	30 °C, medium 1612: acetobacterium medium, anaerobic	
Pathogenicity	Class 1	

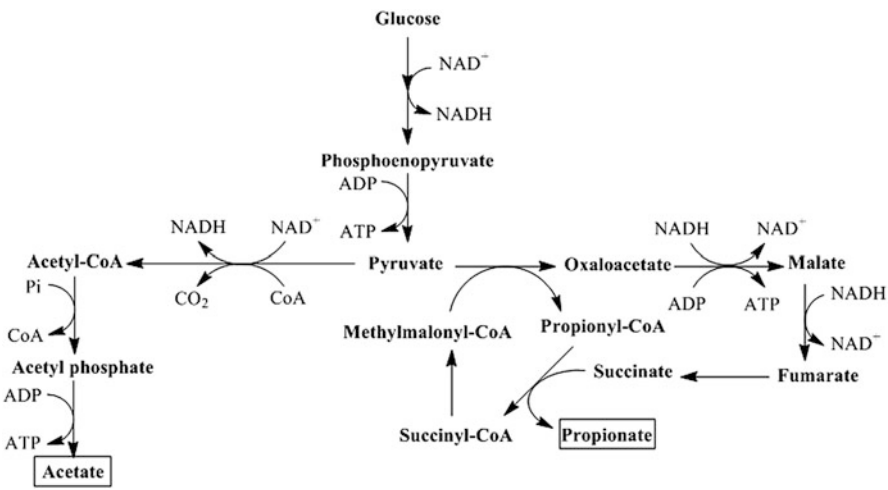
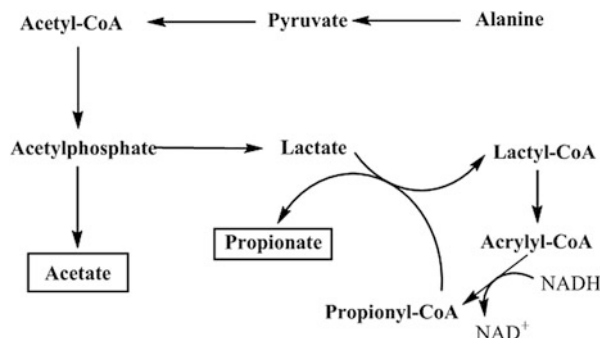


Fig. 1 Dicarboxylic acid pathway

Various bacteria own the ability to produce propionic acid within their metabolic pathways. Present-day research is focused on strains of Propionibacteriaceae and Clostridiaceae. Propionibacteria are using the dicarboxylic acid pathway (methylmalonyl coenzyme A-pathway) to produce the desired product. These gram-positive, anaerobic bacteria are able to use glucose, sucrose, lactate, lactose and glycerol as carbon source. The metabolic end products are propionate, succinate, carbon dioxide and acetate. Professionals acknowledge *Propionibacterium*

Fig. 2 Acrylic acid pathway



acidipropionici, *P. shermanii* and *P. freudenreichii* the highest potential to achieve an economic, big-scale process. The optimal pH value is between 6.5 and 7.0, and the best growth temperature lies at 30–32 °C (Fig. 1).

Another highly potential production species is the gram-positive bacteria *Clostridium propionicum*. This microorganism is able to utilise lactate, glycerol and alanine as substrate. Propionate, acetate, formate, n-propanol and succinate are produced. The optimal pH value is 6.8 and the best temperature is 30 °C. *C. propionicum* uses the acrylic acid pathway to produce the desired product (Fig. 2).

Lactate is able to start both pathways. If three molecules of lactate enter the pathway, two will be reduced to propionate and one will be oxidised to acetate and carbon dioxide. Because of the fact that these microorganisms ferment the product of an earlier fermentation process, they are called secondary fermenters (Tables 6 and 7).

The biotechnological production of propionic acid has never passed the pilot plant level (Abbas and Adolfo 2000; Balamurugan et al. 1999). Feasible reasons are the fastidious fermentation process, the long cultivation time, the end-product inhibition, the low final titer, the product purity and especially the high costs of fermentation and product recovery processes (Colomban et al. 1993; Liang et al. 2012). In order to be competitive, the biotechnological production process needs to have higher productivity and reduced production costs (Sabra et al. 2013) (Table 8).

The most important step will be the decrease of substrate costs. Green biomass depicts a common and cheap source of raw materials. Because of the broad experiences within the silage process including the formation of high amounts of lactic acids, green biomass would be highly suitable. Within a co-cultivation, there are no costs of lactate recovery which is an advantage.

Table 6 Characteristics of *Propionibacterium acidipropionici*

<i>Propionibacterium acidipropionici</i>		Johnson and Cummins (1972), Skerman et al. (1980)
Systematic classification	Kingdom	Bacteria
	Phylum	Actinobacteria
	Class	Actinobacteria
	Order	Actinomycetales
	Family	Propionibacteriaceae
	Genus	Propionibacterium
	Species	<i>P. acidipropionici</i>
Synonyms	<i>Propionibacterium pentosaceum</i> , <i>P. arabinosum</i> , <i>P. acidipropionici</i> , <i>Bacillus acidipropionici</i> , ATCC 25562	
Source of first isolation	Dairy products	
Characteristics	Gram-positive, non-spore forming	
Morphology	Rod shaped	
Growth conditions	37 °C, medium 602: E medium for anaerobes, anaerobic	
Pathogenicity	Class 1	
DNA GC content	68.8	
Special characteristics	High GC content	

Table 7 Characteristics of *Propionibacterium freudenreichii* subsp. *shermanii*

<i>Propionibacterium freudenreichii</i> subsp. <i>Shermanii</i>		van Niel (1928), Moore and Holdeman (1970), Skerman et al. (1980)
Systematic classification	Kingdom	Bacteria
	Phylum	Actinobacteria
	Class	Actinobacteria
	Order	Actinomycetales
	Family	Propionibacteriaceae
	Genus	Propionibacterium
	Species	<i>Propionibacterium freudenreichii</i>
	Subspecies	<i>P. freudenreichii</i> subsp. <i>shermanii</i>
Synonyms	<i>Propionibacterium shermanii</i> , <i>Propionibacterium freudenreichii shermanii</i> , ATCC 9614	
Source of first isolation	Cheese, Weihenstephan, Germany	
Characteristics	Gram-positive, nonmotile, forming	
Morphology	Rod shaped	
Growth conditions	30 °C, medium 593: chopped meat medium, anaerobic	
Pathogenicity	Class 1	
DNA GC content	67.3	
Special characteristics	High GC content	

Table 8 Characteristics of *Clostridium propionicum*

<i>Clostridium propionicum</i>		Cardon and Barker (1946), Ludwig et al. (2009), Janssen (1991)
Systematic classification	Kingdom	Bacteria
	Phylum	Firmicutes
	Class	Clostridia
	Order	Clostridiales
	Family	Clostridiaceae
	Genus	Clostridium
	Species	<i>C. propionicum</i>
Synonyms	ATCC 25522	
Source of first isolation	Black mud, San Francisco Bay, USA	
Characteristics	Gram-positive, obligate anaerobic	
Morphology	Rod shaped	
Growth conditions	37 °C, medium 2210: enriched anaerobe medium, anaerobic	
Pathogenicity	Class 1	
DNA GC content		
Special characteristics		

4.3 *n*-Butyric Acid (C4)

Butyric acid can be conventionally produced by oxidation of butyraldehyde. A variety of anaerobic bacteria are able to produce butyric acid as the major end product during fermentation process. Nevertheless, *Clostridium* species have been used preferentially for butyric acid production because of their plain medium requirements and comparatively high product yields. The major focus in current research is on *C. butyricum* with a pH range between 5.0 and 7.0. But optimisation of *C. tyrobutyricum* within green biomass fermentation could be very worthwhile because of the fact that *C. tyrobutyricum* is able to ferment lactate produced by Lactobacteriaceae. Furthermore, *C. tyrobutyricum* tolerates low pH values down to 4.2 which could be an advantage in big-scale implementation.

The biochemical pathway used by *Clostridia* to produce butyric acid is shown in Fig. 3. The pathway starts with the glycolytic cleavage of glucose to two molecules of pyruvate. After that, pyruvate is oxidised into acetyl coenzyme A. The enzyme which catalyses this step is called pyruvate-ferredoxin oxidoreductase. Two molecules of carbon dioxide and two molecules of hydrogen are released. Starting at acetyl coenzyme A, three possible end products can be formed: ethanol, acetate and butyrate. With the aid of genetic engineering, the formation of side products can be disabled by gene knockout or optimised process control (Tables 9 and 10).

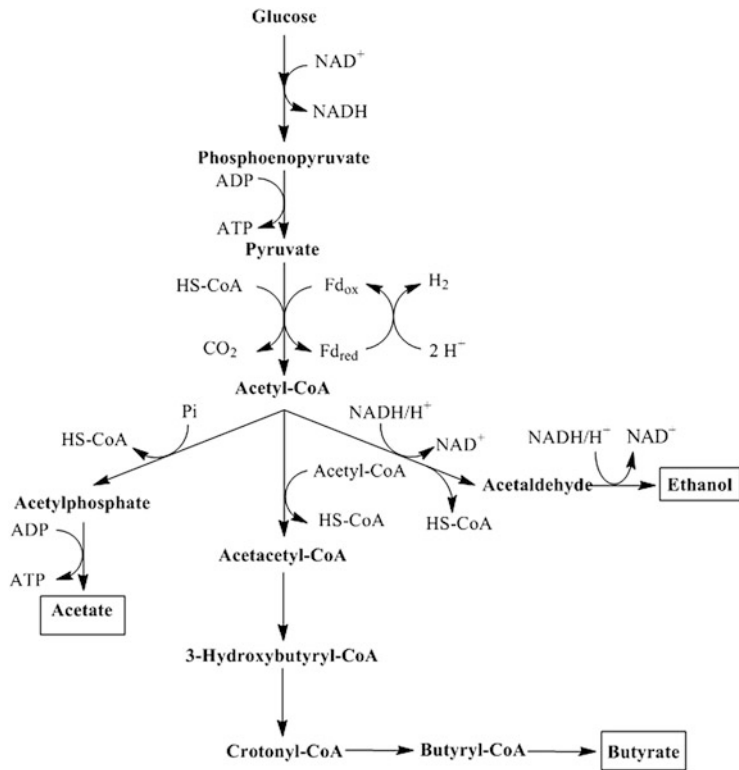


Fig. 3 Butyric acid pathway

Table 9 Characteristics of *Clostridium butyricum*

<i>Clostridium butyricum</i>		Schink and Zeikus (1980), Schink et al. (1981)
Systematic classification	Kingdom	Bacteria
	Phylum	Firmicutes
	Class	Clostridia
	Order	Clostridiales
	Family	Clostridiaceae
	Genus	Clostridium
	Species	<i>C. butyricum</i>
Synonyms	ATCC 19398	
Source of first isolation	Intestine of pig	
Characteristics	Gram-positive, strictly anaerobic endospore-forming bacteria	
Morphology	Rods	
Growth conditions	37 °C, medium 1053: reinforced clostridial medium, anaerobic	
Pathogenicity	Class 2	
DNA GC content	28.8	

Table 10 Characteristics of *Clostridium tyrobutyricum*

<i>Clostridium tyrobutyricum</i>		Skerman et al. (1980)
Systematic classification	Kingdom	Bacteria
	Phylum	Firmicutes
	Class	Clostridia
	Order	Clostridiales
	Family	Clostridiaceae
	Genus	Clostridium
	Species	<i>C. tyrobutyricum</i>
Synonyms	ATCC 25755	
Source of first isolation	Raw milk, Germany	
Characteristics	Gram-positive, spore forming, anaerobe	
Morphology	Rod shaped	
Growth conditions	37 °C, medium 2107: modified reinforced clostridial agar/broth medium, anaerobic	
Pathogenicity	Class 1	
DNA GC content	30.8	
Special characteristics	Heat-resistant spores	

4.4 Isobutyric Acid (C4), Isovaleric Acid (C5), Isocaproic Acid (C6)

Isobutyric acid is used in the production of artificial fibres, plastics and herbicides. It is also used as an intermediate in the production of cosmetics and food additives and in the pharmaceutical industry. There are industrialised chemical syntheses to produce isobutyric acid which does require fossil fuels and harmful chemicals. A biotechnological process based on renewable feedstock is more environmentally friendly and ensures in the long view a cost-effective supply of isobutyric acid.

Isovaleric acid is mainly used for perfumery production and within intensive-care medicine. Valerian is a natural source of isovaleric acid which can be extracted. Mainly, proteolytic bacteria can produce different carboxylic acids during the protein degradation. Several members of the family Clostridiaceae are proficient to use the Stickland fermentation, for example, *Clostridium bifermentans*, *C. sporogenes* and *C. acetobutylicum* (Brooks and Epps 1958).

Clostridium bifermentans is able to produce a broad range of metabolites such as butyric, acetic and formic acids (Wu and Yang 2003), ethanol, butanol, acetone (Khanal 2003), carbon dioxide, hydrogen and nitrogen (Levin et al. 2006). However, the metabolic pathway of *C. bifermentans* has not been investigated in detail so far (Leja et al. 2013).

C. sporogenes can produce acetic, propionic, butyric, isovaleric, isobutyric and isocaproic acid, hydrogen and carbon dioxide. By the current state of scientific knowledge, *C. sporogenes* produces the carboxylic acids applying the Stickland reaction. This reaction is a particular kind of fermentation of amino acids which is characterised by simultaneous oxidation of one amino acid and reduction of another

Fig. 4 Stickland reaction scheme (Stickland 1935; Nisman 1954)

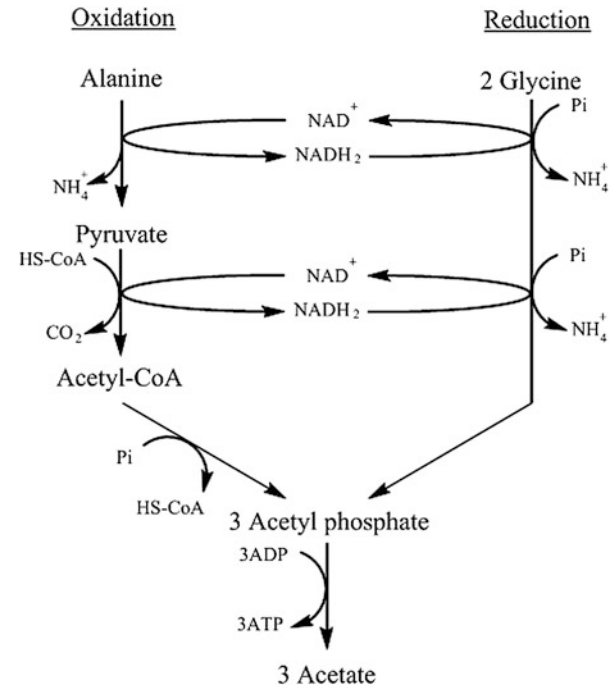


Table 11 Characteristics of *Clostridium sporogenes*

<i>Clostridium sporogenes</i>		Bradbury et al. (2012)
Systematic classification	Kingdom	Bacteria
	Phylum	Firmicutes
	Class	Clostridia
	Order	Clostridiales
	Family	Clostridiaceae
	Genus	Clostridium
	Species	<i>C. sporogenes</i>
Synonyms	ATCC 3584	
Source of first isolation	Cotton, gas gangrene and silage	
Characteristics	Gram-positive, spore forming	
Morphology	Rod shaped	
Growth conditions	37 °C, medium 2107: modified reinforced clostridial agar/broth medium, anaerobic	
Pathogenicity	Class 2	
DNA GC content	28	

amino acid (Nisman 1954). The utilisation of just one amino acid is not possible (Stickland 1935). Valine is the starting substance for isobutyric acid; leucine is converted to isovaleric acid.

Table 12 Characteristics of *Clostridium bifermentans obligat anaerobes*

<i>Clostridium bifermentans obligat anaerobes</i>		Ludwig et al. (2009)
Systematic classification	Kingdom	Bacteria
	Phylum	Firmicutes
	Class	Clostridia
	Order	Clostridiales
	Family	Peptostreptococcaceae
	Genus	Clostridium
	Species	<i>C. bifermentans</i>
Synonyms	Bacillus bifermentans sporogenes, Bacillus biferm., ATCC 638	
Source or first isolation	Silage, H.J. Kutzner, Darmstadt, Germany	
Characteristics	Gram-positive, spore forming, anaerobe	
Morphology	Rods, spores are cylindrical to oval, centrally to subterminally located, which do not noticeably swell the rods	
Growth conditions	37 °C, medium 2107: modified reinforced clostridial agar/broth medium, anaerobic, microaerophilic	
Pathogenicity	Class 2	
DNA GC content	28.4	

Due to the fact that the initial concentration of valine and leucine is crucial for the successful, high product yield fermentation, the engaged green biomass has to be optimised in the future. This represents an important milestone on the way to big-scale implementation (Fig. 4).

The biological high-yield production of isobutyric, isovaleric and isocaproic acid is not commercially established yet (Tables 11 and 12).

5 Sequence Chemical Products and Applications from Organic Acids

The application of biotechnological methods will be highly important with the development of biorefineries for the production of platform chemicals, intermediate chemicals, speciality chemicals and polymers.

The two-carbon short-chain acetic acid is start material for the production of vinyl acetate monomer (VAM). This application consumes one third of the world's production of acetic acid (Cheung et al. 2005). The product of the condensation of two molecules of acetic acid is acetic anhydride. The worldwide production of acetic anhydride is a further major application and uses approximately 25–30 % of the global production of acetic acid. The main process involves dehydration of acetic acid to give ketene at the temperature of 700–750 °C. Ketene is thereafter reacted with acetic acid to obtain the anhydride (Held et al. 2005). Acetic anhydride is an acetylation agent. As such, its major application is for cellulose acetate, a

synthetic textile also used for photographic film. Acetic acid owns a wide field of application. Also acetic acid and various corresponding salts are authorised food additives and increasingly used as preservatives.

The tri-carbon short-chain propionic acid is an important building block chemical and finds a variety of applications in organic synthesis for the production of polymers, such as cellulose acetate propionate, plastic dispersions, textile and rubber auxiliaries, dye intermediates as well as flavours and fragrances. This acid can be used for the synthesis of propionic ether and benzyl propionate, which can be used as additives in cosmetics. Furthermore, sodium 2,2-dichloropropionate is applied as herbicide. Propionic acid anhydride serves as a pharmaceutical intermediate (Kumar and Babu 2006). Also, propionic acid is applied in animal feed and as a grain preservative. The application possibilities cover the affordable preservation of animal feed (especially in cattle husbandry) and foodstuffs for human consumption. The US Food and Drug Administration (FDA) lists the acid and the Na^+ , Ca^+ and K^+ salt of it as preservatives in their summary of generally recognised as safe (GRAS) (Colomban et al. 1993).

The four-carbon short-chain n-butyric acid and its derivatives have numerous potential applications in chemical, textile, plastic, food, beverage, dairy and pharmaceutical industries. They are used as solvents, diluents, drugs, plasticisers, perfumes, fibres and additives (Jha et al. 2014). The main field of application of butyric acid is the food industry. The dairy industry is using the pure acids; the esters are used as food additives to amplify the fragrance of tropic fruits (Centeno et al. 2002; He et al. 2005; Watson 2002).

The four-carbon short-chain isobutyric acid is applied as esters for solvents, polymers, flavour and fragrances. Isobutyric acid is suitable as polar solvent for different chemical reactions. Two molecules of isobutyric acid form isobutyric anhydride. The pyrolysis of isobutyric anhydride produces dimethylketene. Dimethylketene is absorbed into certain carboxylate ester solvents which function as process solvents for subsequent dimerisation of the dimethylketene to 2,2,4,4-tetramethylcyclobutandione followed by the catalytic hydrogenation of the dione to the diol products (Sumner et al. 1992). This four-ring diol is a valuable comonomer for the production of a new family of copolyesters (Kelsey et al. 2000).

The five-carbon short-chain isovaleric acid is applied for the production of flavours and perfumes. Also, this acid is broadly used as intermediate for synthesis of insecticides, fungicides and depressants. The esters serve as plasticisers (Römpf 2005).

6 Future Perspectives

Currently, only few industrial products are produced from green biomass as carbon source, such as lactic acid and ammonium lactates (see chapter [Microorganisms for Production of Lactic Acid and Organic Lactates](#)), lysine (see chapter [Microorganisms for Biorefining of Green Biomass](#)) or 2,3 butanediol. The use of green biomass

could have advantages, if more platform chemicals would be developed. These advantages include using of a variety of microorganisms and potential use of carbon, nitrogen and inorganics, which contain in green biomass. In the case of the production of carboxylic acids, the fermentative use of organic nitrogen compounds like amino acids from proteins by appropriate *Clostridia* would be a rewarding approach.

By the conservation step silage, the green biomass would be available throughout the whole year. Furthermore, by the acid impact, a soft pretreatment happens on the celluloses. Simultaneously, the degradation of the proteins to amino acids continues.

The utilisation of green biomass could open new perspectives for the development of ecologically better adapted biorefineries. For this, it is necessary to work in research and development in order to improve the following areas:

- Improvement of fractionation/hydrolysis/separation methods of green raw feedstock in order to increase the amount of useful and fermentable substances, decrease the amount of inhibitors and lower process costs.
- DNA sequencing of microorganisms and their genome analysis—identify key genes responsible for the expression of useful properties.
- Metabolic engineering of microorganisms in order to broaden their substrate range, increase product tolerance/maximal concentration and increase yield and product specificity. Alternatively, target genes can be transferred into well-known, robust and user-friendly microorganisms (e.g. *E. coli*, *S. cerevisiae*).
- Large-scale screening of microbial genomes for genes or products with market potential.
- Appropriate optimisation of fermentation processes according to the properties of individual microorganisms and products.

References

- Abbas S-A, Adolfo Q-C (2000) Kontinuierliches Fermentationsverfahren für die optimale gleichzeitige Herstellung von Propionsäure und Vitamin B 12, DE69424765T2
- Andrews JH, Kinkel LL, Berbee FM, Nordheim EV (1987) Fungi, leaves, and the theory of island biogeography. *Microb Ecol* 14:277–290
- Andrews JH, Harris RF (2000) The ecology and biogeography of microorganisms on plant surfaces. *Annu Rev Phytopathol* 35:145–180
- Austin B, Goodfellow M (1979) *Pseudomonas mesophilica*, a new species of pink bacteria isolated from leaf surfaces. *Int J Syst Bacteriol* 29:373–378
- Balamurugan K, Venkata Dasu V, Panda T (1999) Propionic acid production by whole cells of *Propionibacterium freudenreichii*. *Bioprocess Eng* 20(2):109–116. doi:10.1007/PL00009039
- Balandreau J, Viallard V, Cournoyer B, Coenye T, Laevens S, Vandamme P (2001) *Burkholderia cepacia* genomovar III is a common plant-associated bacterium. *Appl Environ Microbiol* 67:982–985
- Behrendt U, Stauber T, Müller T (2004) Microbial communities in the phyllosphere of grasses on fenland at different intensities of management. *Grass Forage Sci* 59:169–179

- Brandl MT, Mandrell RE (2002) Fitness of *Salmonella enterica* serovar Thomson in the cilantro phyllosphere. *Appl Environ Microbiol* 68:3614–3621
- Bradbury M, Greenfield P, Midgley D, Li D, Tran-Dinh N, Vriesekoop F, Brown JL (2012) Draft genome sequence of *Clostridium sporogenes* PA 3679, the common nontoxicogenic surrogate for proteolytic *Clostridium botulinum*. *J Bacteriol* 194:1631–1632
- Braun K, Gottschalk G (1981) Effect of molecular hydrogen and carbon dioxide on chemoorganotrophic growth of *Acetobacterium woodii* and *Clostridium aceticum*. *Arch Microbiol* 128:294–298
- Breeze EM, Dix NJ (1981) Seasonal analysis of the fungal community of *Acer platanoides* leaves. *Trans Br Mycol Soc* 77:321–328
- Brooks ME, Epps HBG (1958) Taxonomic studies of the genus *Clostridium*: *Clostridium bifermentans* and *C. sordellii*. *J Gen Microbiol* 21:144–155. doi:10.1099/00221287-21-1-144
- Bunster L, Fokkema NJ, Schippers B (1989) Effect of surface-active *Pseudomonas* spp. on leaf wettability. *Appl Environ Microbiol* 55:1340–1345
- Cardon BP, Barker HA (1946) Two new amino-acid-fermenting bacteria, *Clostridium propionicum* and *Diplococcus glycinophilus*. *J Bacteriol* 52:629–634
- Carlsson R (1985) An ecological better adapted agriculture. Wet fractionation of biomass as green crops, macro-alga, and tuber crops. In: Proceedings of 2nd international conference on leaf protein research, Nagoya, 19–23
- Centeno JA, Tomillo FJ, Fernández-García E, Gaya P, Nuñez M (2002) Effect of wild strains of *Lactococcus lactis* on the volatile profile and the sensory characteristics of ewes' raw milk cheese. *J Dairy Sci* 85(12):3164–3172. doi:10.3168/jds.S0022-0302(02)74404-4
- Cheung H, Tanke RS, Torrence GP (2005) Acetic acid. In: Ullmann's Encyclopedia of industrial chemistry, vol 1. Wiley, Weinheim. doi:10.1002/14356007.a01_045
- Cho JJ, Schroth MN, Kominos SD, Green SK (1975) Ornamental plants as carriers of *Pseudomonas aeruginosa*. *Phytopathology* 64:425–431
- Colomban A, Roger L, Boyaval P (1993) Production of propionic acid from whey permeate by sequential fermentation, ultrafiltration, and cell recycling. *Biotechnol Bioeng* 42:1091–1098
- Dogi CA, Armando R, Pribull R, Pribull B, Soares de Souza MM, da Silva CI, de Melo DA, Dalcero A, Cavaglieri L (2013) Selection of lactic acid bacteria to promote an efficient silage fermentation, capable of inhibiting the activity of *Aspergillus parasiticus* and *Fusarium graminearum* and mycotoxin production. *J Appl Microbiol* 114:1650–1660. doi:10.1111/jam.12173
- Damgaard PH, Abdel-Hameed A, Eilenberg J, Smits PH (1998) Natural occurrence of *Bacillus thuringiensis* on grass foliage. *World J Microbiol Biotechnol* 14:239–242
- Dickinson CH (1967) Fungal colonization of *Pisum* leaves. *Can J Bot* 45:915–927
- Dickinson CH (1976) Fungi on the aerial surfaces of higher plants. In: Dickinson CH, Preece TF (eds) Microbiology of aerial plant surfaces. Academic, London, pp 293–324, 669 pp
- Driehuis F, Oude Elferink SJWH (2000) The impact of the quality of silage on animal health and food safety: a review. *Vet Q* 22:212–217
- Dunière L, Sindou J, Chaucheyras-Durand F, Chevallier I, Thévenot-Sergentet D (2013) Silage processing and strategies to prevent persistence of undesirable microorganisms. *Anim Feed Sci Technol* 182:1–15
- Duveiller E (1994) A study of *Xanthomonas campestris* pv. *undulosa* populations associated with symptomless wheat leaves. *Parasitica* 50:109–117
- FAO (2012) <http://faostat.fao.org/>
- Fokkema NJ, den Houter JG, Kosterman YJC, Nelis AL (1979) Manipulation of yeasts on field-grown wheat leaves and their antagonistic effect on *Cochliobolus sativus* und *Septoria nodorum*. *Trans Br Mycol Soc* 72:19–29
- Gillis M, de Ley J (1980) Intra- and intergeneric similarities of the ribosomal ribonucleic acid cistrons of *Acetobacter* and *Gluconobacter*. *Int J Syst Bacteriol* 30:7–27
- Glushakova AM, Chernov IY (2004) Seasonal dynamics in a yeast population on leaves of the common wood sorrel *Oxalis acetosella* L. *Microbiology* 73:184–188

- Gottschalk G, Braun M (1981) Revival of the name *Clostridium aceticum*. Int J Syst Bacteriol 31:476
- Govan JR, Hughes JE, Vandamme P (1996) *Burkholderia cepacia*: medical, taxonomic and ecological issues. J Med Microbiol 45:395–407
- Green PN, Bousfield IJ (1983) Emendation of *Methylobacterium* Patt, Cole, and Hanson 1976; *Methylobacterium rhodinum* (Heumann 1962) comb. nov. corrig.; *Methylobacterium radiotolerans* (Ito and Iizuka 1971) comb. nov. corrig.; and *Methylobacterium mesophilicum* (Austin and Goodfellow 1979) comb. nov. Int J Syst Bacteriol 33:875–877
- Hafner SD, Howard C, Muck RE, Franco RB, Montes F, Green PG, Mitloehner F, Trabue SL, Alan Rotz C (2013) Emission of volatile organic compounds from silage: compounds, sources and implications. Atmos Environ 77:827–839
- Hansen BM, Damgaard PH, Eilenberg J, Pedersen JC (1998) Molecular and phenotypic characterization of *Bacillus thuringiensis* isolated from leaves and insects. J Invertebr Pathol 71:106–114
- He GQ, Kong Q, Chen QH, Ruan H (2005) Batch and fed-batch production of butyric acid by *Clostridium butyricum* ZJUCB. J Zhejiang Univ (Sci) 6(11):1076–1080. doi:10.1631/jzus.2005.B1076
- Held H, Rengstl A, Mayer D (2005) Acetic anhydride and mixed fatty acid anhydrides. In: Ullmann's Encyclopedia of industrial chemistry. Wiley, Weinheim. doi:10.1002/14356007.a01_065
- Holland MA, Long RLG, Polacco JC (2002) *Methylobacterium* spp.: phylloplane bacteria involved in cross-talk with the plant host? In: Lindow SE, Hecht-Poinar EI, Elliot VJ (eds) Phyllosphere microbiology. APS Press, St. Paul, pp 125–135
- Hutchison ML, Johnstone K (1993) Evidence for the involvement of the surface active properties of the extracellular toxin tolaasin in the manifestation of brown blotch disease symptoms by *Pseudomonas tolaasii* on *Agaricus bisporus*. Physiol Mol Plant Pathol 42:373–384
- Hutchison ML, Tester MA, Gross DC (1995) Role of biosurfactant and ion channel-forming activities of syringomycin in transmembrane ion flux: a model for the mechanism of action in the plant pathogen interaction. Mol Plant Microbe Interact 8:610–620
- Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. Appl Environ Microbiol 70:2667–2677
- Inacio J, Pereira P, de Carvalho M, Fonseca A, Amaral-Collaco MT, Spencer-Martins I (2002) Estimation and diversity of phylloplane mycobiota on selected plants in a Mediterranean-type ecosystem in Portugal. Microb Ecol 44:344–353
- Janssen PH (1991) Isolation of *Clostridium propionicum* strain 19acry 3 and further characteristics of the species. Arch Microbiol 155:566–571
- Jha AK, Li J, Yuan Y, Baral N, Ai B (2014) A review on bio-butyric acid production and its optimization. Int J Agric Biol 16:1019–1024
- Johnson JL, Cummins CS (1972) Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria, and strains of *Arachnia propionica*. J Bacteriol 129:1047–1066
- Johnson KB, Stockwell VO (1998) Management of fire blight: a case study in microbial ecology. Annu Rev Phytopathol 36:227–248
- Kadivar H, Stapleton AE (2003) Ultraviolet radiation alters maize phyllosphere bacterial diversity. Microb Ecol 45:353–361
- Kamm B, Kamm M, Narodoslawsky M, Kromus S (2006) The green biorefinery concept—fundamentals and potentials. In: Kamm B, Kamm M, Gruber P (eds) Biorefineries—biobased industrial processes and products. Status quo and future directions, vol 1. Weinheim, Wiley, pp 253–294. ISBN 3-527-31027-4
- Kang YS, Kim J, Shin HD, Nam YD, Bae JW, Jeon CO, Park W (2007) *Methylobacterium platani* sp. nov., isolated from a leaf of the tree *Platanus orientalis*. Int J Syst Environ Microbiol 57:2849–2853

- Karlsson JL, Volcani BE, Barker HA (1948) The nutritional requirements of *Clostridium acetum*. J Bacteriol 56:781–782
- Kelsey DR, Scardino BM, Grebowicz JS, Chuah HH (2000) High impact, amorphous terephthalate copolyesters of rigid 2,2,4,4-tetramethyl-1,3-cyclobutandiol with flexible diols. Macromolecules 33:5810–5818
- Khanal S (2003) Biological hydrogen production: effects of pH and intermediate products. Int J Hydrogen Energy 29:1123–1131. doi:[10.1016/j.ijhydene.2003.11.002](https://doi.org/10.1016/j.ijhydene.2003.11.002)
- Kinkel LL (1997) Microbial population dynamics on leaves. Annu Rev Phytopathol 35:327–347
- Klerks MM, Franz E, van Gent-Pelzer M, Zijlstra C, van Bruggen AHC (2007) Differential interactions of *Salmonella enterica* serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency. ISME J 1:620–631
- Kong GA, Kochman JK, Brown JF (1997) Phylloplane bacteria antagonistic to the sunflower pathogen *Alternaria helianthi*. Aust. Plant Pathol 26:85–97
- Kuan T-L, Minsavage GV, Schaad NW (1986) Aerial dispersal of *Xanthomonas campestris* pv. *campestris* from naturally infected *Brassica campestris*. Plant Dis 70:409–413
- Kumar S, Babu BV (2006) A brief review on propionic acid: a renewable energy source. In: Proceedings of national conference on environmental conservation, pp 459–464
- Lambais MR, Crowley DE, Cury JC, Bull RC, Rodrigues RR (2006) Bacterial diversity in tree canopies of the Atlantic forest. Science 312:1917
- Lamsal BP (2004) Alfalfa soluble leaf proteins: extraction separation, concentration and characterisation. Ph.D. Dissertation, Department of Biological Systems Engineering, University of Wisconsin, Madison
- Leisinger TH (1965) Untersuchungen zu Systematik und Stoffwechsel der Essigsäurebakterien. Zentralbl Bakteriell II Abt 119:329–376
- Leja K, Myszk K, Czaczky K (2013) The ability of *Clostridium bifermentans* strains to lactic acid biosynthesis in various environmental conditions. Springerplus 2(1):44. doi:[10.1186/2193-1801-2-44](https://doi.org/10.1186/2193-1801-2-44)
- Levin D, Islam R, Cicek N, Sparling R (2006) Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. Int J Hydrogen Energy 31(11):1496–1503. doi:[10.1016/j.ijhydene.2006.06.015](https://doi.org/10.1016/j.ijhydene.2006.06.015)
- Li Y, Nishino N (2013) Changes in the bacterial community and composition of fermentation products during ensiling of wilted Italian ryegrass and wilted guinea grass silages. Anim Sci J 84:607–612. doi:[10.1111/asj.12046](https://doi.org/10.1111/asj.12046)
- Liang Z-X, Li L, Li S, Cai Y-H, Yang S-T, Wang J-F (2012) Enhanced propionic acid production from Jerusalem artichoke hydrolysate by immobilized *Propionibacterium acidipropionici* in a fibrous-bed bioreactor. Bioprocess Biosyst Eng 35(6):915–921. doi:[10.1007/s00449-011-0676-y](https://doi.org/10.1007/s00449-011-0676-y)
- Lindow SE, McGourty G, Elkins R (1996) Interactions of antibiotics with *Pseudomonas fluorescens* strain A506 in the control of fire blight and frost injury to pear. Phytopathology 86:841–848
- Lindow SE, Brandl MT (2003) Microbiology of the Phyllosphere. Appl Environ Microbiol 69:1875–1883. doi:[10.1128/AEM.69.4](https://doi.org/10.1128/AEM.69.4)
- Ludwig W, Schleifer K-H, Whitman WB (2009) Revised road map to the phylum Firmicutes. In: De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn, The firmicutes. Springer, New York, pp 1–13
- Madhaiyan M, Poonguzhali S, Kwon SW, Sa TM (2009) *Methylobacterium phyllosphaerae* sp. nov., a pink-pigmented, facultative methylotroph from the phyllosphere of rice. Int J Syst Evol Microbiol 59:22–27
- Manulis S, Haviv-Chesner A, Brandl MT, Lindow SE, Barash I (1998) Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophila*. Mol Plant Microbe Interact 11:634–642

- McCarter LL, Showalter RE, Silverman MR (1992) Genetic analysis of surface sensing in *Vibrio parahaemolyticus*. *Biofouling* 5:163–175
- McCarter LL, Silverman M (1989) Iron regulation of swarmer cell differentiation of *Vibrio parahaemolyticus*. *J Bacteriol* 171:731–736
- McGarvey JA, Franco RB, Palumbo JD, Hnasko R, Stanker L, Mitloehner FM (2013) Bacterial population dynamics during the ensiling of *Medicago sativa* (alfalfa) and subsequent exposure to air. *J Appl Microbiol* 114:1661–1670. doi:[10.1111/jam.12179](https://doi.org/10.1111/jam.12179)
- Moore WEC, Holdeman LV (1970) Propionibacterium, Arachnia, Actinomyces, Lactobacillus and Bifidobacterium. In: Cato EP, Cummins CS, Holdeman LV, Johnson JL, Moore WEC, Smibert RM, Smith LDS (eds) *Outline of clinical methods in anaerobic bacteriology*, 2nd edn. Virginia Polytechnic Institute Anaerobe Laboratory, Blacksburg, pp 15–21
- Miyamoto T, Kawahara M, Minamisawa K (2004) Novel endophytic nitrogen-fixing clostridia from the grass *Miscanthus sinensis* as revealed by terminal restriction fragment length polymorphism analysis. *Appl Environ Microbiol* 70:6580–6586
- Müller T, Ruppel S (2014) Progress in cultivation-independent phyllosphere microbiology. *FEMS Microbiol Ecol* 87:2–17. doi:[10.1111/1574-6941.12198](https://doi.org/10.1111/1574-6941.12198)
- Nisman B (1954) The stickland reaction. *Bacteriol Rev* 74. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC180783/>
- O'Brien RD, Lindow SE (1989) Effect of plant species and environmental conditions on epiphytic population sizes of *Pseudomonas syringae* and other bacteria. *Phytopathology* 79:619–627
- Ott EM, Muller T, Muller M, Franz CM, Ulrich A, Gabel M, Seyfarth W (2001) Population dynamics and antagonistic potential of *enterococci* colonizing the phyllosphere of grasses. *J Appl Microbiol* 91:54–66
- Preston GM, Bertrand N, Rainey PB (2001) Type III secretion in plant growth-promoting *Pseudomonas fluorescens* SBW25. *Mol Microbiol* 41:999–1014
- Quigley NB, Gross DC (1994) Syringomycin production among strains of *Pseudomonas syringae* pv. *syringae*: conservation of the syrB and syrD genes and activation of phytotoxin production by plant signal molecules. *Mol Plant Microbe Interact* 7:78–90
- Rasche F, Marco-Noales E, Velvis H, van Overbeek LS, Lopez MM, van Elsas JD, Sessitsch A (2006a) Structural characteristics and plant-beneficial effects of bacteria colonizing the shoots of field grown conventional and genetically modified T4-lysozyme producing potatoes. *Plant Soil* 289:123–140
- Rasche F, Trondl R, Naglreiter C, Reichenauer RG, Sessitsch A (2006b) Chilling and cultivar type affect the diversity of bacterial endophytes colonizing sweet pepper (*Capsicum annuum* L.). *Can J Microbiol* 52:1036–1045
- Reiter B, Sessitsch A (2006) Bacterial endophytes of the wildflower *Crocus albiflorus* analysed by characterisation of isolates and by a cultivation-independent approach. *Can J Microbiol* 52:140–149
- Römpf (2005) (Römpf Online, 25.08.2014)
- Ruppel S, Müller T (2012) Die Phyllosphäre - ein mikrobielles Domizil. *BIOspektrum* 18:479–481. doi:[10.1007/s12268-012-0215-7](https://doi.org/10.1007/s12268-012-0215-7)
- Sabra W, Dietz D, Zeng AP (2013) Substrate-limited co-culture for efficient production of propionic acid from flour hydrolysate. *Appl Microbiol Biotechnol* 97:5771–5777. doi:[10.1007/s00253-013-4913-y](https://doi.org/10.1007/s00253-013-4913-y)
- Seyfarth W, Müller M (1997) Changing of the fraction of water soluble carbohydrates of feed grass in the course of vegetation period and the microbial use of fructans by plant associated lactic acid bacteria. In: Soye K, Kamm B, Kamm, M (eds) *The green biorefinery*, Verlag Gesellschaft für ökologische Technologie und Systemanalyse, pp 82–88
- Schink B, Ward JC, Zeikus JG (1981) Microbiology of wet wood: importance of pectin degradation and Clostridium species in living trees. *Appl Environ Microbiol* 42:526–532
- Schink B, Zeikus JG (1980) Microbial ethanol formation: a major end product of pectin metabolism. *Curr Microbiol* 4:387–389

- Shao T, Zhang ZX, Shimojo M, Wang T, Masuda Y (2005) Comparison of fermentation characteristics of Italian ryegrass (*Lolium multiflorum* Lam.) and guinea grass (*Panicum maximum* Jacq.) during the early stage of ensiling. *Asian Aust J Anim Sci* 18(12):1727–1734
- Skerman VBD, McGowan V, Sneath PHA (ed) (1980) Approved lists of bacterial names. *Int J Syst Bacteriol* 30:225–420
- Stickland L (1935) Studies in the metabolism of the strict anaerobes (genus *clostridium*): the reduction of proline by *Cl. sporogenes*. *Biochem J* 29:288–290. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1266487/>
- Sumner CE Jr, Gustafson BL, Knight JR (1992) Process for the manufacture of 2,2,4,4-Tetramethylcyclobutanediol US 5,169,994 (20.08.1991/08.12.1992)
- Thompson IP, Bailey MJ, Ellis RJ, Lilley AK, McCormack PJ, Purdy KJ, Rainey PB (1995) Short-term community dynamics in the phyllosphere microbiology of field-grown sugar beet. *FEMS Microbiol Ecol* 16:205–212
- Thompson IP, Bailey MJ, Fenlon JS, Fermor TR, Lilley AK, Lynch JM, McCormack PJ, McQuilken MP et al (1993) Quantitative and qualitative seasonal changes in the microbial community from the phyllosphere of sugar beet (*Beta vulgaris*). *Plant Soil* 150:177–191
- Uppe CD, Hirano SS, Dodd KK, Clayton MK (2003) Factors that affect spread of *Pseudomonas syringae* in the phyllosphere. *Phytopathology* 93:1082–1092
- van Niel CB (ed) (1928) The propionic acid bacteria. Uitgeverszaak and Boissevain and Co, Haarlem, pp 1–187
- Wang C, Han H, Gu X, Yu Z, Nishino N (2014) A survey of fermentation products and bacterial communities in corn silage produced in a bunker silo in China. *Anim Sci J* 85:32–36. doi:10.1111/asj.12076
- Watson R (2002) Influence of harvest date and light integral on the development of strawberry flavour compounds. *J Exp Bot* 53(377):2121–2129. doi:10.1093/jxb/erf088
- Weller DM, Saettler AW (1980) Colonization and distribution of *Xanthomonas phaseoli* and *Xanthomonas phaseoli* var. *fuscans* in field-grown navy beans. *Phytopathology* 70:500–506
- Whipps JM, Hand P, Pink D, Bending GD (2008) Phyllosphere microbiology with special reference to diversity and plant genotypes. *J Appl Microbiol* 105:1744–1755. doi:10.1111/j.1365-2672.2008.03906.x
- Wieringa KT (1936) Over het verdwijnen van waterstof en koolzuur onder anaerobe voorwaarden. Antonie van Leeuwenhoek. *J Microbiol Serol* 3963–273
- Wildman HG, Parkinson D (1979) Microfungal succession on living leaves of *Populus tremuloides*. *Can J Bot* 57:2800–2811
- Wipat A, Harwood CR (1999) The *Bacillus subtilis* genome sequence: the molecular blueprint of a soil bacterium. *FEMS Microbiol Ecol* 28:1–9
- Wu Z, Yang S-T (2003) Extractive fermentation for butyric acid production from glucose by *Clostridium tyrobutyricum*. *Biotechnol Bioeng* 82(1):93–102. doi:10.1002/bit.10542
- Yang C-H, Crowley DE, Borneman J, Keen NT (2001) Microbial phyllosphere populations are more complex than previously realized. *PNAS* 98(7):3889–3894

Microorganisms in Biorefineries

Kamm, B. (Ed.)

2015, X, 369 p. 71 illus., 20 illus. in color., Hardcover

ISBN: 978-3-662-45208-0