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3-Hydroxypropionaldehyde: applications and perspectives of biotechnological production

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Abstract 3-Hydroxypropionaldehyde (3-HPA) forms, together with HPA-hydrate and HPA-dimer, a dynamic, multi-component system (HPA system) used in food preservation, as a precursor for many modern chemicals such as acrolein, acrylic acid, and 1,3-propanediol (1,3-PDO), and for polymer production. 3-HPA can be obtained both through traditional chemistry and bacterial fermentation. To date, 3-HPA has been produced from petrochemical resources as an intermediate in 1,3-PDO production. In vivo, glycerol is converted in one enzymatic step into 3-HPA. The 3-HPA-producing *Lactobacillus reuteri* is used as a probiotic in the health care of humans and animals. The biotechnological production of 3-HPA from renewable resources is desirable both for use of 3-HPA in foods and for the production of bulk chemicals. The main challenge will be the efficient production and recovery of pure 3-HPA.

Elucidation of the HPA system

The very first observation leading eventually to the elucidation of the synthesis pathway of 3-hydroxypropionaldehyde (3-HPA) dates back to as early as 1741. Geoffrey (1742) described that a new water-soluble syrup was formed from fat after saponification. In 1779, Scheele isolated the same syrup from a diverse array of substrates, including almonds, linseed, rape oils, olive oil, butter and lard. Because of its sweet taste he named it “Ölsüss” (see Scheele 1931). About 40 years later, the French pioneer of organic chemistry Chevreul noted that, after saponification, the neutral fats were transformed into solid fatty acids and the sweet component of Scheele. He renamed this

liquid, believed to be an alcohol, glycerine (Chevreul 1823; Matagrín 1925).

Chevreul's discoveries had some very practical implications for everyday life. The removal of glycerol from the raw material of candles both greatly increased their hardness and illuminating power, and eliminated the unpleasant odour of burning candles that resulted from glycerol decomposition (Matagrín 1925). Brandes was the first to describe this decomposition product and name it acrolein, thus reflecting both its smell (acris) and consistency (oleum), whereas Berzelius was the first to identify it as an aldehyde (Berzelius 1839). Meanwhile, in Liebig's laboratory, Redtenbacher noted that acrolein was formed by the combustion of fats, but not of fatty acids. Furthermore, he suspected that glycerol could be the source of acrolein. During the distillation of glycerol he obtained acrolein, and confirmed that it is an aldehyde since upon further oxidation it formed a new acid, which he named acrylic acid. Thus, Redtenbacher established that acrolein can be formed from glycerol through the loss of four molecules of water (Redtenbacher 1843).

Some years later, Nef heated acrolein in the presence of water, which resulted in the formation of hydracrylaldehyde, or 3-HPA (Fig. 1); this was the first formal description of the compound (Nef 1904). The kinetics of acrolein hydration and the formation of 3-HPA were studied later (Pressman and Lucas 1942). Redtenbacher established that not only chemical, but also biological reactions could use glycerol as a “substrate”, he described the formation of propionic acid from glycerol by bacterial fermentation (Redtenbacher 1846), and Pasteur discovered that glycerol could also be formed during alcoholic fermentation (Pasteur 1858a, 1858b). A few years later, Napoleon asked Pasteur to study the origin of different types of wine spoilage, referred to as “les maladies de l'amertume des vins”. During this period, France produced about 50 million hectolitres of wine worth about 500 million Francs annually, thus making the wine industry the most profitable economic sector in France. Considerable losses from different plant diseases, as well as from wine spoilage itself, prompted this research.

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Pasteur found that bacteria were responsible for the formation of bitter tastes in wine leading to its spoilage (Pasteur 1866).

However, only after 1910 was the connection made between the formation of acrolein from glycerol by bacterial fermentation and the spoilage of wine (Voisenet 1910). Moreover, Voisenet found that propanolal, now referred to as 3-HPA, is formed as an intermediate in this synthesis pathway (Fig. 1). He also identified trimethylene glycol [1,3-propanediol (1,3-PDO)] as another product of this fermentation process (Voisenet 1910, 1914a, 1914b) (Fig. 1). Although Voisenet could identify the presence of acrolein and 3-HPA by a colorimetric test, it was only in 1945 that a quantitative test using tryptophan was developed for acrolein (Circle et al. 1945). The same test was later used to determine the concentration of 3-HPA.

In 1950, during the first systematic study of 3-HPA by Hall and Stern, the importance of 3-HPA as an intermediate of great potential value in the solvent or plasticiser field was mentioned. In their work, Hall and Stern described a dimeric form of 3-HPA in equilibrium with the monomeric form commonly found in solution (Hall and Stern 1950). Finally in 1981, using NMR, Nielsen et al. were able to identify HPA-hydrate as the last component of the HPA system. In summary, the work of over 100 years of research outlined above has resulted in the formulation of a complex reaction model in which 3-HPA plays a central role (Fig. 1). In vivo, 3-HPA is produced by dehydration of glycerol by a B_{12} -dependent glycerol dehydratase, and further reduced to 1,3-PDO by an NAD^+ -dependent oxidoreductase (Toraya 2000). In aqueous solution, 3-HPA undergoes reversible dimerisation and hydration, thus resulting in an equilibrium of 3-HPA, HPA-hydrate, and HPA-dimer, referred to here as HPA or the HPA-system. During thermal dehydration, acrolein is formed in HPA-containing solutions (Fig. 1). Detailed descriptions for the enzymatic pathway are given by Biebl et al. (1999) and Lüthi-Peng et al. (2002a), and a detailed description of acrolein formation is given by Hall and Stern (1950) and Nielsen et al. (1981).

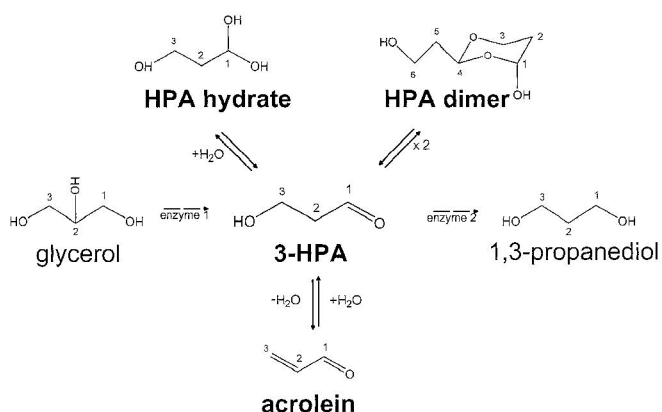


Fig. 1 Formation of hydroxypropionaldehyde (HPA), 1,3-propanediol (1,3-PDO), and acrolein. $\rightarrow$ Enzymatic reaction; $\rightleftharpoons$ equilibrium reactions; enzyme 1 glycerol dehydratase; enzyme 2 1,3-propanediol oxidoreductase

As illustrated by this historical outline, many components of the HPA system can be obtained both through traditional chemistry and bacterial fermentation (Mickelson and Werkman 1940; Otsuka 2003; Sobolov and Smiley 1960). The current use and future prospects of the HPA system in modern biotechnology, in particular as an alternative to its production from fossil resources by the petrochemical industry, will be discussed below.

Production of 3-HPA

Petrochemically derived 3-HPA

The raw materials for the multistep synthesis are obtained from the petrochemical industry. Two chemical processes are known to produce 3-HPA as an intermediate in 1,3-PDO production: the Degussa and Shell processes. The Degussa process begins with the catalytic transformation of propylene into acrolein, which is hydrated to 3-HPA and further reduced to 1,3-PDO (Arntz and Wiegand 1991; Brossmer and Arntz 2000). Although propylene is inexpensive, the separation of the different molecules is expensive and acrolein is toxic. The 1,3-PDO yield of this process is only 43%. The Shell process begins with ethylene, forming ethylene oxide, which is transformed by a hydroformylation reaction under high pressure (150 bar) with syngas to 3-HPA (Knifton et al. 2003). Ethylene is inexpensive and the intermediate products are not toxic, but 3-HPA has to be extracted from the organic phase. The 1,3-PDO yield is about 80%. No information on the yields for 3-HPA production is available in the literature.

3-HPA from renewable resources

Biotechnological production has several advantages compared to the chemical process. First, 3-HPA production can be carried out in glycerol-containing aqueous solution at room temperature or at 37°C under normal pressure. The transformation from glycerol to 3-HPA is a one-step enzymatic catalysis (Fig. 1) and the yields (85–87% mol 3-HPA/mol glycerol) have been shown to be higher than those achieved by chemical synthesis (Arntz and Wiegand 1991; Brossmer and Arntz 2000; Ulmer et al. 2002; Vollenweider et al. 2003).

To date, six genera of bacteria that are able to ferment glycerol into 3-HPA have been identified: *Bacillus* (Voisenet 1914a); *Klebsiella* (*Aerobacter*) (Abeles et al. 1960; Reymolds et al. 1939; Slininger et al. 1983); *Citrobacter* (Mickelson and Werkman 1940); *Enterobacter* (Barbirato et al. 1996); *Clostridium* (Humphreys 1924); and *Lactobacillus* (Mills et al. 1954; Serjak et al. 1954). The enzyme responsible for the formation of 3-HPA from glycerol, glycerol dehydratase, is known to be cobamide-dependent (Abeles et al. 1960; Smiley and Sobolov 1962; Sobolov and Smiley 1960; Toraya 2000). 3-HPA is normally an intracellular intermediate that does not accumulate but is reduced by an NAD^+ -dependent

oxidoreductase to 1,3-PDO, the end-product of glycerol fermentation (Biebl et al. 1999; Daniel et al. 1999; Thiman 1955; Voisenet 1914b; Zeng and Biebl 2002).

The regulation of this pathway is dependent on the availability of fermentable carbohydrates, in particular glucose. In fact, the presence of glycerol and its transformation into 1,3-PDO enables the cells to recover the NAD^+ used during glycolysis. Under conditions of unlimited glucose, 1,3-PDO will be formed. With enterobacteria, the presence of glucose will therefore reduce the yield of 3-HPA production. With lactobacilli, a molar ratio of glucose to glycerol no greater than 0.33 will favour the accumulation of 3-HPA. In non-growing medium (H_2O), addition of glucose reduces accumulation of 3-HPA. Therefore, biotechnological production of 3-HPA should be performed without glucose, to favour accumulation of 3-HPA (El-Ziney et al. 1998; Lüthi-Peng et al. 2002a; Mills et al. 1954; Sauvageot et al. 2000; Sobolov and Smiley 1960). At high concentrations 3-HPA is secreted into the medium and can easily be concentrated by lyophilisation (Slininger et al. 1983; Talarico and Dobrogosz 1989; Vollenweider et al. 2003). However, the toxicity of 3-HPA towards bacteria limits the accumulation of this compound in the fermentation broth medium (Table 1, studies 1–5).

Two methods have been attempted to overcome this toxicity and increase the yield for biotechnological production. In the first method, 3-HPA is entrapped by semicarbazide (Ulmer et al. 2002), such that 3-HPA becomes unavailable. This reduces its toxic affect and prevents its further reduction to 1,3-PDO, increasing the yield of 3-HPA production. This method resulted in a very high total production of 621 mM 3-HPA (Table 1, studies 6–8). Although this method is very promising, the recovery of 3-HPA has never been reported. The second method uses *Lactobacillus reuteri*, a species shown to sustain large amounts of 3-HPA (Table 1, studies 9–10) produced from glycerol. However, although *L. reuteri* is very resistant to high concentrations of 3-HPA, its viability does decrease when 3-HPA is produced in large quantities.

To further optimise biotechnological production, bacterial strains that are both more efficient and capable of tolerating large amounts of 3-HPA need to be developed. To increase efficiency, undesirable by-product formation should be prevented. Genetic engineering of bacterial strains might be necessary, as was carried out in some studies to increase the yield of 1,3-PDO production (Zeng and Biebl 2002; Cameron et al. 1998). Two enzymatic targets appear to be of interest to increase the accumulation of 3-HPA. First, the elimination of 1,3-PDO oxidoreductase activity, which prevents the formation of 1,3-PDO (Fig. 1); and second, the elimination of glycerol dehydrogenase (GDH), which forms dihydroxyacetone in a side reaction (Zeng and Biebl 2002). The development of bacterial strains capable of transforming glycerol into 3-HPA via a non-cobamide-dependent glycerol dehydratase activity also deserves further development (Zeng and Biebl 2002).

To decrease the inhibitory effect of accumulating 3-HPA, the use of semicarbazide to entrap 3-HPA (Vancauwenberge et al. 1990) warrants further development, in particular, development of a method to recover the 3-HPA. The use of semicarbazide for entrapment would have several advantages. For example, when 3-HPA is in its semicarbazone form, its inhibitory effect on cells is reduced, and there is no need to eliminate 1,3-PDO oxidoreductase activity because the substrate is not available. The recovery of 3-HPA from semicarbazide could also be developed as a purification step. Physical methods for 3-HPA purification such as distillation have proved difficult because of the high distillation point of HPA in a concentrated form and the formation of resins (Hall and Stern 1950; Stepanow and Schtschukin 1927).

Table 1 Biotechnological production of 3-hydroxypropionaldehyde (3-HPA) and 1,3-propanediol (PDO) from glycerol under different conditions

Study	Strain	Glycerol (mM)	3-HPA (mM)	PDO (mM)	Strain	Reference
1	<i>Lactobacillus</i> sp.	2.34	+ ^a	2	NRRL B-1720	Sobolov and Smiley 1960
2	<i>Lactobacillus</i> sp.	326	95	nd ^b	NRRL B-1720	Slininger et al. 1983
3	<i>Enterobacter agglomerans</i>	725	30		CNCM1210	Barbirato et al. 1996
		830		237		
4	<i>Citrobacter freundii</i>	760	17	400	ATCC 8090	
		740				
5	<i>Klebsiella pneumonia</i>	760	24		ATCC 25955	
		780		429		
6	<i>K. pneumonia</i>	333	177 ^c	nd	ATCC 8724	Slininger et al. 1983
7	<i>K. pneumonia</i>	326	220 ^c	nd	ATCC 8724	Slininger and Bothast 1985
8	<i>K. pneumonia</i>	760	621 ^c	nd	ATCC 8724	Vancauwenberge et al. 1990
9	<i>Lactobacillus reuteri</i>	250	140	50	1063	Talarico et al. 1988
10	<i>L. reuteri</i>	200	170 ^d	nd	ATCC 53608	Lüthi-Peng et al. 2002b; Vollenweider et al. 2003

^aPresent

^bNot determined

^cProduction in buffered semicarbazide

^dProduction in water

Biotechnological applications

L. reuteri

Probiotics are live microbial feed supplements, which beneficially affect the host by improving its intestinal microbial balance (Tannock 1999). They can inhibit pathogenic bacteria due to the production of antimicrobial compounds. Axelsson et al. (1988) were the first to report an antimicrobial compound produced by *L. reuteri*, a bacterial strain known to inhabit the gastrointestinal tract of all vertebrates and mammals (Casas and Dobrogosz 2000). The antimicrobial agent was described as an anti-fungal, anti-parasite, anti-viral and anti-cancer agent as well as an antibacterial agent (Dobrogosz and Lindgren 1988; Dobrogosz et al. 1989) and named reuterin, after the producing strain. Reuterin has a broad spectrum of antimicrobial activity against prokaryotes (both Gram⁺ and Gram⁻) and eukaryotes (*Saccharomyces cerevisiae* and *Trypanosoma cruzi*). Further characterisation of reuterin revealed the presence of 3-HPA, HPA-hydrate, and HPA-dimer (Axelsson et al. 1988; Talarico and Dobrogosz 1989). Today the name reuterin is used as a synonym for the HPA system, mostly in the field of food preservation and health care.

A detailed review validating the use of *L. reuteri* as a probiotic has been published (Casas and Dobrogosz 2000), and the company, BioGaia, established a web-site on this topic (<http://www.reuteri.com>). Some of this information is summarised below. *L. reuteri* has been shown to survive the effects of gastric acid and bile salts in the stomach and upper intestine; bind to the gut mucus and epithelial cells; and to colonise the intestine. *L. reuteri* is expected to reduce the risk of diarrhoea among children and can significantly lower the incidence of watery diarrhoea among children suffering from rotavirus diarrhoea. It may also reduce *Helicobacter pylori* infections (Mukai et al. 2002; Shornikova et al. 1997). Finally, trials have shown that *L. reuteri* has a positive influence on the development of ileal tissue, thereby enhancing absorption of nutrients. Safety studies have been conducted on healthy newborns, small children, adults and immunodeficient adults (HIV positive) (Wolf et al. 1995, 1998). In all studies, *L. reuteri*

has been shown to be free from side effects, even when administered in doses largely exceeding the levels normally contained in products containing *L. reuteri* (Casas and Dobrogosz 1997); a few individuals experienced a temporary increase in flatulence.

In animal studies, a cholesterol-lowering effect and significant strengthening of the immune defence have also been documented. With animals raised for meat production, administration of *L. reuteri* to newly hatched/newborn animals led to better feed utilisation and growth than in the control groups. Sickness and mortality rates were also lower. Additionally *L. reuteri* has been shown to modulate immune responses in a series of animal models; such immuno-modulation may be one of the basic mechanisms by which *L. reuteri* exerts its probiotic effects in man (Valeur et al. 2002). In conclusion, supplementing food products such as tablets and milk products with *L. reuteri*, the only species shown to accumulate high amounts of 3-HPA in vitro, potentially offers a wide range of benefits to the consumer (Klantschitsch et al. 1996; Reid 1999). However, it is not yet known whether reuterin production occurs in vivo, and if this production is responsible for the health effects of *L. reuteri*. BioGaia is currently the main producer and sells *L. reuteri*-containing products through licensed partners worldwide (Table 2).

HPA system as the antibiotic reuterin

Biotechnologically produced HPA could be used to protect food against microbial spoilage. The anti-bacterial activity of reuterin has been shown to inhibit the growth of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in meat products, milk and cottage cheese (Table 3).

For health care or pharmaceutical applications, reuterin has also been shown to be a useful agent in the sterilisation and fixation of biological tissues. Indeed, the use of reuterin for fixation is more efficient than using glutaraldehyde, and is less cytotoxic (Table 3). Treatment of *Trypanosoma brucei* infected mice with reuterin for 7 days led to a 61% reduction in bacterial numbers and to an increased survival of the mice (Table 3). HPA can therefore be described as a potent agent that shows

Table 2 Use of *L. reuteri* as a probiotic in different countries

Country	Company	Products
Sweden	BioGaia AB	Tablets, capsules, powder and infant formula; milk, yogurt, juice, fresh cheese and ice-cream
Finland	Ingman Foods Oy Ab	Yoghurts, fermented milks, cottage cheese, ice-cream, and fruit drinks
Great Britain	Farm Produce Marketing Ltd.	Yogurt drink
Japan	Chichiyasu milk products company Ltd.	Milk products
Spain and Portugal	Kraft Jacobs Suchard Iberia	Fresh cheese
South Korea	Lotte Ham & Milk Co., Ltd.	Dairy products
Sweden	Milko	Dairy products
USA	Stonyfield Farm	Yoghurt products

Table 3 Use of reuterin as an antimicrobial and fixation agent. *MIC* Minimal inhibitory concentration, *AU* Reuterin activity unit defined as reciprocal of the highest dilution that did not permit growth of the indicator strain, *U* 1 U = 5 µg reuterin/ml

Application	Concentration used	Reference
Bacteria, yeast, fungi, protozoa	2–42 MIC	Dobrogosz et al. 1989
Bacteria		
<i>Salmonella typhimurium</i>	0.35–0.42 ^a (0.7–0.84) ^b mM	
Bacteria	50–100 U/g ground beef	Daeschel 1989
Coliforms		
Protozoa	<35 ^a (70) ^b µM	Yunmbam and Roberts 1992
<i>Trypanosoma brucei brucei</i>		
Vertebrates, mice	LD ₅₀ 1,500 U (7.5 mg)	Yunmbam and Roberts 1993
Protozoa in vertebrate		
<i>Trypanosoma brucei brucei</i> in mice	300 U/ml (1.5 mg)	
Bacteria <i>E. agglomerans</i> , <i>C. freundii</i> , <i>K. pneumonia</i>	30 mM	Barbirato et al. 1996
Bacteria	50–250 AU/g (cottage cheese)	El-Ziney and Debevere 1998;
<i>Listeria monocytogenes</i>	150 AU/ml (UHT skim milk)	El Ziney et al. 1999
<i>Escherichia coli</i> O157:H7	100–500 AU/g (meat surface)	
Bacteria	0–4 AU/ml	Rasch 2002
<i>E. coli</i>		
Bacteria	20–35 ppm	Chen et al. 2002;
<i>E. coli</i> ; <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i>		Sung et al. 2002, 2003
Fixation	0.068 M	

^aCalculation based on HPA dimer

^bCalculation based on HPA monomer

potential as a food preservative while also offering pharmaceutical benefits.

For the use of HPA, or reuterin, in food preparations *L. reuteri*, a probiotic strain, could be used to produce HPA in glycerol-containing water (Vollenweider et al. 2003). HPA concentrations reached by this method (170 mM) are sufficient to achieve an inhibitory effect, and the supernatant can be used directly without further purification after production when food-grade culture medium is used (El-Ziney and Debevere 1998; El Ziney et al. 1999). For pharmaceutical application, a purified preparation of HPA would be needed (Chen et al. 2002). The purified extract should contain only solvents, which could be totally eliminated after purification (Vollenweider et al. 2003).

Mode of action and biological safety

Given the complexity of the HPA/reuterin system (Fig. 1) the question of which molecule (3-HPA, HPA-dimer or HPA-hydrate) is the active substance conferring antimicrobial activity arises. Dobrogosz (Dobrogosz and Lindgren 1988) made a first attempt to identify the active compound and proposed a carbohydrate-like structure for reuterin. A three-dimensional molecular model of the hemiacetal revealed a molecule that closely resembled a pentose such as D-ribose. It was then postulated that reuterin was able to compete with ribonucleotides for binding to the ribose recognition site of ribonucleotide reductase, the first enzyme involved in DNA synthesis. Thus, reuterin might inhibit the conversion of ribonucleotides into deoxyribonucleotides and hence exert its anti-

microbial effect (Dobrogosz and Lindgren 1988; Talarico and Dobrogosz 1989). However, the observed inhibition of ribonucleotide reductase and thioredoxin by HPA might also be explained by another hypothesis involving the functional groups of the aldol 3-HPA. The hydroxyl and the aldehyde group might be responsible for the relative reactivity of the HPA monomer, which might cause it to react with unstable sulfhydryl groups of ribonucleotide reductase and thioredoxin (Schauenstein et al. 1977). Using quantitative ¹³NMR Vollenweider et al. (2003) recently found that the composition of the HPA system was concentration dependent (Fig. 2).

At high concentrations (4.9 M), the HPA system was predominantly composed of HPA-dimer along with unspecified HPA derivatives. At intermediate concentrations (1.2 M) equal amounts of HPA-dimer and HPA-

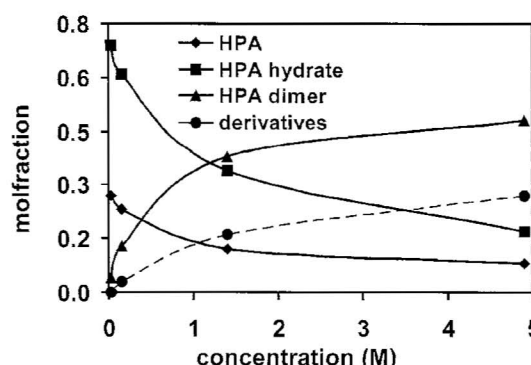


Fig. 2 Concentration-dependent distribution of the three forms of the HPA system in aqueous solution at 20°C measured by quantitative ¹³C NMR (Vollenweider et al. 2003)

hydrate were observed. At further dilution the fraction of dimer decreased rapidly and was replaced mainly by HPA-hydrate. Equal amounts of HPA-dimer and 3-HPA were present at a concentration of 0.4 M. At the very low concentration of 0.03 M, the system was composed mainly of HPA-hydrate (69%), followed by 3-HPA (27%) and HPA-dimer (4%), whereas no derivatives could be detected. Further extrapolation of the curve suggests that the HPA system is likely to have a similar composition at concentrations below 0.03 M.

In some studies where HPA was used as an anti-microbial agent, reported molar concentrations were calculated using the molecular mass of the dimer form (Table 3). However, Fig. 2 clearly shows that HPA occurs mainly in its monomeric form at the concentrations used. This implies that the concentrations used in these studies were actually twice as high as those reported (Table 3).

The composition of HPA has also been reported to be pH-dependent, forming mainly aldol dimer and polymers, or hemiacetal dimer, acetal trimer and polymers (Chen et al. 2002; Sung et al. 2003). All these molecules have aldehyde groups and have been reported to react with the amino groups in biological tissues (Sung et al. 2003). In contrast, in organic solutions the dimeric and polymeric forms seem to be predominant (Talarico and Dobrogosz 1989; Vollenweider et al. 2003). Hence, the composition of the HPA system is highly dynamic, which greatly affects its biological activity.

Detailed studies are needed to elucidate the mode of action of reuterin and the active component *in vivo*. A first step would be to accurately determine the concentration of HPA in aqueous solutions. Until now, no standardised unit is used to express the concentration of HPA (Table 3). The method for acrolein determination, using the acid-catalysed formation of a coloured complex between acrolein/HPA and tryptophan (Circle et al. 1945) has been adopted by several authors to accurately determine the molar concentration of HPA (Barbirato et al. 1996; Lüthi-Peng et al. 2002b; Sauvageot et al. 2000; Slininger et al. 1983; Smiley and Sobolov 1962; Vollenweider et al. 2003). Indeed, the use of molar concentrations would enable researchers to compare the anti-microbial activity of HPA in different biological systems as well as compare its activity to that of other molecules.

Another important consideration for biotechnological applications of HPA in foods is the accumulation of acrolein due to thermal dehydration (Nef 1904; Schütz and Radler 1984; Voisenet 1914a). Acrolein reacts with phenolic compounds, especially tannins present in alcoholic beverages, resulting in a bitter taste (Mills et al. 1954; Rentschler and Tanner 1951; Serjak et al. 1954). This spoilage of alcoholic beverages has been known since the eighteenth century, yet it remains a problem today and is an important topic of research (Claisse and Lonvaud-Funel 2001; Noble 1999; Sauvageot et al. 2000; Sponholz 1993). Acrolein is an α,β -unsaturated aldehyde, which reacts with other molecules especially those containing sulfhydryl groups (e.g. proteins containing cysteine), causing the depletion of glutathione—one of the most

important antioxidants—and/or general enzyme inhibition. Most human contact with acrolein occurs during exposure from incomplete combustion, such as that generated by cigarettes, automobiles, industrial processes, heating of animal and vegetable cooking oils, and burning (Ghilarducci and Tjeerdema 1995). Mucosal irritation has been observed at 1–2 mg/m³. Death in humans has occurred at 351 mg/m³. Fortunately, the odour perception of acrolein is 0.07 mg/m³ (Ghilarducci and Tjeerdema 1995).

HPA is stable at 4°C, hence its use in foods such as milk products, which are stored at or below 4°C and are not heated, may be considered safe (El-Ziney and Debevere 1998; Vollenweider et al. 2003). However, at higher temperatures HPA becomes unstable and may react with other components present (Lüthi-Peng et al. 2002b; Sung et al. 2003). Therefore in heated food preparations containing HPA, the formation of acrolein is a risk requiring careful consideration. In-depth studies need to be carried out to demonstrate the safety of HPA in different foods (Ghilarducci and Tjeerdema 1995; Kehrer and Biswal 2000; Lüthi-Peng et al. 2002b; Sung et al. 2003).

HPA as a biotechnological precursor in the production of bulk chemicals

The value of HPA as a precursor in the production of useful industrial chemicals, for example plastics, was mentioned as early as 1950 (Hall and Stern 1950), and later for the production of acrylic acid. Slininger and colleagues (Slininger and Bothast 1985; Slininger et al. 1983) described the first attempt to produce HPA from renewable resources using modern biotechnology as an alternative for the production of major bulk chemicals.

Acrolein

Acrolein is produced chemically from propylene by direct catalytic oxidation, but the highest reported yield in the oxidation process is only 8% (Hess et al. 1978). The main uses of acrolein are as an intermediate in the synthesis of numerous chemicals, in particular acrylic acid and its lower alkyl esters, and DL-methionine, an essential amino acid. The most significant direct application of acrolein is as a herbicide to control the growth of aquatic plants. It kills plant cells by its reactivity with biological molecules and the destruction of cell membrane integrity (Ashton and Crafts 1981), as well as by its affinity for sulfhydryl groups, which causes the elimination of vital enzymatic pathways (WSSA 1983).

Acrolein is very polar and highly soluble in water. An aqueous acrolein solution has been reported to consist predominantly of HPA-hydrate, with about 92% under laboratory conditions, or even higher in river water (Bowmer and Higgins 1976; Bowmer and Sainty 1977; Ghilarducci and Tjeerdema 1995; O'Loughlin and Bowmer 1975). Other biological degradation products include 3-hydroxypropionic acid, allyl alcohol, acrylic acid,

propionic acid, HCO_3^- , glyceric acid, 1,3-PDO, oxalic acid, propanol and butyric acid (Mao et al. 1994). All of these products are polar and highly soluble in water. Bioassays have shown that aqueous acrolein solutions become biocidally inactive after 120–180 h, probably as a result of volatilisation of the compounds from water (Kissel et al. 1978; WHO 1992).

The formation of HPA from acrolein-containing solutions is reversible, as was mentioned previously for alcoholic beverages. Acrolein is accumulated by thermal dehydration during glycerol fermentation after the nearly complete disappearance of fermentable carbohydrates (El-Ziney et al. 1998; Lüthi-Peng et al. 2002a; Mills et al. 1954; Sauvageot et al. 2000; Sobolov and Smiley 1960). Probiotic strains such as *L. reuteri* produce 3-HPA in the bacterial supernatant in concentrations up to 170 mM (Table 1). For use as a herbicide, concentrations of 6–10 mg/l (0.11–0.17 mM) acrolein are required. Thus, biotechnological fermentation of glycerol into 3-HPA followed by heating of the bacterial supernatant, thus producing acrolein, could provide an inexpensive alternative, requiring no further purification, to chemically-produced, high quality acrolein.

Acrylic acid

Acrylic acid is a petrochemical that is modified and polymerised into a broad range of versatile chemicals and resins for the production of viscosity modifiers and flocculants. Roughly three-quarters of its world production is converted directly into acrylate esters, the remainder being purified into glacial acrylic acid (Burridge 2002). The demand for acrylic acid has been rapidly increasing from a mere 400,000 t in 1980, to 3.4 million t in 2002. This increase is mainly due to the increasing use of super absorber polymers in the hygiene industry. World capacity is predicted to rise to 4 million t/year by 2009 (Burridge 2002).

Acrylic acid is made via acrolein by gas-phase catalytic oxidation of propylene; it is a hydrocarbon gas, and a by-product of oil refineries. The process is not very selective (Burridge 2002; Danner and Braun 1999). Several attempts have been made using different bacterial species to produce acrylic acid biotechnologically (Danner and Braun 1999; Danner et al. 1998). So far, acrylic acid has been produced by bacteria from acrylonitrile (Nagasawa et al. 1990; Narayanasamy et al. 1990) and from acrylamide (Nawaz et al. 1993, 1994), the highest yield being obtained from acrylonitrile (390 g/l). Both the aforementioned substrates are secondary products that must be produced from other raw materials. Therefore, as suggested by several researchers, oxidation of HPA to acrylic acid may present a new alternative with great potential for the production of acrylic acid (Lüthi-Peng et al. 2002b; Slininger et al. 1983; Ulmer et al. 2002). However, no method has been described to date.

1,3-PDO and hydroxypropionic acid

1,3-PDO is used as a bulk chemical for polymer production, especially in the manufacture of the aromatic polyester PTT (polytrimethylene terephthalate) sold by Shell and DuPont under the trademarks CORTERRA and Sorona, respectively. This polymer exhibits properties desirable in carpets and textiles. Compared with other synthetic fibers like nylon, PTT fibers feel softer, dye more easily, retain vibrant colours longer, stretch and recover better, clean easily and dry quickly (http://www.eurekalert.org/pub_releasees/2003-02/d-cfc012903.php). The demand for PTT is therefore expected to exceed 1 million metric tonnes by the year 2010 (<http://search.shellchemicals.com>). Currently 1,3-PDO is made using two different petroleum-based processes. Both methods use 3-HPA as an intermediate, which is hydrogenated with metal catalysts under high pressure (see section on petrochemically derived 3-HPA).

1,3-PDO is one of the oldest known fermentation products. It was first identified in 1881 by August Freund in a glycerol-fermenting mixed culture containing *Clostridium pasteurianum* (Freund 1881). Although the great potential of 1,3-PDO has long known been recognised, the expensive chemical synthesis has prevented its commercial production (Morris and Snider 1950). Over the past few years, extensive work has been done to increase the productivity and yield of biotechnological processes able to generate 1,3-PDO (Cameron et al. 1998). The biological system developed by DuPont and Genencor, based on transformed *E. coli*, produces 129 g/l 1,3-PDO after 74 h, using sugar from corn and other crops (Emptage et al. 2003). However, the yield of 34% (g 1,3-PDO/g glucose) is quite low. 1,3-PDO can also be obtained by the fermentation of glycerol, with a theoretical yield of 67%, since additional products need to be formed to generate sufficient NADH^+ for the reduction of 3-HPA to 1,3-PDO by enzymes (Wittlich 2001). Depending on the price of glycerol, the latter process could still be favoured over the former.

The biotechnological production of 3-HPA followed by its chemical reduction to 1,3-PDO offers great potential (Ulmer et al. 2002; Valerius et al. 1996). As stated above, *L. reuteri* can accumulate up to 12.5 g/l (170 mM) 3-HPA with a yield of 0.68 g HPA/g glycerol, under unoptimised conditions (Lüthi-Peng et al. 2002b; Vollenweider et al. 2003). The theoretical yield would be 0.8 g 3-HPA per gram glycerol (Slininger et al. 1983). In addition, the purity of 3-HPA produced in water containing glycerol reaches 87%. The 3-HPA thus generated could be easily hydrogenated using existing chemical methods, with yields of up to 99% (Brossmer and Arntz 2000; Etzkorn et al. 2000). Alternatively, in the presence of semicarbazide, *Klebsiella* was shown to accumulate up to 46 g/l (621 mM) 3-HPA from glycerol (Vancauwenberge et al. 1990). Although perhaps offering great potential for the future, so far no method has been reported for the recovery of HPA from its semicarbazide form.

3-Hydroxypropionic acid (3-HP), a molecule that has recently attracted considerable attention, can be chemically produced from sodium hydroxide and ethylene cyanohydrine (Read 2002). 3-HP will be used in the manufacture of coatings, adhesives, water-treatment chemicals and personal-care products. Cargill (<http://www.cargill.com>) and the United States Department of Energy will invest over US \$6 million to produce 3-HP biotechnologically (CEP 2003; Gokarn et al. 2002). Additionally, 3-HP is used as a substrate by specialised bacteria and transgenic plants to produce polyhydroxyalkanoates, a raw material for biodegradable plastics (Mittendorf et al. 1998). 3-HP has been described by several authors to be produced from 3-HPA during glycerol fermentation leading to equal molar concentrations of 1,3-PDO and 3-HP (Slininger et al. 1983; Sobolov and Smiley 1960; Talarico et al. 1988).

Polymers

The last two decades have shown a steady increase in polymer research. In 1980, less than 50 new patents concerned polymers, while in 2001 over 1,100 patents on polymers were published. Of these patents, only 25–30% dealt with petrochemical polymers; the vast majority concerned biopolymers, both starch polymers and natural polyesters. Today about 200 million tonnes are used compared with about 60 million tonnes in 1980. This demand is predicted to increase to about 260 million tonnes by 2010 (Lörcks 2000). As already discussed, polymers can be generated from HPA via 1,3-PDO; however, HPA can also transform itself into polymeric derivatives and resins (Fig. 3) (Hall and Stern 1950; Nielsen et al. 1981; Sung et al. 2003).

To date, no study has addressed the issues of controlled production or the various uses of polymeric derivatives. In theory, careful manipulation of experimental conditions such as concentration, solvent, pH, and temperature may provide an important tool to control the synthesis of specific polymers.

Economical considerations

The petrochemical industry is still one of the world's most important industries, with a steadily rising demand for its products. Current estimates predict a 5-fold increase in demand for chemicals and consumables by 2020, and another 5-fold increase between then and the year 2050 (Inverizon International 1998). The availability of petrochemicals is largely determined by political forces, and the source of raw material is limited. Consequently, the price of petroleum has steadily increased since 1970 from about US \$3/barrel to US \$30/barrel today.

Renewable resources from plants, as well as agricultural wastes transformed by bacteria, can provide many of the same chemical building blocks as the petrochemical industry, as well as some new ones. The costs of plant-derived materials are largely dependent on weather conditions. Of great importance is the ecological impact. Plant-derived materials may lead to increased monoculture in agriculture and increased plant production may only be possible with genetically modified plants. However, it is expected that the use of fossil fuels will stabilise at approximately current levels and that the increasing demand for chemicals will be met through bio-transformation and biotechnological engineering using renewable resources (de Boer and Schmidt-Dannert 2003; Inverizon International 1998). Already, the increasing number of

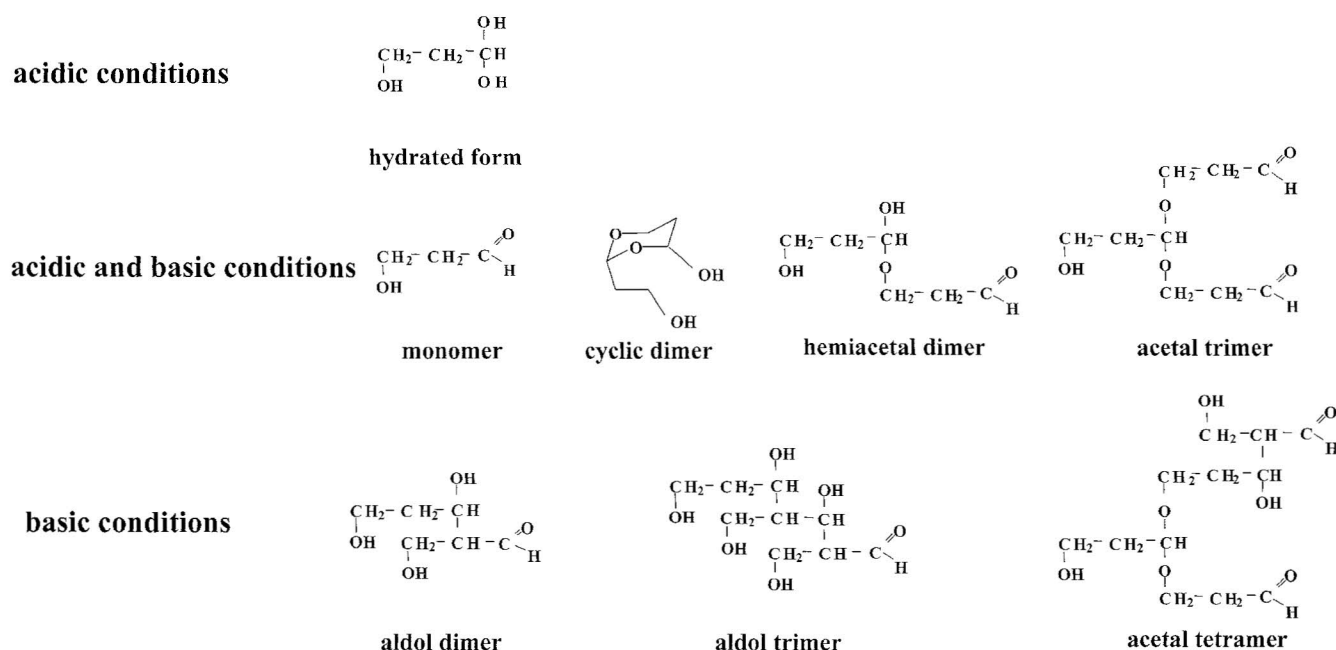


Fig. 3 Different forms of 3-HPA in acidic and/or basic aqueous solutions; adapted from Sung et al. (2003)

patents in this area is indicative of the increasing trend in this industry.

As presented above, 3-HPA can be produced by bacteria by the fermentation of glycerol. Theoretically, the development of strains using fermentable carbohydrates such as glucose as substrate, which cost 3–5 times less than glycerol, is desirable. The example of 1,3-PDO shows the feasibility of this approach. However, since glucose can also be transformed into a wide range of products other than those of interest, the resulting yield and purity of the final product is low. The theoretical yield of the one-step enzymatic transformation process from glycerol to 3-HPA is 1 mol HPA/mol glycerol. Production can be accomplished in water, into which 3-HPA is secreted. This results in increased purity and yield. Bacterial strains using a non-cobamide-dependent glycerol dehydratase may be a valuable tool for decreasing the cost of 3-HPA production (Zeng and Biebl 2002).

Glycerol is formed as a side-product during the production of soap, fatty acids, and biodiesel. Experts predict a tremendous increase in the annual production of biodiesel from 2.7 million tonnes today to about 7–9 million tonnes by 2010 (Widmer and Müller 2003). Roughly 10% of this production will be in the form of glycerol. The expansion of the biodiesel market will therefore lead to an increased glycerol production, concomitant with a decrease in price (Fig. 4).

An additional source for glycerol may be the increased production during ethanol fermentation without changing the ethanol yield. As cited by Zeng and Biebl (2002) High Plains Corporation (York, Neb.) plans to recover glycerol (99.7%) from ethanol fermentation broth by a novel filtration process at costs below US \$300–400/t, which is well below the cost of glycerol on the market (Fig. 4). Production of 600,000 t/year would be possible, which would have a significant economic impact on the glycerol market and price.

The use of 3-HPA as a preservation agent in food or as a disinfectant in the pharmaceutical field is not much affected by these economic considerations. However, as a potential source of bulk chemicals, the price of

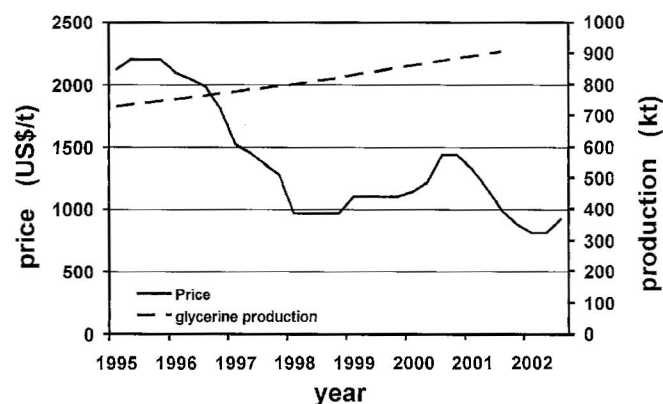


Fig. 4 Development of glycerol price and production since 1995. The price is for refined glycerol. (Procter & Gamble Chemicals, 2003 personal communication; HB International, 2003 personal communication)

Table 4 Current price of chemicals. PTT Polytrimethylene terephthalate

Chemical	Price US \$/metric ton (2003)
Acrolein	1.400 ^a
Acrylic acid	1.345–1.500 ^b
1,3-Propanediol	n.i.a. ^c
PTT	1.000 ^d to <1.500 ^e

^aMarket research, ^bCelanese AG, ^cNo information available, ^dZeng and Biebl (2002), ^eSchoenberger (2003)

biotechnologically produced 3-HPA and thus of glycerol will be of major importance to the viability of this industry, as it has to compete with the chemical industry (Table 4).

HPA biotechnology—conclusions and future trends

The HPA system is composed of different derivatives that create the basis for its outstanding chemical properties. Biotechnologically produced HPA may find applications in health care, in the food industry, and in the chemical industry (Fig. 5).

Due to its broad antimicrobial activity, the 3-HPA-producing bacterium *L. reuteri* is used as a probiotic strain in human and animal diets to positively influence the microbial balance in the intestine. *L. reuteri* was shown to produce two other molecules with antimicrobial activity: reutericyclin (Gänzle and Vogel 2003; Holtzel et al. 2000) and reutericin (Kabuki et al. 1997; Tobal et al. 1991). However, the production of both of these molecules in vivo has not yet been tested. The interaction of HPA with reutericyclin and reutericin warrants further research because these molecules may play a synergistic role in the protection of *L. reuteri*, and therefore of the host, against pathogenic bacteria.

The “antibiotic” properties of reuterin are known to inhibit the growth of several human pathogens, including protozoa, viruses, and bacteria (Dobrogosz and Lindgren 1988), which make it attractive for use as a food

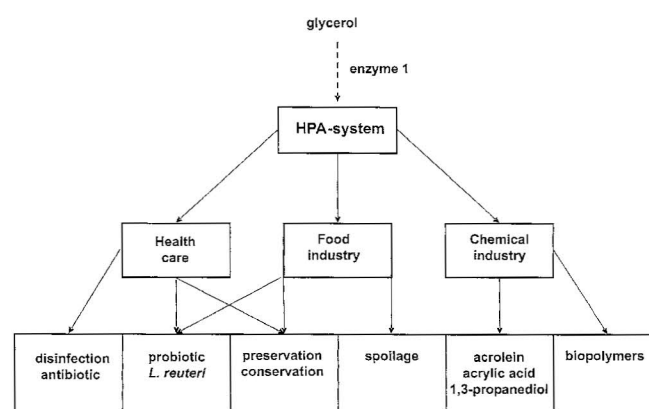


Fig. 5 Potential applications of biotechnologically produced HPA in industry

preservation and conservation agent (Fig. 5). HPA is produced by a probiotic strain and is excreted into the medium. In the future, productivity may be improved by optimising the fermentation strategy, culture conditions, and purification strategy, as well as by genetic engineering. An HPA-enriched medium could be used directly for applications in foods (El-Ziney and Debevere 1998; El Ziney et al. 1999); alternatively HPA may also be purified from bacterial supernatants by existing methods if necessary (Vollenweider et al. 2003).

In combination with other antimicrobial molecules, such as bacteriocins, HPA may also reveal synergistic effects that could help to overcome the increasing resistance of bacteria against antibiotics. HPA was also reported to be a useful chemical in the fixation of biological tissues, with equal fixation possibilities but less cytotoxic effects than glutaraldehyde, a chemical frequently used for this purpose (Sung et al. 2002, 2003). The proven low cytotoxic effect of HPA makes it an ideal molecule for future use in biological systems. However, HPA has been shown to react with other molecules in complex media, particularly when heated (Lüthi-Peng et al. 2002b; Sung et al. 2002, 2003). Under certain conditions, acrolein, a very toxic aldehyde, may be formed, leading to food spoilage (Lüthi-Peng et al. 2002b; Mills et al. 1954; Reymolds et al. 1939; Sauvageot et al. 2000; Serjak et al. 1954). Time-temperature combinations, physicochemical characteristics and the chemical composition of foods may affect these reactions, but very little information is available on this important issue. Therefore heat-treated foods containing HPA should be carefully surveyed, and detailed studies are needed to elucidate the mode of action of HPA in biological systems. Finally, HPA can be used as a precursor in enzymatic and chemical reactions for the preparation of bulk chemicals such as acrolein, acrylic acid, 1,3-PDO and in the formation of biopolymers. However, large-scale synthesis of these HPA-derived compounds will also require optimisation of biotechnological production and purification in order to produce HPA with high yields and low costs.

We may thus anticipate that, after the optimisation of its biotechnological production, HPA will probably achieve a biotechnological importance similar to that of 1,3-PDO.

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