Notice of Retraction

Santosh Kumar N[a]

[a]Editor in-chief

Retracted on 22nd April 2011

During the processing of this article, the corresponding author had sent a signed statement of authorship responsibility stating that the manuscript had not been published and was not under consideration for publication elsewhere and he also signed a document that transferred all copyright ownership to our journal. As per the corresponding author communication and statement the article was published in our journal.

Later on, It has come to our attention that the article published in our journal entitled, "THE STUDY OF RELEVANCE OF MICROBIAL COCULTURE FERMENTATIONS IN BIOTECHNOLOGY," by Vaibhav Changediya (Volume 5/ issue 3, article 002: 5–13), published in the December 2010, is nearly identical to an article published in the *Journal of Applied Microbiology,* with the title "Relevance of microbial coculture fermentations in biotechnology" by J. Bader, E. Mast-Gerlach, M.K. Popovic, R. Bajpai and U. Stahl (109-2010, 371–387, doi:10.1111/j.1365-2672.2009.04659.x).

For this reason *The International Journal of Pharmaceutical Sciences Review and Research* has notified the authors that their article will be retracted. We regret any problems the duplicate publication may have caused.

For any further information please contact at editor@globalresearchonline.net.

Review Article

THE STUDY OF RELEVANCE OF MICROBIAL COCULTURE FERMENTATIONS IN BIOTECHNOLOGY

Vaibhav Changediya*
Yash Institute of Pharmacy, Aurangabad, India.
***Corresponding author's E-mail:** v.changediya@rediffmail.com

Received on: 15-10-2010; **Finalized on:** 15-12-2010.

ABSTRACT

The purpose of this article is to review coculture fermentations in industrial biotechnology. Examples for the advantageous utilization of cocultures instead of single cultivations include the production of bulk chemicals, enzymes, food additives, antimicrobial substances and microbial fuel cells. Coculture fermentations may result in increased yield, improved control of product qualities and the possibility of utilizing cheaper substrates. Cocultivation of different microorganisms may also help to identify and develop new biotechnological substances. The relevance of coculture fermentations and the potential of improving existing processes as well as the production of new chemical compounds in industrial biotechnology are pointed out here by examples.

Keywords: Coculture, fermentation, biotechnology.

INTRODUCTION

Chemical substances worth several billion Euros are produced each year by biotechnological processes as fuels, bulk and fine chemicals and pharmaceuticals using renewable resources.[1] Because sterile cultivation enables an easy way of controlling microbial milieu, growth and product formation, most of the products in industrial biotechnology today are formed using processes involving a single microbial strain. On the other hand, there are many instances where the utilization of cocultures appears to be advantageous over a single micro-organism because of the potential for synergistic utilization of the metabolic pathways of all involved strains in a coculture situation. Most biotransformations in nature take place by the combination of metabolic pathways from different micro-organisms.[2,3] Some examples for the coexistence of different micro-organisms are the forest soils, compost piles, the aerobic and the anaerobic zones of water, spontaneous fermentations of sugar-containing saps and the human skin. Mammalian intestine with involvement of up to 500 strains is another example for a very complex, natural mixed microbial system; the interactions between supply of substrates and the utilization of metabolites have formed the basis of analysing behaviour of the human gut.[4]

Definitions used in this text:-

Coculture:-

Anaerobic or aerobic incubation of different specified microbial strains under aseptic conditions.

Mixed culture:-

Anaerobic or aerobic incubation of different sometimes unspecified micro-organisms; may be conducted under septic conditions.

In cocultures, degradation and metabolization of substrates occur by the combined metabolic activity of the known microbial strains under aseptic conditions. Mixed cultivations are often found in nature under septic conditions with unspecified microbial strains. In a habitat, different micro-organisms may compete for substrates as well as act symbiotically. Micro-organisms have evolved mechanisms to protect their substrates and to defend their habitat against competitors. *Xanthomonas campestris* synthesizes the carbohydrate polymer xanthan as a storage substance that is degraded by only a few other micro-organisms. Acidogenic bacteria produce organic acids that suppress acid-intolerant organisms by reducing medium pH as well as by causing growth inhibition in micro-organisms.[5-7] Some strains of the genus Lactobacillus defend their habitat against other Gram-positive bacteria by the secretion of growth-inhibiting substances such as nisin or lactain F. In other cases, there may be a symbiosis among different micro-organisms caused by synergies of their different enzymatic systems and metabolic pathways. Lichens, including more than 1500 species consisting of cyanobacteria and yeasts, are an example of symbiotic relationship between different micro-organisms.[8-11] This symbiosis has lasted for over 600 million years. This long survival can be viewed as evidence of the great benefit for partners in this symbiosis. The natural cooperation of different micro organisms is utilized in only a few applications in industrial biotechnology.. Examples of the utilization of cocultures in food industry are the production of cheese, yoghurt, sauerkraut, sourdough, kefir, African fermented dairy products, salami, whisky, cacao beans and Belgian beer such as Lambic. A mixed culture of different yeasts and several bacteria is also important in wine production wherein the involved micro-organisms grow during fermentation in a special succession influencing the aroma and flavour profile of the wine.[12,13] Modification of raw materials during food production by cocultures results in improved texture, taste and flavour, and in microbial stabilization. This

protection may be caused by a decreased pH-value or by the formation of growth-inhibiting substances such as lactic acid, acetic acid or ethanol. Further stabilization may be achieved by the reduction of available carbohydrates as well as by the secretion of bacteriostatic or bactericidal substances such as nisin. Growth and product formation are not effected through external regulation but by modification of internal conditions such as oxygen availability, pH and substrate and product concentrations during the given examples of fermentation processes. A further advantage of cultivation of cocultures is the possibility of utilizing secondary products (e.g. whey, molasses) cheaper than glucose as substrates for biotechnological production of chemicals[14,15] Using substrates other than glucose offers potential to develop biological production processes at competitive costs. Furthermore, cocultivation processes can help find new substances of industrial interest, because a number of secondary metabolites are produced during cocultivation. Besides having the industrial importance, coculture systems have medical implications as well.[16]

Interactions between micro-organisms in coculture systems:-

Cells present in a medium communicate with each other either by direct cell-to-cell-interactions or through the signal substances in the fermentation broth[17] An example of the chemical trigger substances is the acetylated homoserine lactone (AcylHSL). Production of bioluminescence protein, Lux 1, triggered by intracellular binding of AcylHSL to LuxR-proteins that are homologous to the transcription factor.Low concentrations of AcylHSL do not trigger the bioluminescence response that occurs only when a significant number of cells are present in a colony to cumulatively produce AcylHSL to concentration levels enough to cause expression of Lux-1 protein. An elevated concentration of Lux-1 protein in the cell leads to an increased production of AcylHSL, causing a positive feedback regulation. The communication by secreted chemical substances such as AcylHSL is an example of quorum sensing (the process in which single-cell organisms, usually bacteria, determine population density by detecting the concentration of small, diffusible signal molecules). Induction of responsible genes may occur dependent on the concentration of micro-organisms with respect to that of the signal substances.[18,19]

Serratia plymuthica, controlled by quorum sensing. Intracell communication by AcylHSLs takes place even between microbes of different species and genera. reported communication between the eukaryotic algae Enteromorpha zoospores and the prokaryotic bacteria *Vibrio anguillarum*. *Aspergillus giganteus* produces increased amounts of the antifungal protein (AFP) in cocultivation with *Fusarium oxysporum*. Further examples for reactions controlled by quorum sensing include production of antibiotics and the development of virulence factors. In contrast to a pure culture, interactions between the different micro-organisms play a critical role in a coculture.[20-22] Growth of cells of one strain may be enhanced or inhibited by the activities of other micro-organisms present in the medium. The same is also true for the formation of primary and secondary metabolites and when triggered by the presence of cocultivating cells, it may be a unique characteristic of the cocultivation processes. Activation of microbial promoters that could not be observed in pure cultures indicates the potential of production of new substances, possibly of industrial interest, in cocultures. Although examples dealing with negative control of growth of cells in a mixed culture by the production of inhibitory primary and secondary metabolites abound, an interactive promotion of growth also occurs in many instances. [23]

Potential utilization of coculture fermentations in industrial biotechnology:-

Biological production of fine chemicals for the chemical industry using renewable resources has increasing relevance. Energy consumption and the use of environmentally hazardous substances can often be reduced by biotechnological production processes. Further advantages may be the production of pure enantiomers, reduced steps required in synthesis of products, and less stringent security needs resulting in reduced production costs.[24] The risk of accidents decreases as a result of lower process temperatures and normally low pressures in biotechnological processes in contrast to many chemical processes. Moderate process conditions result in lower required charge in the field of process security and approval procedures.[25]

Bulk chemicals, Fine chemicals and Biofuels:-

Ethanol:-

The production of ethanol by fermentation of starches and cellulosic materials is gaining increasing interest because of the increasing economy of bioethanol production caused by the high oil price[26] The utilization of inulin from artischoke as a substrate for ethanol production by a coculture of *Z. mobilis* and *Kluyveromyces fragilis*. They achieved a conversion of 94% of the theoretical maximum. In case of sorghum as a substrate for the ethanol production, suggested a coculture fermentation process with *Saccharomyces cerevisiae* and *Fusarium oxysporum*. Hydrolysis of cellulose and fermentation of the released sugars occurs simultaneously in this example. Another combination of a mould and a yeast for the production of ethanol. *Kluyveromyces marxianus* and *Talaromyces emersonii* were cocultivated at a temperature of 45_C in this example. Utilization of cellulosic materials for the production of ethanol is hampered by lack of adequate progress in producing monosaccharides from cellulose and in efficient utilization of all the sugars formed.[27,28] Because products of hydrolysis often cause feedback inhibition of enzymes used for hydrolysis, simultaneous hydrolysis and fermentation has been proposed. An advantage of simultaneous hydrolysis and fermentation of cellulose lies in avoiding the accumulation of glucose

and disaccharides. Hence, no product inhibition of the cellulolytic enzymes occurs. Up to now, no single micro-organism (wild type or recombinant) with a high cellulolytic activity and a simultaneous high yield and production rate of ethanol is known. Considerable improvement in this area has been observed using cocultivation of different micro-organisms for ethanol production from cellulose. They explained this improved enzyme production by the consumption of all available oxygen and metabolic degradation of inhibiting substances by aerobic organisms, creating better conditions for *Clostridium straminisolvens*. Process development for coculture fermentation is crucial for successful application.[29,30]

Hydrogen:-

Hydrogen has lately been in the news as a source of clean energy. It is especially interesting because of its use in fuel cells in space applications where production of any extra waste is highly undesirable. Hydrogen can be produced from renewable resources using obligate anaerobes and fermentative microbes such as Clostridium, Enterobacter and Escherichia. These organisms produce hydrogen from carbon sources such as glucose in pure culture rapidly but with low yields. Requirements of strict anaerobic conditions for the cultivation of obligate anaerobe. Clostridium have been avoided by coculturing it with facultative Enterobacter that acts as scavengers of oxygen in the medium have reported using a coculture of *Clostridium butyricum* with a photosynthetic bacterium and achieved a net production of 7 moles of hydrogen per mole of glucose achieved similar molar yields of hydrogen from glucose using a coculture of *Lactobacillus delbrueckii* with a photosynthetic microbe, *Rhodobacter spheroids*. Here, Lactobacillus forms lactic acid, which is rapidly converted into hydrogen by the photosynthetic microbe. Another attempt to produce hydrogen was made by using molasses as substrate. A 12– 220% increase in hydrogen formation is described by the utilization of a coculture of *Clostridium pasteurianum* F40 or *Clostridium tyrobutyricum* F4 with *Clostridium sporosphaeroides* in comparison with single cultivation. An explanation for this improved hydrogen production might be the utilization of glutamate for the hydrogen production by *C. sporosphaeroides*. Another cheap substrate for hydrogen production is sugarcane distillery effluent. used a coculture of *Citrobacter freundii*, *Enterobacter aerogenes* and *Rhodopseudomonas palustris* in a pilot plant with a volume of 100 m3.[31-33]

Acetic acid:-

The worldwide annual production of biologically produced acetic acid is more than 190 000 tons. A part of this organic acid is obtained biotechnologically using strains of Acetobacter and Gluconobacter for the oxidation of ethanol in submerged fermentations. Most of the acetic acid thus produced is utilized in food industry, but some of the acid is used also in the chemical and pharmaceutical industry and in the production of environmentally friendly road salt in the form of Ca and Mg salts. In modern, industrially applied production processes, acetic acid concentrations up to 20% and space time yields up to 100 g / (l h) can be achieved. Yields of 94% are obtained in industry reported the utilization of a coculture of *Zymomonas mobilis* and Acetobacter sp. For the production of acetic acid from glucose with a yield of 95Æ5% of theoretical maximum. Glucose is fermented to ethanol that is oxidized to acetic acid nearly simultaneously by Acetobacter sp in the same bioreactor. This alternative fermentation process reaches comparable yields with industrially applied processes offering the advantage of ethanol formation and acetic acid production simultaneously in one single bioreactor achieved a comparable yield of 96% of theoretical maximum using a coculture of *Clostridium thermolacticum* and *Moorella thermoautotrophica* for the production of acetic acid using lactose as substrate.[34,35]

Lactic acid:-

Lactic acid can be used for the synthesis of polylactate (PLA) besides its utilization in the food, pharmaceutical and textile industry. PLA has a wide range of applications. In the form of foils, it can be applied for the production of packages; as a fibre, it is used in the production of clothing's . It can also be applied in medicine for nontoxic implants and injectable carriers of tissue cultures. Dow Chemical Inc. and Cargill Inc. have already built a plant with a capacity of 140 000 tons PLA per year. Lactic acid is produced in fermentation processes using glucose or glucose-producing polymers as substrate. In case of substrates containing cellulose, the addition of cellulases and amylases has been found to be beneficial. Simultaneous saccharification and fermentation (SSF) utilizing microbial cocultures saves the addition of these enzymes. Furthermore, substrates in high concentrations can be utilized decreasing production volume and processing costs. Cheaper substrates may be used in lactic acid production when coculture fermentations are utilized as described previously. Utilization of cheaper substrates is critical because carbon source is often the greatest contributor to the cost of microbial products and the price of PLA has to compete with plastics based on mineral oil. Cocultivation processes offer the utilization of lignocellulose hydrolysates in the production of lactic acid.[36-40]

Biopolymers:-

Polyhydroxyalkanoates (PHAs) are of great interest among the biopolymers. This group consists of over 125 different kinds of polymers, some of which are already produced industrially. The price of microbial PHA is strongly dependent on the price of substrates. To reduce this cost, suggest the utilization of propionate-containing wastewaters as a substrate for mixed culture fermentations to produce PHAs. The application of mixed culture fermentation processes, utilizing agricultural and / or industrial wastes, could be very promising in increasing

financial attractiveness of PHA production. Lactic acid bacteria can be used not only for the production of lactic acid as substrate for PLA, but also for the production of other biopolymers. *Lactobacillus kefiranofaciens* is able to form the polydextrin, kefiran. This polymer consists of equal amounts of glucose and galactose and has an average molecular mass of 7.6 105 g mol)1. In animal tests, the intake of kefiran resulted in strongly decreased hypertension and blood fat. Hence, the authors suggest the application of kefiran in functional foods.[41] The yield of kefiran can be increased by cocultivation of lactic acid bacteria and the yeast *Saccharomyces cerevisiae* produced kefiran by a cocultivation of *Lactobacillus delbrueckii* ssp. bulgaricus and *S. cerevisiae*. In contrast to the pure cultures, the yields could be increased by 70%. A part of this strong increase may have resulted from the consumption of the produced growth-inhibiting lactic acid by the yeast. Biotechnological production of another important biopolymer, the cellulose, by a coculture fermentation process consisting of *Gluconacetobacter xylinus* and *Lactobacillus mali*. The ecological advantage of fermentative production of cellulose over wood. Possible uses of this product could be the medical and pulp and paper industries. Biopolymer succinoglycan production by coculture fermentation process involving *Cellulomonas cellulans* and *Agrobacterium tumefaciens*. This biopolymer is a potential flocculation additive that does not exhibit noxious or environmentally hazardous effects that are associated with many currently used flocculation additives that contain aluminium.[42] Use of succinoglycan in flocculation additives would enable reduction of subsequent wastewater treatment costs. Utilization of coculture fermentation processes may improve product quality as well, besides possible reduction of substrate costs and replacement of environmentally hazardous substances. Reported production of dextrans having lower molecular mass by cocultivation of *Leuconostoc mesenteroides* and *Lipomyces starkeyi* than by *Leuconostoc mesenteroides* alone in pure culture. The low-molecular-mass product is potentially more clinically useful for the application in blood plasma extenders and blood flow improvers.[43,44]

Enzymes:-

Cocultures of different micro-organisms may be also advantageous for the production of enzymes. One example is the production of laccases (EC 1.10.3.2). These enzymes are able to hydrolyze the polymer lignin and may allow the utilization of this complex biopolymer for the production of fine chemicals. Further applications of laccases may be the decolourization of textile dyes or the production of biosensors. Transition elements such as manganese or phenolic compounds are often necessary for the expression of laccases in moulds.[45-48] Utilization of both compounds results in cost-intensive wastewater treatment. The natural induction of laccase production led to a 40-fold increase in the production of laccase during a cocultivation of *Trichoderma harzianum* and *T. versicolor* compared with single cultivation and reported a strong increase in laccase production. The laccase production using Trametes sp. AH28-2 in cocultivation with Trichoderma sp. ZH1 is comparable to that using induction with toxic compounds. Additionally, the formation of a laccase only produced with contact to the other microorganism during cocultivation was reported. These biological approaches may be an environmentally friendly and cost-saving alternative for the production of laccases.[49-50]

Production of food additives:-

Carotenoids:-

Carotenoids are used in food industry as colouring agents, as nutraceuticals and as antioxidants. Carotenoids may be extracted from plants or produced by chemical synthesis or produced from biotechnological processes. Biological production processes offer the opportunities of reducing seasonal dependence on the supply of raw materials and utilization of cheaper substrates. Production of carotenoids by a coculture of the lactose-negative yeast *Rhodutorula rubra* and *Lactobacillus casei* ssp. casei has also been reported. Here, whey filtrate was used as carbon source. Lactose was hydrolyzed by *Lactobacillus casei*, enabling the growth of *Rh. rubra* and product formation have also reported carotenoid production by cocultures of *Rh. rubra* and *Lactobacillus casei ssp. casei.*[51-53]

Aroma and flavour substances:-

The demand for aroma and flavour substances is ever increasing. Their extraction from natural resources such as fruits and vegetables is often expensive. Biotechnological production of aroma components may be a good alternative to extraction from natural resources. The flavours thus produced can be declared as 'natural flavour' if the raw materials / precursors for fermentation or enzymatic biotransformation and the product are found in nature or in traditional foods. Precursors suitable for the production of flavour substances can be carotenoids. The enzymatic cleavage of carotenoids occurs in plants naturally and contributes to their characteristically aromatic compounds. Some aromatic compounds can be produced in pure culture. In other cases, a coculture fermentation process may be advantageous or even necessary, for example during the production of components of tobacco aroma.[54,55]

Production of antimicrobial substances:-

The discovery of antibiotics by Alexander Flemming in 1929 revolutionized the treatment of bacterial diseases such as scarlet fever, gonorrhea, infected wounds and pneumonia. Today, more efforts are being made to the research and development of new antibiotic substances because of the increasing number of pathogenic antibiotic-resistant strains. A very promising group of substances are the more than 500-member family of antimicrobial peptide, many of them showing a high antibacterial activity.[56,57] Production of these peptides by many micro-organisms has been reported, wherein their

expression may be constitutive or induced by the presence of signal substances formed by micro organisms.[58-60] A well-known example of antimicrobial peptides is the antibiotic nisin. It has been approved for microbial stabilization of food in England for over 50 years. Further applications are cosmetics and pharmaceuticals. Nisin acts by increasing permeability of membranes of Gram-positive bacteria resulting in growth inhibition or even cell death. It is produced by *Lactococcus lactis*, but its production can be increased by cocultivation of the lactic acid bacterium with *Saccharomyces cerevisiae* or *Kluyveromyces marxianus*. Production of antibacterial peptides by cocultures may be advantageous in areas where no recombinant micro-organisms should be used. The induction of plantaricin production by the cocultivation of *Lactobacillus plantarum* NC8 and *Lactococcus lactis*. These authors hypothesized a quorum-sensing mechanism responsible for the induction. Besides the production of antimicrobial peptides, coculture fermentation processes may lead to the discovery and characterization of new antimicrobial peptides also attributable to the unique induction phenomenon observed in cocultivation.[61-63] In this context, report of the formation of a bactriocide by *Lactobacillus plantarum* J23 in coculture with *Oenococcus oeni*, Lactobacillus ssp. or Pediococcus species only is noteworthy. The number of antimicrobially active substances produced by strains of the genus Streptomyces is estimated to be as many as 100 000. Today, only 3–5% of these substances are known. Besides bacteria, various fungi also produce and secrete antimicrobial substances. The antifungal protein (AFP) from *Aspergillus giganteus* inhibits the growth of human and plant pathogen filamentous fungi by permeabilizing their cellular membrane. On the other hand, bacteria, yeasts and endothelian cells are not influenced by AFP. The possibility of the application of AFP in medicine and plant protection. During the cocultivation of *Aspergillus giganteus* and *Fusarium oxysporum*, an increased production of AFP was observed. Direct cell–cell interactions between the micro-organisms might be responsible for the enhanced production.[64]

Microbial fuel cells (MFC) represent a very innovative field of research. Recent review articles dealing with the development of MFCs indicate the great interest and high potential of the microbial power generation. The production of electricity in MFCs enables direct conversion of biomass to electricity without the circuitous route of e.g. ethanol or biogas production. Nearly every organically degradable compound may be utilized for the production of electricity. In the MFCs, the capability of special micro-organisms, the so-called exoelectrogenes, is utilized to transfer electrons to solid substrates (anode) under anaerobic conditions. The simultaneously produced protons bind to oxygen at the cathode consuming electrons in the aerobic chamber of the MFC. The utilization of dilute solutions in MFCs is advantageous. Reported about the production of electricity from medium containing cellulose, by a coculture of Clostridium cellulolyticum and *Geobacter sulfurreducens*. Also, a biofilm-forming coculture of *Acetobacter aceti* and *Gluconobacter roseus* was applied for the current production developed a complex mixed culture for the utilization of cellulose during the electricity production.[65-67]

Bioremediation:-

Harmful chemical substances as organophosphate esters, alkanes, polycyclic aromatic hydrocarbons (PAH) and polychloride biphenyls (PCB) may be set free during accidents, incautious handling, application in agricultural pesticides or in waste and faeces. If these substances are not eliminated from the atmosphere, they will cause serious damage to ecology and human health. Therefore, processes have to be developed to remove the harmful chemicals.[68] In many cases, the degradation can be achieved at lower costs. Many microorganisms involving strains of Rhodococcus sp., Burkholderia sp., Mycobacterium sp., Stenotrophomonas sp., Alcaligenes sp., Sphingomonas sp., Phanerochaete sp., Pleurotus sp., Trametes sp., Penicillium sp. and Cunninghamella sp. are known to degrade PAHs containing four benzene rings in single cultivation. However, during the degradation of PAHs containing five benzene rings by single cultivations only particular steps of the mineralization are observed. The utilization of a coculture of the fungus *Penicillium janthinellum* and the bacterium *Stenotrophomonas maltophilia* offers the possibility for the degradation of these mutagenic and carcinogenic PAHs. They assumed a first oxidizing step catalysed by fungal enzymes resulting in an improved solubility of the PAHs. Afterwards, the bacterium is able to catalyse further oxidizing steps. An actual overview of more than 20 microorganisms degrading aromatic compounds. Another large group of hazardous pollutants are the organophosphate esters including tris (2-chloroethyl) phosphate (TCEP) and tris (1,3-dichloro- 2-propyl) phosphate (TDCPP). These chemicals are used worldwide in pesticides, flame retardants and plasticizers in large amounts.[69-71]

Utilization of Lignocellulose in bioconversion:-

Lignocellulose is the major structural component of all plants and consists mainly of cellulose, hemicellulose and lignin. To utilize the lignocellulose for the production of biofuels or biobased chemicals, discussed in previous sections, an efficient hydrolysis of the different branched polysaccharides followed by the conversion of glucose and xylose is required. Coculture fermentation processes offer the possibility to implement all necessary enzymatic conversions in one bioreactor.[72-77]

Table 1: presents an overview of the mixed culture and coculture fermentation processes described in this article.

Product / process	Applied micro-organisms	Reference
Food industry Cheese	Yeast, bacteria, moulds	Martin et al. 2001
Yoghurt	*Lactobacillus sp., Streptococcus sp.*	Sodini et al. 2000
Kefir	*Candida kefyr, Lactobacillus sp., Kluyveromyces sp., Saccharomyces sp*	Lopitz-Otsoa et al. 2006
African fermented dairy products	*Candida sp., Saccharomyces sp., lactic acid bacteria,*	Narvhus and Gadaga 2003
Sourdough	*Lactobacillus sp., Saccharomyces sp.*	Kariluoto et al. 2006
Salami	*Lactobacillus sp., Pediococcus sp., Micrococcus sp., Staphylococcus sp.*	Dicks et al. 2004
Whisky	*Streptococcus sp., Lactobacillus sp., Saccharomyces sp.*	Van Beek and Priest 2002
Lambic	*Lactobacillus sp., Brettanomyces sp.*	De Cort et al. 1994
Wine	*Saccharomyces sp.* *Brettanomyces sp., Pichia sp., Gluconobacter sp.,*	Clemente-Jimenez et al. 2005;
Cacao beans	*Yeasts, lactic acid bacteria, acetic acid bacteria*	Schwan and Wheals 2004
Bulk chemicals, fine chemicals and biofuels		
Ethanol	*Zymomonas mobilis, Saccharomyces sp. Zymomonas mobilis, Kluyveromyces fragilis Saccharomyces cerevisiae, Fusarium oxysporum Kluyveromyces marxianus, Talaromyces emersonii*	Abate et al. 1996 Szambelan et al. 2004 Mamma et al. 1996 Ward et al. 1995
Butanol	*Different Clostridium strains*	Bergstrom and Foutch 1985
Hydrogen	*Clostridium sp., Enterobacter sp. Clostridium butyricum, Rhodopseudomonas sp. Lactobacillus delbrueckii; Rhodobacter spheroids Clostridium pasteurianum Clostridium tyrobutyricum, Clostridium sporosphaeroides Citrobacter freundii, Enterobacter aerogenes,* *Rhodopseudomonas palustris Clostridium kristjanssonii, Clostridium saccharolyticus Clostridium pasteurianum, Different bacteria Clostridium thermocellum, Thermoanaerobacterium sp.*	Miyake et al. 1984 Yokoi et al. 1998 Asada et al. 2006 Hsiao et al. 2009 Vatsala et al. 2008 Zeidan and van Niel 2009 Cui et al. 2009 Levin et al. 2009
Acetic acid	*Zymomonas mobilis / Acetobacter sp Clostridium thermolacticum / Moorella thermoautotrophica Clostridium thermolacticum, Moorella thermoautotrophica Methanothermobacter thermoautotropicus*	Kondo and Kondo 1996 Talabardon et al. 2000 Collet et al. 2005
Lactic acid	*Enterococcus casseliflavus, Lactobacillus casei Different rec. Escherichia coli strains*	Taniguchi et al. 2004 Eiteman et al. 2008
Gallic acid	*Aspergillus foetidus, Rhizopus oryzae*	Banerjee et al. 2005
2-Keto-L-gluconic acid	*Gluconobacter oxydans, Bacillus megaterium*	Bremus et al. 2006
Polyglutamate	*Bacillus subtilis, Corynebacterium glutamicum*	Xu et al. 2002
Polyhydroxyalkanoate	*Azotobacter chroococcum, Bacillus megaterium Ralstonia eutropha, Lactobacillus delbrueckii*	Zhang et al. 2003 Patnaik 2009
Kefiran	*Saccharomyces cerevisiae, Lactobacillus sp.*	Cheirsilp et al. 2003; Frengova et al. 2002
Polydextrans	*Rhodutorula rubra, Streptococcus thermophilus, Lactobacillus bulgaricus*	Simova et al. 2004a
Cellulose	*Gluconacetobacter xylinus, Lactobacillus mali*	Seto et al. 2006
Biopolymer	*Cellulomonas cellulans, Agrobacterium tumefaciens*	Kurata et al. 2003
Dextrans	*Leuconostoc mesenteroides, Lipomyces starkeyi*	Kim and Day 1994
Laccase	*Rhizoctonia solani, Pseudomonas fluoreszenz Trichoderma harzianum, T. versicolor Pleurotus ostreatus, Phanerochaete chrysosporium Trametes sp., Trichoderma sp*	Crowe and Olsson 2001 Baldrian 2004, Verma and Madamwar 2002 Zhang et al. 2006
Tannase	*Penicillium glaucum, Aspergillus niger Rhizopus oryzae, Aspergillus foetidus*	Aguilar et al. 2007 Banerjee et al. 2005
Cellulase	*Aspergillus ellipticus, Aspergillus fumigates Aspergillus niger, Trichoderma reesei*	Gupte and Madamwar 1997 Ahamed and Vermette 2008
Food additives		
Carotenoid	*Rhodutorula glutinis, Debaromyces castellii Rhodutorula rubra, Lactobacillus casei*	Buzzini 2001
Tobacco aroma	*Trichosporon asahii, Paenibacillus amylolyticus Geotrichum sp., Bacillus sp.*	Frengova et al. 2003; Simova et al. 2004b
Antimicrobial substances		
Nisin	*Lactobacillus sp., Saccharomyces cerevisiae Kluyveromyces cerevisiae*	Liu et al. 2006
Antifungal Protein (AFP)	*Aspergillus giganteus; Fusarium oxysporum*	Meyer and Stahl 2003
Antibacterial Protein (AlpP	*Pseudoalteromonas tunicate; Alteromonas sp.*	Rao et al. 2005
Antimicrobial Proteins	*Serratia plymuthica; Escherichia coli*	Moons et al. 2006
Plantaricin	*Lactobacillus plantarum, Lactococcus lactis*	Maldonado et al. 2004
Antibiotics	*Streptomyces sp.; different marine bacteria*	Slattery et al. 2001
Microbial fuel cell (MCF)	*Clostridium cellolyticum, Geobacter sulfurreducens Geobacter sp., Desulfuromonas sp., Alcaligenes faecalis Acetobacter aceti, Gluconobacter roseus*	
Electricity	*Penicillium janthinellum, Stenotrophomonas maltophilia; Sphingomonas sp. and Aquabacterium sp. Cladosporium sp., Mycobacterium*	Kargi and Eker 2007 Karthikeyan et al. 2009
Lignocellulose for bioconversion		
Lignocellulose degradation	*Zymomonas mobilis, Pichia stipidis Clostridium thermocellum, Thermoanaerobacterium saccharolyticum*	Fu et al. 2009 Maki et al. 2009

CONCLUSION

The given examples of possible applications of coculture fermentation processes illustrate the increasing importance of this kind of fermentation in industrial biotechnology. Coculture fermentations can be utilized in the production of foods, food additives, pharmaceuticals, enzymes, bulk and fine chemicals, bioremediation and degradation of lignocelluloses. They offer the opportunity to use cheap substrates, increase yields and product quality. Further potential of cocultures rests in the discovery of new substances with industrial or pharmaceutical interest such as fine chemicals or antibacterial active substances and other secondary metabolites that are produced in cocultivation only. The controlled cultivation of cocultures enables the synergistic utilization of the metabolic pathways of the participating micro-organisms under industrial, reproducible and controlled conditions. The optimal values of process parameters (pH, temperature and oxygen demand) and the acceptable ranges of substrate and product concentrations have to be known and considered to achieve the controlled fermentation, as in pure culture cultivation. In coculture fermentation processes, the complexity of possible interactions (positive or negative) has to be taken into account. All aspects, the process parameters, the produced and secreted substances and possibly the occurring biotransformations, may provide an opportunity to control growth and product formation during coculture fermentation processes. Parameters have to be found enabling the utilization of the desired part of the metabolic pathway of every single strain in coculture to achieve the development of a controlled coculture fermentation process and to form the favoured product.

REFERENCES

1. Cui MJ, Yuan ZL, Optimization of biohydrogen production from beer lees using anaerobic mixed bacteria, Int J Hydrogen Energy, 34, 2009, 7971–7978.
2. Abate C, Callieri D, Ethanol production by a mixed culture of flocculent strains of *Zymomonas mobilis* and *Saccharomyces sp*., Appl Microbiol Biotechnol, 45, 1996, 580–583.
3. Appelbaum PC, The emergence of vancomycin intermediate and vancomycin-resistant *Staphylococcus aureus*, Clin Microbiol Infect, 12, 2006, 16–23.
4. Asada Y et. al, Hydrogen production by co-cultures of Lactobacillus and a photosynthetic bacterium, *Rhodobacter spheroides* RV, Int J Hydrogen Energy, 31, 2006, 1509–1513.
5. Belenguer A et.al, Two routes of metabolic cross-feeding between *Bifidobacterium adolescentis* and butyrate-producing anaerobes from the human gut, Appl Environ Microbiol, 72, 2006, 3593–3599.
6. Ahamed A, Vermette P, Enhanced enzyme production from mixed cultures of *Trichoderma reesei* RUT-C30 and *Aspergillus niger* LMA grown as fed batch in a stirred tank bioreactor, Biochem Eng J, 42, 2008, 41–46.
7. Benkerroum N et. al, Lyophilized preparations of bacteriocinogenic *Lactobacillus curvatus* and *Lactococcus lactis* subsp. lactis as potential protective adjuncts to control *Listeria monocytogenes* in dry-fermented sausages, J Appl Microbiol, 98, 2005, 56–63.
8. Chaudhry GR, Chapalamadugu S, Biodegradation of halogenated organic compounds, Microbiol Rev, 55, 1991, 59–79.
9. Clemente-Jimenez JM et.al, Influence of sequential yeast mixtures on wine fermentation, Int J Food Microbiol, 98, 2005, 301–308.
10. Crowe JD, Olsson S, Induction of laccase activity in *Rhizoctonia solani* by antagonistic *Pseudomonas fluorescens* strains and a range of chemical treatments, Appl Environ Microbiol, 67, 2001, 2088–2094.
11. Brede DA, Heterologous production of antimicrobial peptides in *Propionibacterium freudenreichii*, Appl Environ Microbiol, 71, 2005, 8077–8084.
12. Dalmau M et.al, Bacterial membrane injuries induced by lactacin F and nisin, Int Microbiol, 5, 2002, 73–80.
13. Demain AL, Elander RP, The beta-lactam antibiotics: past, present, and future, Antonie Leeuwenhoek, 75, 1999, 5–19.
14. Demain AL, Newcomb M, Cellulase, clostridia, and ethanol, Microbiol Mol Biol Rev, 69, 2005, 124–154.
15. Deslouches B et.al, De novo generation of cationic antimicrobial peptides: influence of length and tryptophan substitution on antimicrobial activity, Antimicrob Agents Chemother, 49, 2005, 316–322.
16. Dicks LM, Mellett FD, Use of bacteriocin-producing starter cultures of *Lactobacillus plantarum* and *Lactobacillus curvatus* in production of ostrich meat salami, Meat Science, 66, 2004, 703–708.
17. Du Z, Li H, A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy, Biotechnol Adv, 25, 2007, 464–482.
18. Fang HH, Liu H, Effect of pH on hydrogen production from glucose by a mixed culture, Bioresour Technol, 82, 2002, 87–93.
19. Faraco V, Giardina P, Metal-responsive elements in *Pleurotus ostreatus* laccase gene promoters, Microbiology, 149, 2003, 2155–2162.
20. Farid MA, El-Enshasy HA, Alcohol production from starch by mixed cultures of *Aspergillus awamori* and immobilized *Saccharomyces cerevisiae* at different agitation speeds, J Basic Microbiol, 42, 2002, 162–171.

21. Frengova GI, Simova ED, Exopolysaccharides produced by lactic acid bacteria of kefir grains, Z Naturforsch, 57, 2002, 805–810.
22. Frengova GI, Emilina SD, Carotenoid production by lactoso-negative yeasts co-cultivated with lactic acid bacteria in whey ultrafiltrate, Z Naturforsch, 58, 2003, 562–567.
23. Eggert C et.al, Molecular analysis of a laccase gene from the white rot fungus *Pycnoporus cinnabarinus*, Appl Environ Microbiol, 64, 1998, 1766–1772.
24. Tada S et.al, Fed-batch coculture of *Lactobacillus kefiranofaciens* with *Saccharomyces cerevisiae* for effective production of kefiran, J Biosci Bioeng, 103, 2007, 557–562.
25. Bremus C et.al, The use of microorganisms in L-ascorbic acid production, J Biotechnol, 124, 2006, 196–205.
26. Bullen RA, Arnot TC, Biofuel cells and their development, Biosens Bioelectron, 21, 2006, 2015–2045.
27. Fu N, Peiris P, A novel co-culture process with *Zymomonas mobilis* and *Pichia stipidis* for efficient ethanol production on glucose / xylose mixtures, Enz Microb Tech, 45, 2009, 210–217.
28. Fukushima M, Kawai S, Behavior of organophosphoric acid triesters in Japanese riverine and costal environment, Water Sci Technol, 25, 1992, 271–278.
29. Fuqua C, Parsek MR, Regulation of gene expression by cell-to-cell communication: acylhomoserine lactone quorum sensing, Annu Rev Genet, 35, 2001, 439–468.
30. Gupte A, Madamwar D, Production of cellulolytic enzymes by coculturing of *Aspergillus ellipticus* and *Aspergillus fumigatus* grown on bagasse under solid state Fermentation, Appl Biochem and Biotech, 62, 1997, 267–274.
31. Hagen S, Marx F, The antifungal protein AFP from *Aspergillus giganteus* inhibits chitin synthesis in sensitive fungi, Appl Environ Microbiol, 73, 2007, 2128–2134.
32. Harazono K, Nakamura K Decolorization of mixtures of different reactive textile dyes by the white-rot basidiomycete *Phanerochaete sordida* and inhibitory effect of polyvinyl alcohol, Chemosphere, 59, 2005, 63–68.
33. Hsiao CL et.al, Clostridium strain co-cultures for biohydrogen production enhancement from condensed molasses fermentation solubles, Int J Hydro Energy, 34, 2009, 7173–7181.
34. John RP, Nampoothiri KM, Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives, Appl Microbiol Biotechnol, 74, 2007, 524–534.
35. Joint I et.al, Cell-to-cell communication across the prokaryote-eukaryote boundary, Science, 298, 2002, 1207.
36. Kang SW et.al, The use of poly (lactic-co-glycolic acid) microspheres as injectable cell carriers for cartilage regeneration in rabbit knees, J Biomater Sci Polym, Ed 17, 2006, 925–939.
37. Kariluoto S et.al, Effects of yeasts and bacteria on the levels of folates in rye sourdoughs, Int J Food Microbiol, 106, 2006, 137–143.
38. Kato S, Haruta SC, Effective cellulose degradation by a mixed-culture system composed of a cellulolytic Clostridium and aerobic non-cellulolytic bacteria, FEMS Microbiol Ecol, 51, 2004, 133–142.
39. Khanal SK et.al, Biohydrogen production in continuous-flow reactor using mixed microbial culture, Water Environ Res, 78, 2006, 110–117.
40. Khanna S, Srivastava AK, Recent advances in microbial polyhydroxyalkanoates, Proc Biochem, 40, 2005, 607–619.
41. Kim JD, Lee CG, Microbial degradation of polycyclic aromatic hydrocarbons in soil by bacterium-fungus co-cultures, Biotech Bioproc Eng, 12, 2007, 410–416.
42. Kleerebezem R, Van Loosdrecht MC, Mixed culture biotechnology for bioenergy production, Curr Opin Biotechnol, 18, 2007, 207–212.
43. Kondo T, Kondo M, Efficient production of acetic acid from glucose in a mixed culture of *Zymomonas mobilis* and Acetobacter sp, J Ferm Bioeng, 81, 1996, 42–46.
44. Lai R, Lomas LO, Two novel non-cationic defensin-like antimicrobial peptides from haemolymph of the female tick, *Amblyomma hebraeum*, Biochem J, 379, 2004, 681–685.
45. Hancock RE, Patrzykat A, Clinical development of cationic antimicrobial peptides: from natural to novel antibiotics, Curr Drug Targets Infect Disord, 2, 2002, 79–83.
46. Maldonado-Robledo G et.al, Production of tobacco aroma from lutein. Specific role of he microorganisms involved in the process, Appl Microbiol biotechnol, 62, 2003, 484–488.
47. Lemos PC, Serafim LS, Synthesis of polyhydroxyalkanoates from different short-chain fatty acids by mixed cultures submitted to aerobic dynamic Feeding, J Biotechnol, 122, 2006, 226–238.
48. Levin DB, Carere CR, Challenges for biohydrogen production via direct lignocellulose fermentation, Int J Hydro Energy, 34, 2009, 7390–7403.
49. Li YQ et.al, Diesel pollution biodegradation: synergetic effect of mycobacterium and filamentous fungi, Biomed Environ Sci, 21, 2008, 181–187.
50. Liu C, Hu B, An Stimulation of nisin production from whey by a mixed culture of *Lactococcus lactis* and *Saccharomyces cerevisiae*, Appl Biochem Biotechnol, 129–132, 2006, 751–761.
51. LeBel GL, Williams DT, Gas chromatographic determination of trialkyl / aryl phosphates in drinking

water, following isolation using macroreticular resin, J Assoc Off Anal Chem, 64, 1981, 991–998.

52. Maldonade IR et.al, Selection and characterization of carotenoid reducing east from Campinas region, Brazil, Braz J Microbiol, 38, 2007,65–70.
53. Rojo-Bezares B et.al, Coculture-inducible bacteriocin activity of *Lactobacillus plantarum* strain J23 isolated from grape must, Food Microbiol, 24, 2007, 482–491.
54. Miller MB, Bassler BL, Quorum sensing in bacteria, Annu Rev Microbiol, 55, 2001, 165–199.
55. Rehm BH, Steinbuchel A, Biochemical and genetic analysis of PHA synthases and other proteins required for PHA synthesis, Int J Biol Macromol, 25, 1999, 3–19.
56. Mamma D et.al, Bioethanol from sweet sorghum: simultaneous saccharification and fermentation of carbohydrates by a mixed microbial culture, Proc Biochem, 31, 1996, 377–381.
57. Mapari SA, Nielsen KF, Exploring fungal biodiversity for the production of water-soluble pigments as potential natural food colorants, Curr Opin Biotechnol, 16, 2005, 231–238.
58. Martin N, Berger C, Aroma compound production in cheese curd by coculturing with selected yeast and bacteria, J Dairy Sci, 84, 2001, 2125–2135.
59. Meyer V, Stahl U, The influence of co-cultivation on expression of the antifungal protein in *Aspergillus giganteus*, J Basic Microbiol, 43, 2003, 68–74.
60. Miyake J, Mao XY, Photo production of hydrogen from glucose by a co-culture of a photosynthetic bacterium and *Clostridium butyricum*, J Ferment Technol, 62, 1984, 531–535.
61. Moller S et.al, In situ gene expression in mixed-culture biofilms: evidence of metabolic interactions between community members, Appl Environ Microbiol, 64, 1998, 721–732.
62. Roble ND, Ogbonna JC, L-Lactic acid production from raw cassava starch in a circulating loop bioreactor with cells immobilized in loofa (*Luffa cylindrica*), Biotech letters, 25, 2003, 1093–1098.
63. Narvhus JA, Gadaga TH, The role of interaction between yeasts and lactic acid bacteria in African fermented milks: a review, Int J Food Microbiol, 86, 2003,51–60.
64. Oh DC, Kauffman CA Induced production of emericellamides A and B from the marine-derived fungus Emericella sp. in competing co-culture, J Nat Prod, 70, 2007, 515–520.
65. Patnaik PR, Perspectives in the modeling and optimization of PHB production by pure and mixed cultures,Crit Rev Biotechnol, 25, 2005,153–171.
66. Patnaik PR, Cognitive optimization of microbial PHB production in an optimally dispersed bioreactor by single and mixed cultures, Bioprocess Biosyst Eng, 32, 2009,557–568.
67. Qian M et.al, Ethanol production from dilute-acid softwood hydrolysate by co-culture, Appl Biochem Biotechnol, 134, 2006, 273–284.
68. Ren Z, Ward TE, Electricity production from cellulose in a microbial fuel cell using a defined binary culture, Environ Sci Tech, 41, 2007, 4781–4786.
69. Renouf V et.al,. Interactions between *Brettanomyces bruxellensis* and other yeast species during the initial stages of winemaking, J Appl Microbiol, 100, 2006, 1208–1219.
70. Rikkinen, J. Oksanen, I. Lichen guilds share related cyanobacterial symbionts, Science, 297, 2002, 357.
71. Penttinen P et.al, Interactions between *Streptomyces californicus* and *Stachybotrys chartarum* can induce apoptosis and cell cycle arrest in mouse RAW264.7 macrophages,Toxicol Appl Pharmacol, 202, 2005, 278–288.
72. Pham TH et.al, Microbial fuel cells in relation to conventional anaerobic digestion technology, Eng Life Sci, 6, 2006, 285–292.
73. Shimizu H, Mizuguchi T, Nisin production by a mixed-culture system consisting of *Lactococcus lactis* and *Kluyveromyces marxianus*, Appl Environ Microbiol, 65, 1999, 3134–3141.
74. Rodriguez-Bustamante E, Sanchez S, Microbial production of C(13)-norisoprenoids and other aroma compounds via carotenoid cleavage, Crit Rev Microbiol, 33, 2007, 211–230.
75. Xu Z et.al,. Production of polyglutamic acid from mixed glucose and sucrose by co-cultivation of *Bacillus subtilis* and *Corynebacterium glutamicum*, Z Naturforsch, 57, 2002, 805–810
76. Roy JJ, Abraham TE, Biosensor for the determination of phenols based on cross-linked enzyme crystals (CLEC) of laccase, Biosens Bioelectron, 21, 2005, 206–211.
77. Salehizadeh H, Van Loosdrecht MC, Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance, Biotechnol Adv, 22, 2004, 261–279.

****