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Co-Culture: A Promising Method In Enzyme Production

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Abstract : Enzyme production has been increased during the past century as they have immense applications in various industries. Majority of the industrial enzymes are obtained from microorganisms, due to its rapid growth and easy cultivation using low-cost substrates. However, the yield is not satisfactory to meet industrial demand. Several methods are available to enhance enzyme production such as one factor at a time approach, and statistical approaches like response surface methodology by using of improved or mutant strains. Co-culture is another promising approach which involves the cultivation of two or more different microbes. Co-culture appears to be very beneficial because of the synergistic expression of metabolic pathways of all microorganisms involved. Thus, it provides an opportunity to increase the yield and enzyme activity . It is also a promising approach for various industrial products like antibiotics, bulk chemicals, food additives, alcohols and so on. In enzyme production, co-culture is found to be a superior method than monoculture because of the mutual expression of metabolic pathways in substrate utilization. In addition, it allows the expression of silent genes by all microorganisms involved in co-culture. This mini-review highlights the optimistic roles of co-culture in enzymes production.
Key words : Co-culture, enzymes, Fermentations, Microbial interactions, Product yield.

Introduction

Co-cultivation or mixed cultivation is the one that mimics the natural habitation where microbes always co-exist within complex microbial communities. Co-culture enables to undergo a combined metabolic activity in the laboratory condition for better utilization of substrates [1]. Co-cultivation of microbes appears to be very beneficial because of their synergistic expression of metabolic pathways of all microorganisms involved in the co-culture. During co-culturing, microorganisms develop various mechanisms to utilize substrates either by symbiotic [2],or antagonistic interactions[3]. The interactions and stress conditions that are prevailed during co-culturing tends to activate the unexpressed silent biosynthetic genes [4]. One major reason for the stress conditions are due to the competition for limited resources among the microbes.

Microorganisms are found to be more efficient when they are allowed to grow together with other microorganisms of same or different species[5]. During co-culturing, growth of one microorganism existing in the medium inhibits or enhances[6] the activity of other strain. Hence, co-culture fermentations shows great influence on many industrial products like alcohols, enzymes [7], bulk chemicals, organic acids.Co-culture proven to bea potential method to discover novel products of biofuels, bio polymers, and antibiotics (Figure 1). The best part in co-culture is precise quality control and better yield of the product [8] by utilizing low-cost substrates [9].

Ingeneral,low cost substances are used for enzyme production by co- culture method is found to much economical than monocultures for examplexylanase production by solid state fermentation of two fungal mixed cultures using sugarcane bagasse [10]. Likewise, soybean hulls with wheat bran were used for production of cellulolytic enzymes by culturing of *Trichodermareesei*and*Aspergillusoryzae*[11]andtannase production using grape wastes under solid state fermentation by fungal co-culture was explained by Paranthaman et al., (2009). Mixed cultures of *Aspergillusniger*and*Tricodermaviride* by using waste paper was reported to have higher yield of cellulase[12]. Gluco amylase was produced from gelatinized rice flour usingco-culture of *Bacillus* and *Rhizopus*strains [13]. Interestingly, corn cob with pineapple peel was used as a substrate for production of cellulase - free xylanases during co-culture of *Trichodermakoeningi*and*Sclerotiumrolfsii*[14]. Likewise, corn stover from agro-industrial residue was used for the production of exo-glucanases by co-culture of *Trichodermaviride*and*Ganodermalucidum*[15].

Several co-cultivation reports have been documented in recent years to prove as a promising approach to enhance the yield of enzyme production using low-cost substrates. The present review will prove helpful to explore the use of co-culture or mixed culture in enzyme production**.**

Potential applications of co-culture in enzyme production

Enzymes are widely used in numerous applications and it's demands are raising.day by day[16]. Some of the significant industrial applications of enzymes in various processing include coffee processing, candies, dairy, leather, beverages, dry cleaning, laundry, animal feeds, meat processing, paper, pharmaceuticals and clinical, photographic, textiles and so on. The enzymes that were produced using co-cultural condition are represented in Table 1.

Ligninases

Lignin is a phenyl propanoid polymer.The degradation of lignin by micro organisms is very tough, due to its complex three-dimensional structures which are formed by the radical polymerisation, its degradation by the microbes is very tough. The structure becomes so compressed by the lignin in case of woody tissues so that proteins cannot enter into the cell wall. Lignin modifying enzymes comprise oxidative enzymes which are involved in the biodegradation of lignin. Lignin modifying enzymes include laccase, lignin peroxidase, and manganese peroxidise[17].Ligninases acts as a detoxification agent in pollutant degradation, waste water degradation, and also in bleaching of pulps where it increases the utility of kraft lignin,alsodelignified materials can be used in paper industries and animal fed stock[14].

Since wood rotting fungi are the best degraders of lignin, the production of ligninases by co-culturing of two white rot fungi was investigated[17]. Among different pairs of six fungal species, it was found that co-culture of *P.radiata* and *D.squalens* showed considerably increased production of laccases of 120 U/mg after incubation for four days. Other lignin modifying enzymes like lignin peroxidase and manganese peroxidase were increased drastically during co-culture of *P.ostreatus* and *P.radiata.*

In monoculture, fungi was unable to degrade acid precipitated lignin. Where as in co-culture acid insoluble lignin content was degraded by fungal strains. Monocultures of *Physisporinusrivulosus*, *Phanerochaetechrysosporium* disparaged easily acid soluble lignin. In co-culture, the combination of *P. rivulosus* and *P. ostreatus*tend the degradation shift towards the acid precipitated lignin.Co-cultivation of *Cerioporiopsissubvermispora*with*Pleurotusostreatus*, also showed the significant effect on degradation of wood blocks compared with monocultures[18].

Laccases

Laccases contain copper and it belongs to copper oxidases group [19]. This enzyme is extensively dispersed in a very broad range of higher plants, bacteria, insects, ascomycetes and basidiomycetes. Most common producers of laccases are wood rotting fungi. Laccase is a homodimer protein and also group under wider polyphenol oxidases as they all have a common feature to oxidize aromatic compounds. Phenolic subunits of lignin are attacked by laccases. Laccases have pretty low oxidation potential. Various functions of laccases are pigment formation in fruiting bodies, morphogenesis, and lignin biodegradation of wood which was also engaged in microbial and cellular activities. Laccases has a wide application in various industries such as pulp and paper industry[20] waste detoxification and,decolorization of dyes[21], degradation of polyaromatic

hydrocarbon, the transformation of textile dyes, disinfectant and sterilization of drinking water and biosensors. It is also used for bioremediation of waste water from beer and food industries, food processing, fruit juices and wine stabilizing and baking[22]. The production of laccases was demonstrated by co-culturing of wood rotting fungi *Trametes sp*. AH 28-2 with saprophytic fungi *Trichoderma sp*. ZH1. The activity of laccase enzyme by *Trametes sp.* AH 28-2 increased up to 6210 U/l when co-cultured with *Trichoderma sp*. ZH1. 60% to 70 % of laccase activity was observed after co-cultivation for 5 days. The enzyme stability and activity stand at least for 20 days using co-culture but when it was induced by chemicals the activity was reduced within three days[23].

Laccase over production was observed in co-culture of *Rhodotorulamucilaginosa*yeast and *Pleurotus ferulae* JM301. Surface changes also can be observed in fermentation process. The *P.ferulae* rough mycelia surface changed to smooth mycelia when co-cultured with *R.mucilaginosa*[24]*Candida sp. HSD07A* confronted with *Ganodermalucidum*lead to overproduction of laccase. *Candida sp. HSD07A* produces glycerol in the fermentation broth. The released glycerol stimulated the over production of laccase from *Ganodermalucidum* and extended the survival time[25]. A fungal laccaseLcc 9 which was silent during monoculture was induced by co-culturing of *Coprinopsiscinerea*and *Gongronella sp.* The activity of laccase by co-culture was 900 times higher than that of monoculture, with an elevation up to 1800 U/l recorded within two days of fermentation[26].

Lipases

Lipases are omnipresent and are serine hydrolases(Sharma, Chisti et al. 2001). Triacylglycerols Some of the industrial application of lipases are hydrolysis of fats in detergents, dairy foods, flavour enhancer in meat and fish, bakery foods, beverages as aroma enhancer, synthesis of chemicals, pharmaceuticals, as moisturizers in cosmetics, paper and cleaning process. Selection of microbes and designed co-cultures are found be an important criteria for overproduction of desired product. This was well evidenced by a recent study which involves the selection of appropriate combinations of *Lactococcuslactis, Lactobacillus brevis,Lactococcuslactis*and *Lactobacillus plantarum.* The highest lipase activity was obtained by co-culture of *L.brevis*and *L.Plantarum* when compared to pure cultures[27].

Tannase

Tannase is a membrane bound inducible enzyme which catalysis the ester hydrolysis and hydrolysis of tannin such as tannic acid, ethyl gallate,gallic acid and so on[28]. Fungal sources were found to be a very good producer of tannase. The industrial application of tannases in food industry includes preservatives, clarification of wines, beer and fruit juices, instant tea and coffee flavoured drinks. In addition, in the animal feed, it increases digestibility and also used for bio remediation of sewages.

In a study by Mukherjee and Banerjee (2006), an elevation in tannase yield was noticed while co-culturing the soil isolates *Rhizopusoryzae*and *Aspergillusfoetidus.* Engineering microbial consortia to express complex biosynthetic pathways is a promising approach which was evident by co-culturing UV, heat and NTG treated strains of *Rhizopusoryzae*and *Aspergillusfoetidus*. [29]. Various co-culture strategies are followed by many groups to mimic the natural habitations, where microbes exist in complex communities, one such example is grapes,coffee wastes and wheat brawn used as substrates to enhance tannase production by co-culture fermentations [30].

Cellulases

Cellulaseshydrolyze the crystalline polymer cellulose to its monomer glucose and cello-oligosaccharides. For commercial use, cellulases are produced by submerged or solid state fermentation using all three modes of fermentation[31]. Some of the extensive applications of cellulases are deinking and biomechanical pulping in paper and pulp industry, biofuel production, laundry, detergents, clarification of fruit juices in food industry and also in animal feeds. The cellulase production by co-culture of *Trichodermareesei* and *Aspergillusniger*was explained by Ahamed and Vermette, (2008), with 2.1 fold increasedthan mono cultures. Very limited studies had reported trigger molecule for enzyme synthesis and enhancement during co-culture. One such example is, during co-culturing of *Hypocreajecorina*Rut C30 and *Candida bombicola*, the sophorolipids produced by *C.bomicola* are hydrolyzed to sophorose, which acts as an inducer for the enhanced production of cellulose by the fungi *H.jecorina*[33].Mixed cultivation of *Aspergillusniger and Trichoderma*reseei in substrate fermentation produced more amount of cellulase, glucosidase, endoglucanase (CMCase) and

xylanase enzymes when compared to monocultures. It was showed that high amount of cellulase activities by using rice straw and wheat brawn in 3:2 ratios in solid state fermentations[34].

Amylases

Amylases are hydrolytic enzymes which hydrolyze the α-D-(1,4) - glycosidic linkages in starch which is an abundant polysaccharide[35]. It has a very great importance in biotechnology. Most important applications of amylases are starch liquefaction process, their role in detergent to increase its capacity to remove hard stains ,production of ethanol. In food industries,their major application includes like baking, brewing andand also used for coating process to enhance the quality of paper to make its surface strong. Some of the other applications are in textile, pharmaceuticals and in the production of a chemical[36].

Co-cultureof*Clostridium thermohydrosulfuricum* and *Clostridium thermosulfurogenes* reported to have complete degradation of starch, in the case of single cultures, the starch metabolism was very less or even not detectable in one of the strain. The rate of degradation of starch by pure cultures and co-culture were tested with two different concentrations of starch namely 0.65 % and 3% [37].As discussed earlier different microbial interactions occurred in co-culture method. Commensalism interaction helped to increase the amylase production by co-culturing of *S. cerevisiae* and *B. amyloliquefaciens04BBA15*. In monoculture fermentation of *B. amyloliquefaciens04BBA1*5 was $107.5\pm0.5 UmL^{-1}$ of amylase activity in 40-50hours of incubation . In co-culture, it was increased up to $300\pm0.3 UmL^{-1}$ in 30 hours of incubation time. Mutualism interaction between *S. cerevisiae* and *L. fermentum* 04BBA19 in mixed culture assisted amylase production upto $351.1\pm0.4 UmL^{-1}$.Where as, in the pure culture of *L. fermentum*04BBA19, 147.5 ± 0.3 UmL^{-1} of amylase was produced[38].

Glucoamylase

The production of exoamylolytic enzyme glucoamylase in a co-culture system of *Bacillus amyloliquefaciens*and *Rhizopuscohnii* using submerged fermentations was reported by Sato *et al.*, (2011). The production of glucoamylase by co-culture was estimated as 740 U/ml which has higher enzyme activity compared to enzyme production by pure culture .

Xylanases

Xylanases are members of glycoside hydrolases group which hydrolyses the xylosides[39]. Xylan which is a wide spread polysaccharide is an essential substrate for xylanases production. Xylanases are classified based on their profiles of product, specificity of substrates, molecular weight, crystal structure and properties of kinetics. Based on the sequence similarities of amino acids and cluster analysis, they are grouped as GH 10 andGH 11. Some of the potential producers of xylanolytic enzymes are actinomycetes[40], fungi[41] and bacteria. The important applications of xylanases are the conversion of lignocellulosicand agricultural wastes into products using microorganisms, pulp bleaching, rayon and cellophane production, chemicals, and clarification of beer and juices[42]. Increased production of xylanases using co-culture of *Trichodermareesei* with either *Aspergillusniger* or *A. phoenicis* using soy meal as a supplement, showed 7 fold yield increase than monocultures. [10].

Chitinases

Chitinases belong to glycosyl hydrolases group and they are found in wide range like yeast, fungi, bacteria, arthopods, actinomycetes, plants, and human [43]. Chitinases are split into two groups namely exo-chitinases and endo-chitinases. Chitinases hydrolyse the polysaccharide chitin into its monomer N-acetyl glucosamine by breaking the glycosidic bonds[44]. Chitinases have a very broad range of application in various fields as a biocontrol of fungal phytopathogens, bio-pesticides, management of wastes, morphogenesis and also in medical use for topical applications [45]. An interesting study by[46] demonstrated the interaction of bacteria with different mechanism for chitin degradation. Their study highlighted that mixed species of biofilm enhances substrate utilisation and enzyme yield.

Interactions of microbes during co-culture fermentations

Microbes are engaged in various interactions in their natural habitats, the reason behind the interspecific interactions are still not completely explored. Co-culture is one kind of approach to mimic the natural

habitations, however, the selection of microbes and providence of suitable cultural conditions are tedious process[47] . While using the co-culture approach, the type of interactions such as commensalism, mutualism, parasitism and competition between the stains should be completely understood either to explore novel products or to enhance the product yield. A study by Jagmann *et al.,* (2012b) reveals that microbes involving different mechanisms for substrate utilization can co-exists by formation of mixed- species biofilm. Thus understanding the complexity of possible interactions has to be addressed to enable the expression of desired metabolic pathways for the development and enhancement of required product.The comparative enzyme yield beween the monoculture and co-culture fermentations we represented in Table 2.

Conclusion

Even though this review presented limited reports, it clearly showcases that co-cultivation is a significant approach in enzymes production with numerous advantages. The fermentations with mixed microbial communities can utilize different carbohydrates effectively which in turn increases the fermentation rate. In addition, co-culture helps to mangage solid wastes such as agro industrial waste and forest waste namely, ligocellulosics biomass. Moreover, the enhancements in enzyme yield are reported in almost all co-culture fermentations with specific up-regulation of enzyme synthesis. However, the important strategies about co-culture with respect to enzyme production is, understanding the complexity of possible interactions for effective substrate utilization and product formation. In most of the study, the complete details about the positive or negative interactions during co-culture are lacking. Moreover, for controlled fermentations the metabolic pathways of the microbes involved have to be known as in case of pure culture.

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