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Review

Microbial production of raw starch digesting enzymes

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Raw starch digesting enzymes refer to enzymes that can act directly on raw starch granules below the gelatinization temperature of starch. With the view of energy-saving, a worldwide interest has been focused on raw starch digesting enzymes in recent years, especially since the oil crisis of 1973. Raw starch digesting enzymes are ubiquitous and produced by plants, animals and microorganisms. However, microbial sources are the most preferred one for large-scale production. During the past few years, the production of raw starch digesting enzymes by various microorganisms has been studied extensively. This paper reviews the recent development in the microbial production of raw starch digesting enzymes with a biotechnological perspective. This is the first review on microbial raw starch digesting enzymes till date.

Key words: Microbial, raw starch digesting enzymes, production.

INTRODUCTION

Starch is the most abundant form of storage polysaccharides in plants and constitutes an inexpensive source for production of syrups containing glucose, fructose or maltose, which are widely used in food industries (Roy and Gupta, 2004). In addition to that, the sugars produced from starch can be fermented to produce bioethanol, amino acids, organic acids and others (Polakovic and Bryjak, 2004). Conventionally, conversion of starch to glucose requires a two-step process namely liquefaction and saccharification. This process is energy intensive thus increasing the production cost of starch-based products. With the view of reducing the energy consumption, there is considerable research on raw starch degrading enzymes currently.

Raw starch digesting enzymes (RSDE) refer to enzymes that can act directly on raw starch granules below the gelatinization temperature of starch. RSDE are ubiquitous and produced by plants, animals and microorganisms. In spite of the wide distribution of RSDE, microbial sources have many advantages for the industrial production such as cost effectiveness, consistency, less time and space required for production and ease of process modification and optimization. Therefore, microbial sources are the most preferred one for large-scale production. As listed in Table 1, a wide variety of microorganisms, including fungi, yeasts and bacteria have been reported to produce RSDE. This review illustrates an overview of microbial production of RSDE.

PRODUCTION OF RAW STARCH DIGESTING ENZYMES

Carbon sources

Various raw starches and soluble starch are the preferred choices for most microorganisms because of their better induction on RSDE production and low cost. Chiou and Jeang (1995) observed significant difference in the enzyme yield between raw and gelatinized starch as carbon source. When raw starch was used, RSDE activity reached 269.3 U/ml, which was 8-fold of that from gelatinized starch. However, Takao et al. (1986) working with *Corticium rolfsii* reported the same level of RSDE yield in the media containing either raw or gelatinized starch as carbon source. The reason for this disagree-

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Table 1. RSDE-producing microorganisms so far reported.

Microorganism	Reference	Microorganism	Reference
Fungi		**Yeast**	
Acremonium sp.	Marlida et al., 2000b	*Aureobasidium pullulans*	Li et al., 2007
Aspergillus awamori	Amsal et al., 1999	*Candida antarctica*	De Mot and Verachtert , 1987
Aspergillus carbonarius	Okolo et al., 2000	*Cryptococcus* sp.	Lefuji et al., 1996
Aspergillus cinnamomeus	Kurushima et al., 1974	*Lipomyces starkeyi*	Punpeng et al., 1992
Aspergillus ficum	Hayashida et al., 1986	*Saccharomycopsis fibuligera*	Horváthová et al., 2004
Aspergillus niger	Pothiraj et al., 2006		
Aspergillus oryzae	Hata et al., 1997	**Bacteria**	
Aspergillus terreus	Pothiraj et al., 2006	*Anoxybacillus contaminans*	Anders et al., 2006
Chalara paradoxa	Mikuni et al., 1987	*Bacillus alvei*	Achi and Njokuobi , 1992
Cladosporium gossypiicola	Mimaki et al., 1998	*Bacillus amyloliquefaciens*	Demirkan et al., 2005
Corticium rolfsii AHU 9627	Nagasaka et al., 1998	*Bacillus cereus*	Sarikaya et al., 2000
Endomycopsis fibuligera	Ueda and Saha, 1983	*Bacillus circulans*	Kim et al., 1990
Fusidium sp. BX-I	Ohno et al., 1992	*Bacillus firmus*	Gawande et al., 1999
Gibberella pulicaris	Marlida et al., 2000b,c	*Bacillus firmus/lentus*	Wijbenga et al., 1991
Mucor rouxianus	Yamasaki et al., 1977	*Bacillus licheniformis*	Arasaratnam and Balasubramaniam, 1992
Nodilusporium sp.	Marlida et al., 2000b	*Bacillus macerans* BE101	Yamamoto et al., 2000
Penicillium brunneum	Haska and Ohta, 1994	*Bacillus* no. 2718	Higashihara et al., 1987
Penicillium oxalicum	Yamasaki and Suzuki, 1977	*Bacillus polymyxa* TB1012	Itkor et al., 1989b
Penicillium sp. X-1	Sun et al., 2007	*Bacillus* sp. I-3	Nidhi et al. , 2005
Rhizoctonia solani	Singh et al., 1995	*Bacillus* sp. YX-1	Liu and Xu , 2008
Rhizomucor pusillus	Kanlayakrit et al., 1987	*Bacillus stearothermophilus*	Dettori-Campus et al., 1992
Rhizopus niveus	Chandra et al., 1983	*Bacillus subtilis* IFO 3108	Mitsuiki et al., 2005
Rhizopus oryzae	Ashikari et al., 1986	*Clostridium butyricum* T-7	Tanaka et al., 1987
Rhizopus sp. W-08	Wang et al., 2007	*Clostridium thermosulfurogenes*	Saha et al., 1987
Rhizopus stolonifer	Pothiraj et al., 2006	*Cytophaga* sp.	Jeang et al., 2002
Schizophyllum commune	Shimazaki et al. , 1984	*Geobacillus thermodenitrificans*	Ezeji et al. , 2007
Streptomyces limosus	Fairbairn et al. , 1986	*Klebsiella pneumoniae*	Gawande & Patkar , 2001
Streptomyces precox NA-273	Takaya et al. , 1979	*Lactobacillus amylophilus*	Vishnu et al. , 2006
Streptomyces sp.E-2248	Kaneko et al. , 2005	*Lactobacillus amylovorus*	Imam et al. , 1991
Streptomyces thermocyaneoviolaxeus	Mai et al. , 1996	*Lactobacillus plantarum*	Giraud et al. , 1991, 1994
Thermoactinomyces-thalpophilus	Okolo et al. , 1996	*Rhodopseudomonas* sp	Buranakarl et al. , 1985
Thermomucor indicae-seudaticae	Kumar and Satyanarayana, 2003	*Streptococcus bovis* 148	Satoh et al. , 1997
Thermomyces lanuginosus	Odibo and Ulbrich-Hofmann , 2001	*Thermoanaerobacter* sp.	Tae et al. , 1997

ment remains to be determined.

RSDE production is generally subjected to catabolite repression by glucose and other readily metabolizable substrates (Okolo et al., 1996). Only a small amount of RSDE (5.6 U/ml) was produced by *Cytophaga* sp with glucose as carbon source, while the RSDE activity reached 269.3 U/ml when raw starch was used as carbon source. To release carbon repression, a 2-deoxyglucose-resistant mutant of *Rhizopus* sp. MB46 was derived. The productivity of the mutant was over 2-times that of the wild type strain (Tani et al., 1988). Glycerol was also used as an alternative carbon source to release carbon repression caused by glucose, RSDE yield showed a significant increase of 4.5-fold (Wong et al., 2002). Excep-

tionally, a high level of RSDE was observed not only in the culture of *Corticium rolfsii* grown on raw starch, but also in the cultures grown on the monosaccharides, such as glucose, fructose and disaccharides (Takao et al., 1986).

Nitrogen sources

Peptone and yeast extract are the common nitrogen sources for RSDE production. Soybean meal is also a promising nitrogen source for RSDE production due to its low cost and availability. Though organic nitrogen sources were universally used in RSDE production currently, Morita and Fujio (2000) discovered that organic nitrogen sources negatively affected RSDE production by *Rhizopus* sp. MKU 40, because organic nitrogen sources induced the formation of protease, which resulted in the proteolysis of RSDE. $(NH4)_2SO_4$ (Wang et al., 2007), CH_3COONH_4 (Morita et al., 1998) and sodium L-glutamate (Buranakarl et al., 1988) were also proved as effective nitrogen sources for RSDE production. In some cases, a combination of organic and inorganic nitrogen sources produced high yield of RSDE (Marlida et al., 2000a, b, c).

Metal ions

Supplementation of salts of certain metal ions influences the growth of microorganisms and thereby stimulates or inhibits enzyme production. RSDE production by *Rhizopus* sp. A-11 was maximized in the presence of 75 ppm of calcium and 0.7 ppm of zinc ions in liquid medium (Morita et al., 1998). $CaCl_2$ and $LiSO_4$ significantly stimulated RSDE production by *Bacillus* sp. I - 3, while $MgSO_4$, $FeCl_3$, $MnSO_4$, $CuSO_4$, $HgCl_2$, $ZnSO_4$ or AgCl had negative effect on RSDE production (Nidhi et al., 2005). For *Penicillium* sp. X-1, $MgSO_4$, $MnCl_2$, $ZnCl_2$ or $CaCl_2$ stimulated RSDE production while $LiSO_4$ or $NiSO_4$ were inhibitory (Sun et al., 2007). The RSDE yield from *Cytophaga* sp. was at a slightly higher level with the addition of $MgSO_4$, $FeSO_4$ and $ZnCl_2$ suppressed RSDE production (Chiou and Jeang, 1995). Therefore, the effect of metal ions on RSDE production varies from microorganism to microorganism.

Surfactants

Some surfactants can influence the production and secretion of RSDE through changing the permeability of cell membrane. Triton X-100 doubled RSDE production by *Penicillium* sp. X–1 (Sun et al., 2007), while Lin et al. (1998) reported that Triton X-100 and SDS had a lethal effect on RSDE production by *Bacillum* sp.TS-23. Therefore, whether the surfactant can improve RSDE production depends on both the property of the surfactant and physiological properties of the strain.

Moisture

The moisture level is a main factor in SSF which often determines the success of the process. A certain quantity of water is essential for cell growth and enzyme production. The importance of moisture level under SSF and its influence on the biosynthesis of enzymes has been attributed to the interference of moisture in the physical properties of solid particles. Lower moisture level gives a lower degree of swelling and higher water tension, and then reduces the solubility of nutrients. Higher moisture level decreases porosity, changes substrate particle structure, promotes development of stickiness, reduces gas volume and exchange and decreases diffusion, which results in lowered oxygen transfer (Lonsane et al., 1985). Maximum RSDE yield by *Penicillium* sp. X–1 was detected when the initial level of moisture was 65%, which was 4.1-fold of that obtained at a moisture level of 50% (Sun et al., 2007). The optimum initial moisture level for RSDE production by *Aspergillus* sp GP-21 was 75% (Mamo and Gessesse, 1999a). The moisture level of 48 and 45% was used for RSDE production by *Rhizopus* sp. A-11 and *Rhizopus* sp. MB46, respectively (Morita et al., 1998; Tani et al., 1988). Studies indicated that α-amylase titers could be increased significantly by agitation of the medium with high moisture content (Ramesh and Lonsane, 1990). Maybe it is also feasible for RSDE production.

pH

pH is one of the important factors that determine the growth and enzyme secretion of microorganisms as they are sensitive to the concentration of hydrogen ions present in the medium. For example, the RSDE yield by *Acremonium* sp. with the initial pH of 5.0 was 12.5-time higher that obtained in the initial pH of 3.5 (Marlida et al., 2000a). In general, fungi and yeasts required slightly acidic pH (4.0 - 6.5) and bacteria required neutral pH for optimum growth and RSDE production. There were some exceptions: RSDE was produced by *Bacillus* sp. YX-1 with the initial pH of 4.5 (Liu and Xu, 2008). The optimum pH for RSDE production by alkliphilic *Bacillus* sp TS-23 and *Bacillus* sp. IMD 370 were 8.5 and 9.7, respectively (Lin et al., 1998; Mc Tigue et al., 1994). The pH change during the fermentation process also affects enzyme production. For RSDE production by *Penicillium* sp. S-22, RSDE productivity increased to 2.18-fold by the pH-stat strategy of keeping pH at set-point of 5.5 (Sun et al., 2006). In some processes, the buffering capacity of some media constituents eliminates the need for pH control. However, when *Bacillus* sp TS-23 was cultivated in buffered medium (Tris/HCl buffer, pH 8.5), the cell growth was poor and thus no RSDE activity was detected. When pH was uncontrolled during the culture with the initial pH of 8.5, RSDE production recorded the maximum (Lin et al., 1998). Multiple pH optima were observed for RSDE

production by *Thermoactinomyces-thalpophilus* F13, which might be attributed to the enzyme heterogeneity (Okolo et al., 1996).

Temperature

The optimum temperature for most microorganisms to produce RSDE was in the range of 25 - 37°C. RSDE production has also been reported at the optima of 40 - 65°C by the thermophilic microorganisms. Temperature as high as 65°C was used for RSDE production by the thermophile *Bacillus* sp. WN11 (Mamo and Gessesse, 1999b). Sometimes the optimum temperature for cell growth and RSDE production are not identical. The optimum temperature for cell growth and RSDE production of *Lipomyces starkeyi* HN-606 were 25 and 15°C, respectively. Culture at 25°C decreased the RSDE productivity to a half of that at 15°C (Punpeng et al., 1992).

Dissolved oxygen

Agitation intensity influences the mixing and oxygen transfer rates and thus influences cell growth and product formation. Dissolved oxygen is affected by agitation, aeration, viscosity of the medium, shape of fermentor, etc. When RSDE production was carried out in flasks, 100 - 300 rpm were generally adopted. When fermentors were used for RSDE production, agitation of 100 - 600 rpm with aeration rate of 1 - 10 l/min was usually applied. Exceptionally, agitation speed of 900 rpm was used for RSDE production by *Bacillus firmus/lentus* (Wijbenga et al., 1991).

Fermentation mode

RSDE can be produced under submerged fermentation (SmF) or solid-state fermentation (SSF). SmF is widely carried out because of ease of handling and controlling of environmental factors such as temperature and pH (Soni et al., 2003). Morita et al. (1998) made a comparison of RSDE production in liquid and solid cultures by *Rhizopus* strains. It was observed that RSDE productivity of the liquid culture was about 4.4 times higher than that of the solid-state culture, based on the unit starch amount in the liquid and solid media carbon source. However, SSF attained new attention in recent years due to its advantages such as its simplicity and closeness to the natural growth conditions of many microorganisms, especially fungi. Wang et al. (2007) maximized the production of RSDE by *Rhizopus* sp using SSF combined with SmF. The yield of RSDE from the combined system were over 18-fold and 4-fold higher than the results obtained by SmF system and SSF system singly, respectively.

Batch fermentation is used in most cases for RSDE production because of its ease of control. However, fed-batch culture is superior to batch fermentation for RSDE production by *Penicillium* sp. S-22. When partially hydrolyzed raw yam starch and peptone were fed together in a pH-stat mode, RSDE activity and productivity increased by 22.9 and 17.8-fold greater than the values obtained in the batch culture, respectively (Sun et al., 2006).

CONCLUSION

Although much work has been done on RSDE production, a number of points still need improvement. Some practical approaches could be adopted to make RSDE economically attractive: these include the use of over-producing mutant and recombinant strains, cheaper raw materials, optimized medium and culture conditions and efficient recovery processes. Especially the application of DNA recombinant techniques to obtain high-yielding recombinant strains would be the real breakthrough in the development of RSDE.

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