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Applications, Properties and Importance of α-L-Rhamnosidase.

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ABSTRACT

α-L-rhamnosidase [E. C. 3.2.1.40] splits terminal α-L-rhamnose especially from a massive range of herbal goods. The enzyme has a huge incidence in nature and is said from animal tissues, plants, yeasts, fungi, and bacteria. It's a biotechnologically important enzyme because of its solicitations in de-bittering and clearance of citrus fruit juices, enrichment of wine aromas and derhamnosylation of many natural goods containing terminal α-l-rhamnose to compounds of pharmaceutical interests. Even though α-L-rhamnosidase have been researched keenly at some phase in recent years, there is no latest review on α-l-rhamnosidase. An effort has been made to refill this gap in this review. It consists of a brief advent of α-L-rhamnosidase that is followed via a vital description of the methods used for assaying the enzyme diversion. Purifications, characterizations, and properties of α-L-rhamnosidase from unique assets had been mentioned and to be had a structural and molecular organic study at the enzyme have been given. Biotechnological and Pharmaceutical applications of this enzyme in different methods have been in briefly defined. The evaluation determines with the identity of regions which wishes similarly vast research.
Keywords: Debittering Enzyme, α-L-Rhamnosidase, Rhamnose, Biotransformation.

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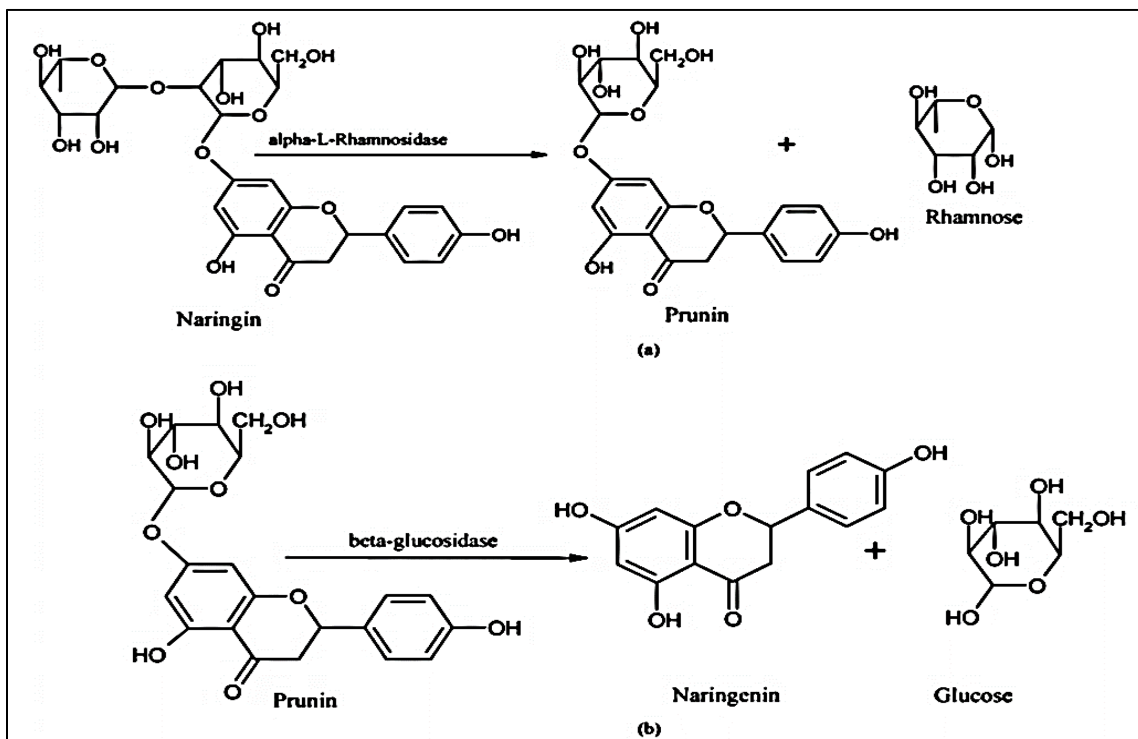
INTRODUCTION

α-L-rhamnosidase [E. C. 3.2.1.40] splits terminal l-rhamnose in particular from a vast collection of natural products which consist of naringin, rutin, quercitrin, hesperidin, diosgene, terpenyl-glycosides and many altered herbal glycosides containing terminal l-rhamnose (Habelt K and Pittner F.1983; Roitner M *et al*, 1984) (response Scheme 1). The enzyme has an extensive prevalence in nature and has been indicated from animal tissues, vegetation, yeasts, fungi, and bacteria.



Reaction Scheme 1. (a) Hydrolysis of naringin to prunin by α-L-rhamnosidase. (b) Hydrolysis of prunin to naringenin by β-d-glucosidase. (V. Yadav *et al*., 2010)

This enzyme has grown to become out to be a biotechnologically important enzyme. Its applications in a ramification of techniques like debittering of citrus fruit juices (Gray GM *et al*, 1981; Busto MD *et al*, 2007). Manufacture of prunin from naringin (Roitner M et al, 1984), production of L-rhamnose by hydrolysis of natural glycosides containing terminal L-rhamnose (Cheetham PSJ, 19910), enhancement of wine aromas by using enzymatic hydrolysis of terpenyl glycosides containing L-rhamnose (Caldini C et al 1994; Spagma G et al 2000), removal of hesperidin crystals from orange juices (Miyake T and Yumoto T 1999), conversion of chloropolysporin B to chloropolysporin C (Sankyo Co,1988) the derhamnosylation of many l-rhamnose containing steroids as an instance, diosgene, desglucoruscin, ginsenosides-Rg2, and so on. Whose derhamnosylated products have their medical significance (Feng B *et al* 2005; Elujoba AA and Hardman R. 1987; Yu H *et al*, 2002). In spite of α-L-rhamnosidases being biotechnologically essential enzymes, assessment article on α-L-rhamnosidases isn't always available within the literature. The α-L-rhamnosidase activities are associated with debittering proteins which are commercially referred to as hesperidinases (Monti D *et al*, 2004) and naringinases (Romero C *et al*, 1985). There are two evaluations on naringinase: the only by using Chandler and Nicol (1975) which covers literature up to 1975 and the alternative through Puri and Banerjee (2000) which covers literature up to 2000. In this text, an attempt has been made to study the current literature on α-L-rhamnosidases

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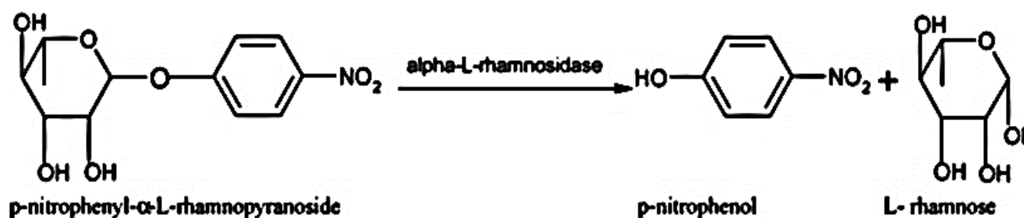
ASSESS METHODS

A handy approach for assaying the activity of α-L-rhamnosidase has been trouble before the investigators working in this enzyme since the very beginning. This problem has been solved in part through Romero *et al*. (1985). The usage of artificial substrate p-nitrophenyl-α-L-rhamnopyranoside and monitoring the liberation of p-nitrophenolate ion spectrophotometrically at 400nm the usage of molar extinction coefficient cost of 21.44mM^{-1} cm^{-1} (Reaction Scheme 2)



Reaction Scheme 2. Hydrolysis of p-nitrophenyl-α-l-rhamnopyranoside by α-l-rhamnosidase. (V. Yadav et al., 2010)

Though the artificial substrate, p-nitrophenyl-α-l-rhamnopyranoside, is commercially available with highly-priced. The method for synthesizing p-nitrophenyl-α-l-rhamnopyranoside triacetate is available inside the literature (Garegg PJ, 1975), but it requires expert synthetic organic chemists to control the experimental situations for its preparation, and it also isn't handy for biochemists, microbiologists, and biotechnologists operating on α-L-rhamnosidase.

Naringin is the maximum usually used natural substrate for assaying the activity of α-L-rhamnosidase using the Davis approach (Davis DW. 1947) α-L-rhamnosidase cleaves terminal L-rhamnose of naringin and converts it to prunin as shown in response Scheme 1(a). If d-glucosidase pastime is present alongside α-L-rhamnosidase interest, prunin is converted into naringenin and glucose as shown in response Scheme 1(b). Naringenin so produced reacts with the Davis reagent (Davis DW. 1947) to shape a lot less color than naringin. Samples of the reaction aggregate taken at intervals show a non-stop lower in coloration on reacting with the reagent. This makes it possible to comply with the course of naringin hydrolysis. The concentration of naringin at different intervals of time might be decided by way of drawing a calibration curve with the known levels of naringin. The problem with this method is that it measures the disappearance of either naringin or prunin or both and therefore it isn't precise for the assay ofα-L-rhamnosidase pastime. However, this is the simplest method which may be done with no trouble.

The α-L-rhamnosidase pastime could also be determined via tracking the formation of prunin from naringin as shown in response Scheme 1(a). The usage of HPLC as achieved through Romero *et al*, (1985) however the method needs change inside the mild of the technique mentioned by using Yusof *et al,* (1990) inside the context of willpower of naringin contents in the citrus result.

The α-L-rhamnosidase pastime can also be assayed by tracking the awareness of L-rhamnose liberated via the enzymatic hydrolysis of glycosides containing terminal L-rhamnose using Nelson and Somogyi colorimetric technique (1944; 1951). However, if other glycosidases are present in α-L-rhamnosidase enzyme sample, this technique can't be used. Several investigators have separated L-rhamnose the usage of TLC and feature quantified it for measuring the pastime of L-rhamnosidase (Victor DB *et al* 1987; Feng B *et al,* 2005) however the technique is inconvenient and time-ingesting. Use of HPLC with carbohydrate column and RI detector is a possibility but has rarely been used (Manzanares P *et al*, 1979). The activity of α-L-rhamnosidase can also be assayed by using tracking the quantity of rhamnose liberated from the enzymatic hydrolysis of a herbal substrate containing terminal L-rhamnose the use of rhamnose dehydrogenase (Bruel SL *et al*, 1979) and NAD+. This approach is expensive due to the costs of the enzyme and NAD+. For this reason a convenient method for assaying.

α-L-rhamnosidase the usage of a reasonably priced substrate is not available, and the research people

within most of the instances should increase their technique depending on the character of the problem and the centers available to them.

SOURCES

Isolation of naringinase, an enzyme complex containing α-L-rhamnosidase and β-d-glucosidase activities, has been stated inside the literature as early as 1938 from celery seeds (Hall DH, 1938). The presence of the same enzyme has been suggested in grapefruit leaves (Hall DH, 1938; Ting SV, 1958.]. The catalyst with the name rhamnodiastase, a mixture of α-L-rhamnosidase and β-d-glucosidase, has been reported from *Rhamnus dahurica* (Suzuki H. 1962). α-L-rhamnosidase has been studied from the seeds of *Fagopyrum esculentum* (Bourbouze R *et al,*1975). However, α-L-rhamnosidase from different plant sources have no longer been mentioned thus far.

The α-L-rhamnosidases from most effective two animal sources, viz. *Turbo Cornutus* liver and pig liver had been reported (Kurosawa Y *et al*, 1973; Qian S *et al,* 2005). The human intestine *Bacteroid* JY-6 and *Fusabacterium* ok-60 have been shown to provide α-L-rhamnosidase (Jang IS, Kim DH, 1996; Park S *et al,* 2005). The manufacturing of α-L-rhamnosidase with the aid of thermophilic anaerobic bacterium *Clostridium stercorarium* has been suggested (Zverlov VV, 2000). A few *Pseudoalteromonas* species and *Ralstonia pickettii*, which have been received from the ocean water of sub-Antarctic environment, display the L-rhamnosidase activity inside the low-temperature variety of −1 to 8◦C (Orrillo AG *et al*, 2007) *Sphingomonas paucimobilis* and *Bacillus* spGL1 display great α-L-rhamnosidase activities in a medium containing gellan as a carbon source Hashimoto W and Murata K, 1998; Hashimoto W *et al,* 1999). *Corticium rolfsii* produces α-L-rhamnosidase that is lively at low pH. Two new thermostable α-L-rhamnosidases from the thermophilic bacterium PRI-1686 were stated by Birgisson et al. (2004). The α-L-rhamnosidases from few Lactobacillus species were stated (Beekwilder J *et al*, 2009, McMahon H *et al*, 1999).

A few yeasts like *Saccharomyces cerevisiae*, *Hanshula anomala*, *Debaryomyces ploymorphus* show low level of α-L-rhamnosidase activities (McMahon H *et al*,1999) But, *Pichia angusta* X349 is a fantastic manufacturer of α-L-rhamnosidase (Yanai T, Sato M. 2000)

Even though a few fungal sources are patented and a few are stored secrets and techniques by way of the industries, even then α-L-rhamnosidase suggested from fungal resources are plentiful in the literature (Romero C *et al*, 1985; Chandler BV and Nicol KJ, 1975). Best two technical preparations of α-L-rhamnosidase, naringinase, and hesperidinase are to be had, and each is from fungal sources. Hesperidinase is from *Aspergillus niger,* and *Penicillium* species [20] and naringinase is from *Penicillium decumbens* (Romero C *et al,* 1985). Monti I *et al*, (2000) have proven the induction of α-L-rhamnosidase manufacturing in the fungal lines. *Acremonium persicinum* CCF 1850, *Aspergillus aculeatus* CCF 108, *A. aculeatus* CCF 3134, *A. aculeatus* CCF 3138, A. niger CCIM K2, *Aspergillus terreus* CCF 3059, *Circinella muscae* CCF 2417*, Emericella nidulans* CCF 2912, *Eurotium amstelodami* CCF 2723, *Fusarium oxysporum* CCF 906, *Mortierella alpina* CCF 2514, *Mucor circinelloides griseo-cyanus* CCIM, *Penicillium oxalicum* CCF 2430, *Rhizopus arrhizus* CCF one hundred, *Talaromyces flavus* CCF 2686 and *Trichoderma harzianum* CCF 2687 the usage of L-rhamnose, naringin, rutin, hesperidin as inducers. Shanmugam and Yadav (1995) have suggested extracellular manufacturing of α-L-rhamnosidase with the aid of *Rhizopus nigricans*. Some of *Aspergillus species* have been reported for the production of α-L-rhamnosidase (Spagma G *et al*, 2000; Yadav S and Yadav KDS, 2001; Koseki T *et al*, 2008]. Feng *et al*. (2007) have reported saponin rhamnosidase from *Curvularia lunata*. Scaroni et al. (2002) have said some mesophilic fungal strains (viz. *Aspergillus flavus*, *Mucor racemosus*, *Fusarium sambucinum*, *Aspergillus kawachii*, *Penicillium aureatiogriseum*, *Trichoderma longibrachiatum, Fusarium solani*) for the production of α-L-rhamnosidases Hughes *et al,* (2004) have characterized a α-L-rhamnosidases from the fungal pathogen of oat leaf, *Stagonospora avenae* which specifically hydrolyses a saponin avenacoside.

The distinct resources of α-L-rhamnosidases are summarized in table 1 in conjunction with a few houses of the enzymes from one-of-a-kind assets. Its miles apparent from the table that only fungal and bacterial assets of the enzyme had been explored to a degree.

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REPRESENTATION AND PURIFICATION

The α-L-rhamnosidase from the liver of *T. cornutus,* a marine gastropod, has been purified to homogeneity the usage of column chromatography with CM-cellulose and Sephadex G-one hundred fifty, warmth treatment, freezing and thawing in acidic pH (Kurosawa Y *et al,*1973). The purification of α-L-rhamnosidase from pig liver worried extraction of the enzyme with the aid of homogenizing pig liver with buffer, fractional precipitation with ammonium sulphate, dialysis and ion exchange chromatography on DEAE-cellulose (Qian S *et al*, 1996). The purification of α-L-rhamnosidase from the seeds of *F. esculentum* involved extraction of the crude enzyme, fractionation with the aid of ammonium sulphate precipitation and chromatography on columns of Sephadex G-seventy five, DEAE-Sephadex and Ultrogel AcA-44 and has been found to be natural consistent with the criteria of discgel electrophoresis (Bourbouze R *et al*, 1976).

Table 1: Sources of α-L-rhamnosidases along with their properties.

S. no.	Source	pH optima	Temperature optima (◦C)	Molecular weight	pI	Reference
1	*Fagopyrum esculentum*	-	-	70,000a	3.7	Bourbouze R *et al*, 1976
2	*Turbo cornutus*	2.8	-	-	-	Kurosawa Y *et al,*1973
3	*Pig Liver*	7	42	47,000b	-	Qian S *et al*, 1996
4	*Bacteroid* JY-6	7	-	120,000b, 240,000a	4.2	Jang IS and Kim DH, 1996
5	*Fusabacterium* K-60	5.5	-	41,000b, 170,000a	5.2	Park S *et al*, 2005
6	*Clostridium stercorarium*	7.5	60	-	-	Zverlov VV *et al,*2000
7	*Pseudoalteromonas species*	6.0	40	-	-	Orrillo AG *et al,* 2007
8	*Ralstonia pickettii*	-	-	-	-	Orrillo AG *et al,* 2007
9	*Bacillus* sp GL1	7	50	100,000b,c	-	Hashimoto W *et al*, 1999
10	PRI-1686 (RhmA & RhmB)	7.9,5-6.9	70	104,000b, 210,000a and 107,000b,	--	Birgisson H *et al,*2004
11	*Lactobacillus plantarum* NCC 245 (RhaB1 and RhaB2)	7,5	50,60	210,000a73,000b, 155,000c,a And 57,000b,100,000c,a	--	Avila M *et al,* 2009
12	*Lactobacillus plantarum*	7	-	-	-	Beekwilder J *et al,* 2009
13	*Lactobacillus acidophilus*	6	-	-	-	Beekwilder J *et al,* 2009
14	Corticium rolfsii	2	-	-	-	Kaji A and Ichimi T, 1973
15	*Pseudomonas paucimobilis* FP2001	7.8	45	112,000b,a	7.1	Miake F *et al,*2000
16	*Pichia angusta* X349	6	40	88,000b, 90,000c	4.9	Yanai Tadn Sato M. 2000
17	*Rhizopus nigricans*	6.5	60-80	-	-	Shanmugam V and Yadav

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						KDS. 1995
18	*Aspergillus aculeatus* (RhaA and RhaB)	4.5-5.0	-	92,000b and 85,000b	-	Manzanares P *et al,*2001
19	*Aspergillus aculeatus* (pnp-rhamnohydrolase and RG-rhamnohydrolase)	5.5,4	60	87,000b and 84,000b	-	Mutter M *et al,* 1994
20	*Aspergillus nidulans*	4.5-6	60	102,000b	5	Manzanares P *et al*, 2000
21	*Aspergillus terreus*	5.5	60	89,000b, 97,000a	-	Soria FF *et al*, 1999
22	*Aspergillus flavus*	6.5	50	-	-	Scaroni E *et al*, 2002
23	*Mucor racemosus*	5.5-6.5	55-60	-	-	Scaroni E *et al*, 2002
24	*Fusarium sambucinum*	5.5-6.5	50-60	-	-	Scaroni E *et al*, 2002
25	*Aspergillus kawachii*	4.5	60	-	-	Scaroni E *et al*, 2002
26	*Penicillium aureatiogriseum*	-	60	-	-	Scaroni E *et al*, 2002
27	*Trichoderma longibrachiatum*	4.5-5.5.5	60	-	-	Scaroni E *et al*, 2002
28	*Fusarium solani*	6.5	-	-	-	
29	*Curvularia lunata*	4	50	66,000b	-	Feng B *et al,* 2007
30	*Absidia sp.*	5	40	53,000b	-	Yu H *et al*, 2002
31	*Aspergillus niger*	4	50	168,000a	-	Puri M and Kalra S, 2005
32	*Aspergillus kawachii*	4	50	90,000b	-	Koseki T *et al,* 2008

a Molecular weight determined by gel filtration. ,**b** Molecular weight determined by SDS-PAGE. **c** Molecular weight determined by native-PAGE.

The α-L-rhamnosidase from the human intestinal bacterium *Bacteroides* JY-6 has been purified via disrupting the bacterial cells suspended in 20mM phosphate buffer of pH 7 by using ultrasonicator, fractionating the consequent extract by way of ammonium sulphate precipitation, column chromatography on DEAE-cellulose, Silica-PAE, Sephacryl S-300, and hydroxyapatite, respectively (Jang IS and Kim DH, 1996). The α-L-rhamnosidase from another human intestinal bacterium *Fusobacterium* k-60 has been purified the usage of a technique which concerned disruption of bacteria by way of ultrasonicator, fractionation of the protein with the aid of ammonium sulphate precipitation and column chromatography on Butyl-Toyopearl, hydroxyapatite, Sephacryl S- three hundred and Q-Sepharose. Hashimoto et al. 1999 have purified α-L-rhamnosidase of Bacillus sp. GL1 with the aid of extracting the enzyme after disrupting the cells by way of ultrasonication, ammonium sulphate precipitation and column chromatography on DEAE-Sepharose CL- 6B, Butyl-Toyopearl 650M, Sephacryl S-200HR, and QAE-Sephadex A-25. Miake *et al*, (2004) have purified and represent an intracellular α-L-rhamnosidase from *Pseudomonas paucimobilis* FP2001. The thermo strong α-L-rhamnosidase Ram A of *C. stercorarium* has been purified and characterized by using Zverlov et al (2000). Beekwilder et al. (2009) have purified α-L-rhamnosidase from *Lactobacillus plantarum* and *Lactobacillu*s acidophilus. Ávila et al. (Hughes HB) have overexpressed α-L-rhamnosidase genes from *L. plantarum* NCC 245 in *Escherichia coli,* and feature purified it.

Up to now only one α-L-rhamnosidase from a yeast supply, i.e., *P. angusta* X349 has been purified to homogeneity the use of ammonium sulphate precipitation and column chromatography on concanvalin A-Sepharose, DEAE Bio-Gel A agarose, Rhamnose-Sepharose 6B and hydroxyapatite (Yanai T and Sato M, 2000).

Roitner *et al*. (1984) have tried to signify α-L-rhamnosidase and β-d-glucosidase activities from the industrial coaching of naringinase of *A. niger* beginning. By way of gel filtration, the enzyme complex can be separated into various oligopeptides which are multiples of the smallest lively subunit with a molecular mass of 95 kDa. The oligomers show either enzymatic activity or little rhamnosidase activity. Protein fractions with glucosidase interest could not be isolated. But, infractions with rhamnosidase activity simplest, the glucosidase pastime may be restored through immobilization of the enzyme. The glucosidase activity changed into related to the concentration of protein within the answer, which disappears in a very dilute response whereas rhamnosidase turned into nevertheless active. This remark desires investigation with different α-L-rhamnosidase activities similarly. Two α-L-rhamnosidase with exceptional substrate specificities have been isolated from a commercial preparation produced by *A. aculeatus* by Mutter et al. (1994) the first was active towards p-nitrophenyl-α-l-rhamnopyranoside, naringin, and hesperidin. The second one α-L-rhamnosidase become energetic towards rhamnogalacturonan (RG fragments) liberating rhamnose. Soria *et al*, (1999) have purified α-L-rhamnosidase from the culture filtrate of *A. terreus* CECT-2663 grown on medium containing both rhamnose and naringin as a carbon source. The purification manner protected ammonium sulphate precipitation, ion exchange chromatography on DEAE-Sepharose CL-6B, gel filtration on Sephadex-G 2 hundred. They had been a hit in setting apart glucosidase interest from α-L-rhamnosidase activity when rhamnose became inducer. α-L-rhamnosidase from *Aspergillus nidulans* CECT 2544 has been purified from the culture filtrate of the fungal strain grown on L-rhamnose as the sole carbon supply via the aggregate of batch adsorption on DEAE A-50, steps of HiLoad 16/10 Q Sepharose FF column, hi Load26/S Sepharose FF column and subsequently by means of gel filtration on Superose 12 HR 10/30 column (Manzanares P *et al,* 2000.) Manzanares et al. (2001) have purified and characterised different α-L-rhamnosidases RhaA and RhaB from the subculture filtrate of *A. aculeatus* grown on hesperidin the use of cation exchange and gel filtration chromatography. Purification and characterization of ginsenoside α-L-rhamnosidase from fungus *Absidia sp.* (EECDL-39) has been said using Yu et al. (2002). The concerned purification elimination of mycelia from the lifestyle filtrate, ammonium sulphate precipitation and fractionation on Bioscale Q-2 column of BioRad. Feng et al. (2007) have purified and characterised a lively saponin rhamnosidase from *C. lunata* using ammonium sulphate precipitation, gel filtration, cation and anion trade chromatography. Purification and characterization of naringinase from *A. niger* MTCC 1344 has been stated through Puri and Kalra (2005). The purification involved awareness of the tradition filtrate through ultrafiltration, precipitation utilizing ammonium sulphate, ion exchange chromatography on Q Sepharose and gel filtration on Sephadex G-two hundred. Koseki et al. (2008) have purified α-L-rhamnosidase from the way of life filtrate of A. kawachii grown on l-rhamnose as a sole carbon source using fractional precipitation by using ammonium sulfate, HPLC on ion exchange and gel filtration columns. It became discovered to be thermostable and retained its more than eighty% pastime at 60 ◦C for one hour. The physicochemical residences of the purified α-L-rhamnosidases are summarized in table 1. The stated α-L-rhamnosidases have pH optima within the variety 2.0–8.0. The α-L-rhamnosidases having pH optima above pH 8.0 have now not been mentioned. The temperature optima of the said α-L-rhamnosidases are in the variety of 40-90◦C although one bacterial α-L-rhamnosidase energetic at 4◦C is reported (Orrillo AG *et al*, 2007). The relative molecular loads of the reported α-L-rhamnosidases are in the range 53.0–240.0 kDa although in a few instances oligomeric forms of the enzyme having relative molecular mass as high as 500 kDa had been mentioned. (Hashimoto W *et al*, 2003)

SUBSTRATE SPECIFICITY

The structure of typically used substrates is given in Fig. 1 and the substrate specificities of α-l-rhamnosidases purified from unique sources are summarized in table 2, wherein the to be had Km values using p-nitrophenyl-α-l-rhamnoside, naringin, hesperidin, rutin, quercitrin, poncirin, saikosaponin C, proscillaridin A and neohesperidin are given. The substrate specificities of the purified α-L-rhamnosidases toward -1, 2; -1, three; -1, 4 and -1, 6 glucosidic linkages are

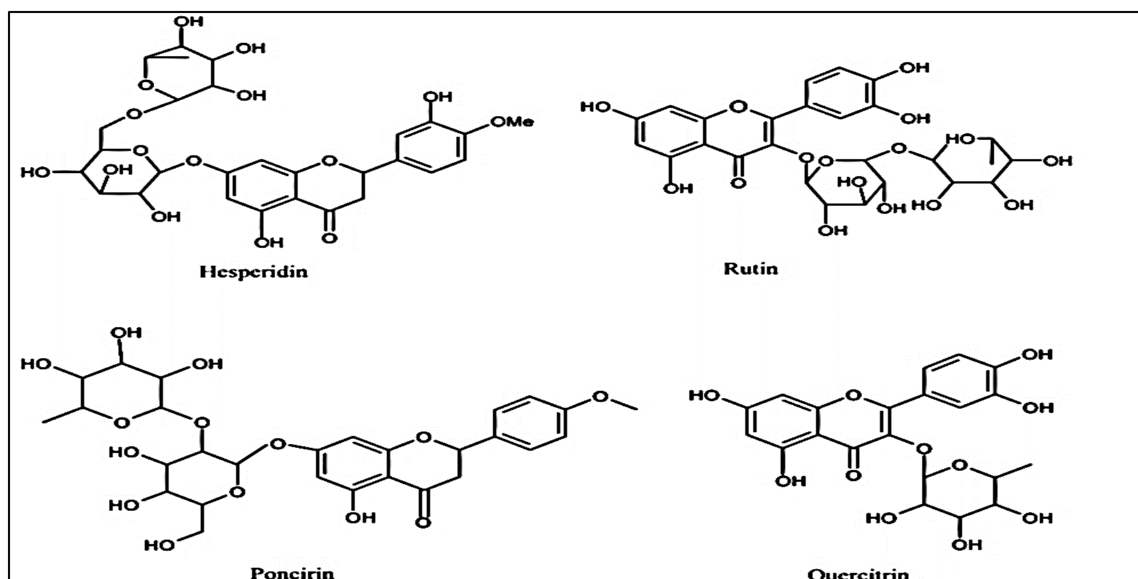

Fig. 1. Structure of some commonly used substrates. (V. Yadav et al., 2010)

Also referred to anywhere to be had. The Km values for p-nitrophenyl- α-L- rhamnoside are inside the range 0.057–2.8mM, for naringin 0.021–1.9mM, for hesperidin zero.02–1.33mM, for rutin zero.028–1.44mM, for quercitrin 0.077–0.89mM and poncirin zero.02–zero.93mM. Majority of α-L-rhamnoside are energetic on -1, 2 glucosidic linkages whereas the number of α-L-rhamnosidases lively on -1, 6 linkages comes second. There are a few α-L-rhamnosidases energetic on -1, four linkages but α-L-rhamnosidases lively on different glycosidic linkages are rare. The glucoamylase from *C. lunata* having steroidal saponin rhamnosidase activity hydrolyses a huge range of spirostanoside and furostanoside (the fascinated readers are cited

S.No	Organism	Substrate	Type Of Linkage	Km Value(mM)	Specificity	References
1	*Fagopyrum esculentum*	p-Nitrophenyl- α-L-rhamnoside, 6-O-alpha-l-rhamnosyl-d-glucopyranose	α-1 α-1,4	0.33 2.2	- -	Bourbouze R *et al*, 1976 -
2	*Bacteroides JY-6*	p-Nitrophenyl-α-L-rhamnoside Neohesperidin Naringin Poncirin Hesperidin Rutin Saikosaponin C	α -1 α -1,2 α -1,2 α -1,2 α -1,6 α -1,6 α -1,4	0.29 0.82 0.89 0.93 1.33 1.44 1.6	162.57 190.21 242.67 174.60 109.31 88.12 2.93	Jang IS and Kim DH. 1996
3	*Pseudomonas paucimobilis* FP2001	Hesperidin Proscillaridin A Rutin Naringin Saikosaponin C Quercitrin p-Nitrophenyl-α-L-rhamnoside	α -1,6 α -1 α -1,6 α -1,2 α -1,4 α -1 α -1	0.06 0.07 0.13 0.17 0.88 0.89 1.18	0.12 1.48 0.17 0.18 2.52 11.20 99.40	Miake F et al, 2000

4	Fusobacterium K-6	p-Nitrophenyl-α-L-rhamnoside Quercitrin Hesperidin Naringin Poncirin Rutin	α -1 α -1 α -1,6 α -1,2 α -1,2 α -1,6	0.057 0.077 0.022 0.021 0.020 0.028	3.40 5.00 0.52 0.34 0.35 0.07	Park S *et al,* 2005
5	Clostridium stercorarium	p-Nitrophenyl-α-L-rhamnoside Naringin Hesperidin	α -1 α -1,2 α -1,6	- - -	82 1.5 0.46	Zverlov VV *et al,* 2000
6	Bacillus spGL1 (RhaA and RhaB)	p-Nitrophenyl-α-L-rhamnoside Naringin Gellan	α -1 α -1,2 α -1,6	0.119, 0.282 - -	0.119, 0.282 - -	Hashimoto W *et al,* 2003
7	Pichia angusta X349	Naringin Rutin Hesperidin Quercitrin	α -1,2 α -1,6 α -1,6 α -1	0.119, 0.282 - - -	- - - -	Yanai T and Sato M. 2000
8	Absidia sp.	20(S)-Ginsenoside, 20(R)-ginsenoside	α -1,2,α -1,2	-	-	Yu H *et al,* 2002.
9	Aspergillus niger	Naringin	α -1,2	1.9	21	Feng B *et al,* 2007
10	Aspergillus aculeatus (RhaA and RhaB)	p-Nitrophenyl α -L-rhamnoside	α -1	0.3, 2.8	24,14	Puri M *et al,* 2005
11	Stagonospora avenae	Avenacoside- α-L -rhamnoside	α -1,4	0.091	-	Hughes HB *et al,* 2004

MECHANISM AND KINETICS

Kinetics and mechanism of α-L-rhamnosidase catalyzed reactions have rarely been studied (Zverlov *et al* 2000; Mutter M *et al,* 2000). Zverlov *et al,* (2000) have determined the form of enzymatic mechanism of RamA by using reading the hydrolytic products of p-nitrophenyl-α-L-rhamnopyranoside through the 1HNMR spectra of the products. The dynamic behavior has indicated an inverting mechanism of hydrolysis wherein β-rhamnose turned into formed from the α-rhamnoside via a single displacement mechanism but was spontaneously transformed to the α-shape by mutarotation. The α-L-rhamnosidase from *A. aculeatus* also acts as an inverting enzyme (Pitson SM *et al,* 1998) but, also studies, paintings on kinetics and mechanisms of reactions catalyzed by using α-L-rhamnosidases from excellent resources are required to recognize the molecular mechanism of catalysis with the aid of this enzyme.

INHIBITORS

Numerous investigators have mentioned inhibition of α-L-rhamnosidase with the assistance of L-rhamnose, glucose, citric acid and various metallic ions (Manzanares P *et al*, 2000, Jang IS and Kim DH, 1996; Yanai T and Sato M, 2000; Orejas M *et al*, 1999; Puri M and Kalra S, 2005). However, activity in synthesizing the α-L-rhamnosidase inhibitors has emerged due to the finding that certain compounds of this kind have displayed activity in opposition to the human immunodeficiency virus and its miles thought that activity of such compounds might also lie in their potential to inhibit glucosidase impairing processing of viral glycoprotein. There are some research papers aimed toward synthesizing robust α-L-rhamnosidase inhibitors which may additionally inhibit the activities of different glucosidases and may have the ability in pharmaceutical industries (Washiyama S *et al,*1993; Hakansson AE *et al,*

2007). Researchers on this area are also in new degree and want substantial efforts.

STRUCTURAL COMPONENTS

The crystal structure of one most effective α-L-rhamnosidase, RhaB from the Bacillus sp. GL1, is to be had at 1.9Å decision (Cui Z *el al*, 2007). The molecular mass of the enzyme is 106 kDa, and it includes 956 amino acid residues. The overall shape is shown in Fig. 2 and consists of 5 domains exact as N, D1, D2, A, and C in order of N-terminal to C-terminal. The secondary elements are shown in Fig. 2(c). Domain names N, D1, D2, and C are β-sandwiched structure whereas domain A is an (/) 6-barrel shape. Tertiary structures similar to RhaB had been searched within the protein records bank by using DALI. It's been found that RhaB shares the sizable structural similarity with chitobiose phosphorylase ChBP from *Vibrio proteolyticus* and maltose phosphorylase MalP from the *Lactobacillus brevis* even though the number one structure of RhaB is notably different from the primary structure of those two enzymes. The structure of rhamnose certain RhaB has also been determined at 2.1 Å resolution. Rhamnose binds to the deep cleft of (/) 6-barrel domain. Numerous negatively charged residues which include Asp567, Glu572, Asp579 and Glu841 interact with rhamnose and RhaB mutants of those residues substantially reduced the enzyme activity indicating that those residues are important for the enzyme catalysis and the substrate binding. There's a scientific want to crystallize α-L-rhamnosidases from other sources and to solve their crystal structures to obtain extensive structural variations in α-L-rhamnosidases

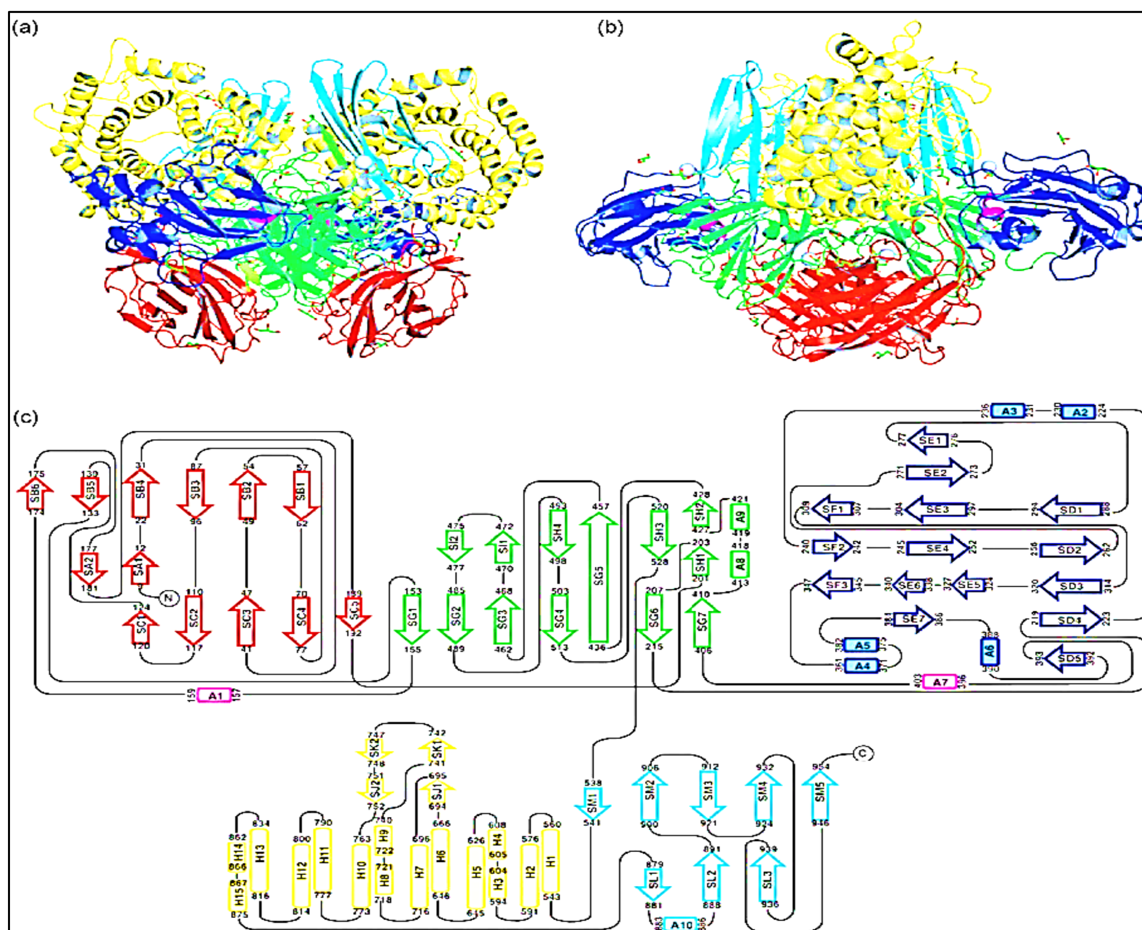

Fig. 2. The Overall structure of RhaB. (a) Forward view of the homodimer structure. (b) Side view of the structure. (c) Topology diagram of RhaB. Cylinder, α-helices; arrows, β-strands; gray ball, calcium ion; green stick and red ball glycerol. Red, domain N; blue, domain D1; green, domain D2; yellow, domain A; cyan, domain C

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MOLECULAR BIOLOGY COMPONENTS

Just a few reports on the isolation, cloning and overexpression of the gene coding for α-L-rhamnosidase are available (Zverlov VV *et al,* 2000; Beekwilder J *et al* 2009; Avila M *et*, *al*,2009; Manzanares P *et al,* 2001; Koseki T *et al*, 2008 and Hashimoto W *et al*, 2003; Miyata T *et al*, 2005). The ram A, which belongs to the new form of glycoside hydrolase family, represents the first cloned α-L-rhamnosidase gene and it changed into obtained from the anaerobe thermophilic bacterium (Zverlov VV *et al,* 2000). Two genes rhaA and rhaB from *A. aculeatus* encoding the α-L-rhamnosidases RhaA and RhaB have been cloned respectively, via the use of polyclonal antibodies (Manzanares P *et al,* 2001). The genes rhaA and rhaB from *Bacillus* sp. GL1 encoding two one of a kind the α-L-rhamnosidases were cloned in E. coli and over expressed. The RhaA of Bacillus sp. GL1 suggests 41% sequence identification with RamA of *C. stercorarium*, 23% identity with RhaB of *Bacillus* sp. GL1, even as RhaB shows the simplest 20% identification with RamA and 24% identification with RhaA and RhaB of *A. aculeatus*. Handiest RhaB becomes produced while *Bacillus* sp. GL1 mobile was grown in gellan medium. The gene ramA, rhaA and rhaB are categorised into own family GH seventy eight (Cui Z *el al*, 2007). The cloning and expression of gene rhaM encoding α-L-rhamnosidase of Sphingomonas paucimibilis FP2001 changed into performed in E. coli. The RhaM protein showed no sizable homology to different α-L-rhamnosidases of glycoside hydrolase circle of relatives seventy eight (Ito T and Takiguchi Y, 1970). Mutants of RhaB of Bacillus sp. GL1 substituting Asp-567, Glu-572, Asp-579 and Glu-841 with Asn, Gln, Asn, and Gln respectively using site-directed mutagenesis have been prepared and discovered to be of decreased enzymatic activity indicating that above amino acids residues are vital for enzymatic catalysis (Cui Z *el al*, 2007). The two α-L-rhamnosidase genes, rhmA and rhmB had been diagnosed in a partially sequenced genome of the bacterium PRI-1686. Complete genes were recovered through amplifying flanking sequences with unmarried precise primers and non-particular walking primers. The recovered genes were then cloned into E. coli (Birgisson H *et al*, 2009). Two putative rhamnosidase genes, ram1LP and ram2LP were identified within the genome of *L.plantarum*, and one rhamnosidase gene ramALA turned into identified in *L. acidophilus* genome (Beekwilder J *et al,* 2009). The two α-L-rhamnosidases genes rhaB1 and rhaB2 have been identified in operon rhaP2B2P1B1, become repressed by using glucose and brought on via L-rhamnose, displaying regulation at transcription level (Avila M *et al*, 2009). Koseki *et al.* (2008) have suggested that *a. kawachii* α-L-rhamnosidase encoding gene (Ak-rhaA) encodes for a 655 amino acid protein. The protein possesses thirteen capacity N-glycosylation recognition websites and reveals 75% of sequence identity with the α-L-rhamnosidases belonging to the glycoside hydrolase own family 78 from *A. aculeatus* and with hypothetical *Aspergillus oryzae* and *Aspergillus fumigatus* proteins. Until now, no attempt has been made to enhance the catalytic efficiency of α-L-rhamnosidase the use of the method of directed evolution or site-directed mutagenesis.

BIOTECHNOLOGICAL FEATURES OF A-L-RHAMNOSIDASE

The bitterness of citrus fruit juices is due to naringin (four, 5, 7-trihydroxy flavonone 7-rhamnoglucoside) and limonin. The bitterness because of naringin may be removed through treating the juice with α-L-rhamnosidase. The α-L-rhamnosidase hydrolyses naringin to prunin and α-L-rhamnosidase. The bitterness of pruning is best one third that of naringin. Some of the tactics for debittering of citrus fruit juices based totally on α-L-rhamnosidase are patented (Ito T and Takiguchi Y, 1970; Tanabe S, 1971; Krasnobaev V, 1973; Krasnobaev V, 1974) and several immobilized α-L-rhamnosidase arrangements for the debittering of citrus fruit juices were posted. The crystallization of soluble hesperidin inside the canned mandarin orange juice causes the turbidity to the juice (Baker RA and Tatum JH, 1986). The hesperidinase enzyme containing α-L-rhamnosidase pastime is used to prevent the turbidity of canned orange juice (Yoshinobu T *et al*, 1995). The α-L-rhamnosidase handled hesperidin and hesperidin glycosides are extraordinarily soluble in water and are free from crystal precipitation even when stored for lengthy time period (Miyake T and Yumoto T, 1999)

The volatile additives such as linalool, geraniol, nerol, citronellol, and α-terpeniol are responsible for the aroma of wines. However, most of them are a gift in the grape skin as odourless diglycosides of terpenes, viz. α-L-arabinofuranosyl-β-d-glucopyranosides, α-L-rhamnopyranosyl-d-glucopyranosides which on the two sequential hydrolysis launch risky terpenol. The immobilized α-L-rhamnosidase alongside glucosidase and -arabinosidase has been used for the aroma enhancement in wine (Caldini C *et al*, 1994). Spagma et al. (2000) have shown that purified α-L-rhamnosidase from *A. niger* will increase the aroma of model wine solution containing aromatic precursors

extracted from muscato grapes. Manzanares *et al*. (2003) have proven that rhaA gene of *A. aculeatus* that codes α-L-rhamnosidase while expressed in industrial wine strains alongside with β-d-glucosidase interest, a vast boom in α-terpeniol, nerol and linalool had been discovered in wine indicating the importance of α-L-rhamnosidase in aroma the enhancement of wine. Orejas *et al*. (1999) have proven that α-L-rhamnosidase activity is simplest slightly tormented by glucose and SO2 and in part inhibited by means of ethanol indicating a potential use of this enzyme in wine aroma release. The α-L-rhamnosidase from *P. angusta* X349 suggests excessive tolerance closer to glucose and ethanol indicating that the enzyme may be used for wine making process (Yanai T and Sato M, 2000)

IMPORTANCE IN THE PHARMACEUTICAL INDUSTRY AND BIOTRANSFORMATION

Rhamnose plays the position of chiral intermediate within the natural synthesis of pharmaceutically essential sellers and plant defensive marketers. The α-L-rhamnosidase cleaves the l-rhamnose from the glycosides that contain terminal l-rhamnose (Cheetham PSJ and Quail MA, 1991; US Patent, 1995. US Patent]. For this reason α-L-rhamnosidase has potential within the manufacture of l-rhamnose. α-L-rhamnosidase may be used in the education of many drugs and drug precursors.

α-L-rhamnosidase hydrolyses the diosgene (a saponin) to provide -l-rhamnose and diosgenin that is used within the synthesis of clinically useful steroid pills together with progesterone (Elujoba AA and Hardman R, 1987), α-L-rhamnosidase produced via C. lunata can cast off L-rhamnose from some of steroidal saponins (Feng B *et al*, 2007). Quercitin is a flavanol that's acquired via the derhamnosylation of quercitrin. It famous antioxidative, anticarcinogenic, anti-inflammatory, antiaggregatory and vasodeilating effects. The anticarcinogenic interest of hesperitin that's received through the motion of α-L-rhamnosidase on hesperidin has been proven within the laboratory animals (Erlund I. 2004). Quercitin-3-glucoside a derhamnosylated product of rutin has been stated to be antioxidant (Ting S, 2007). The ginsenoside-Rh1 obtained via the removal of L-rhamnose from ginsenosides-Rg2 reveals anticancer interest (Yu H *et al,* 2002). The glycopeptide antibiotic chlorosporin C is received via the derhamnosylation of chlorosporin B (Sankyo Co, 1988). Prunin, the derhamnosylated manufactured from naringin, has anti-inflammatory and variable interest in opposition to DNA/RNA viruses (Kaul TN *et al,* 1985).

The enzyme α-L-rhamnosidase is used inside the structural will power of polysaccharides and glycosides (Barker SA *et al*, 1965; Kamiya S *et al,* 1985; Turecek P and Pittner F, 1986). The α-L-rhamnosidase has been used to supply functional beverages (viz.: Black currant juice, orange juice and inexperienced tea infusion) that have improved amount of doubtlessly bioavailable flavonoid glucosides (Barrio RGL *et al,* 2004)

There are only a few reports which shows the pharmaceutical importance related to the derhamnosylation of natural glycoside rutin gives isoquercitrin (quercetin-3-o-b-glucoside) which is a rare product with several biological activities. It is an antithrombotic drug to treat myocardial ischemia, cerebral hypoxia and ischemic disease due to its nonoxidizable, antiinflammatory, anti-mutagenetic, anti-viral properties and other pharmacological effects, α-L-rhamnose is bound to the β-D-glucosidic residue of isoqueratrin via an a-1,6 linkage. It could be inferred that the rhamnosidase selectively hydrolyzes the a-1, 6 linkage of rutin to give isoquercitrin. Similar substrate specificity has been described for the a-L-rhamnosidases from fungi (De Winter et al. 2013; Manzanares et al. 1997) and yeasts (Yanai and Sato 2000). The biotransformation pathway of isoquercitrin by a-L-rhamnosidase breve is shown in Fig. 6. Some enzymes can selectively remove rhamnosyl moieties. Two a-L-rhamnosidases from Asp. *Aculeatus* have been used for preparing isoquercitrin, but possessed potential pathogenicity. A potential candidate rhamnosidase from *Lactobacillus plantarum* was used for biotransformation rutin, but its transformation efficacy was only 13.2 % after 24 h (Beekwilder et al. 2009). a-L-Rhamnosidase and hesperidinase from A. niger and *A. aculeatus*, respectively, were used for the successful removal of rhamnose from rutin; however, they had high b-D-glucosidase activities that decreased the specificity of the rutin biotransformation (Manzanares et al. 1997, 2003). The B. breve strain is a food-grade microorganism showing an outstanding transformation rate of rutin to isoquercitrin of 97 % in 2 h. Recombinant a-L-rhamnosidase isolated from B. breve was expressed as completely void of a-D-glucosidase activity which predestines its use for highly selective derhamnosylation and the production of such compounds as isoquercitrin.

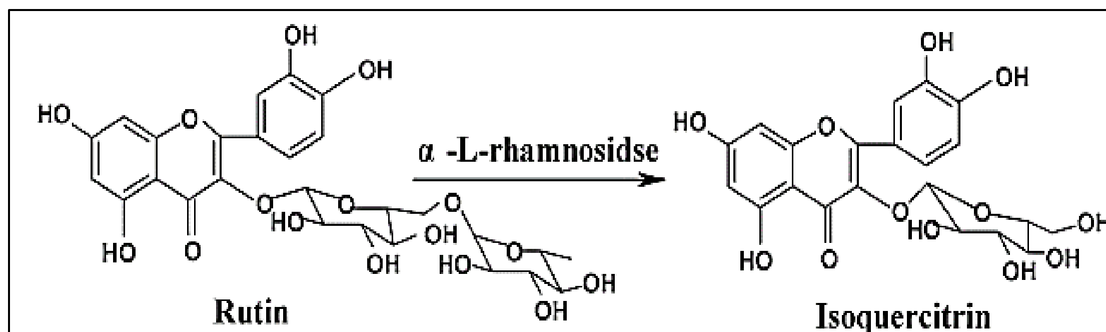

Fig. 3 Biotransformation pathway of rutin to isoquercitrin by α-L-rhamnosidase

CONCLUSION

End even though α-L-rhamnosidase were studied for extra than seven many years, its structural, purposeful and molecular biology aspects have no longer been investigated extensively as done in instances of different enzymes (Fersht A, 1999)For instance, crystal shape of best one α-L-rhamnosidase has been decided (Cui Z *et al*, 2007). Uncommon tries have been made to understand the mechanism of the enzyme catalyzed reaction on molecular degree [41]. The tries to use web page directed mutagenesis for knowledge the molecular mechanism of the catalysis have hardly ever been made (Cui Z *et al*, 2007). No strive has been made to improve the catalytic efficiency of this enzyme the usage of directed evolution strategies (Fersht A, 1999; Arnold EH and Georgion G, 2003). The studies in the above guidelines on α-L-rhamnosidase from exceptional assets are needed to recognize the function of this biotechnologically and pharmaceutically crucial enzyme.

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