Simple screening procedure for microorganisms to degrade amygdalin.

Biotechnology techniques

Brimer, L.; Tunçel, G.; Nout, M.J.R.

<https://doi.org/10.1007/bf00151870>

This publication is made publicly available in the institutional repository of Wageningen University and Research, under the terms of article 25fa of the Dutch Copyright Act, also known as the Amendment Taverne. This has been done with explicit consent by the author.

Article 25fa states that the author of a short scientific work funded either wholly or partially by Dutch public funds is entitled to make that work publicly available for no consideration following a reasonable period of time after the work was first published, provided that clear reference is made to the source of the first publication of the work.

This publication is distributed under The Association of Universities in the Netherlands (VSNU) 'Article 25fa implementation' project. In this project research outputs of researchers employed by Dutch Universities that comply with the legal requirements of Article 25fa of the Dutch Copyright Act are distributed online and free of cost or other barriers in institutional repositories. Research outputs are distributed six months after their first online publication in the original published version and with proper attribution to the source of the original publication.

You are permitted to download and use the publication for personal purposes. All rights remain with the author(s) and / or copyright owner(s) of this work. Any use of the publication or parts of it other than authorised under article 25fa of the Dutch Copyright act is prohibited. Wageningen University & Research and the author(s) of this publication shall not be held responsible or liable for any damages resulting from your (re)use of this publication.

For questions regarding the public availability of this publication please contact openscience.library@wur.nl

SIMPLE SCREENING PROCEDURE FOR MICROORGANISMS TO DEGRADE
AMYGDALIN

L. Brimer¹, G. Tunçel², and M.J.R. Nout^{3*}

¹Department of Pharmacology and Pathobiology, The Royal Veterinary and Agricultural University, 13 Bülowsvej, DK-1870 Frederiksberg C, Copenhagen, Denmark; ²Faculty of Engineering, Ege University, 35100 Bornova-Izmir, Turkey; ³Department of Food Science, Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands.

Summary:

Amygdalin is a cyanogenic glycoside occurring among others in almonds and bitter apricot seeds. Utilization of seeds for human or animal nutrition requires adequate detoxification. The present method for screening microbial cultures for their ability to degrade amygdalin is a convenient micro-titre version based on the picrate reaction principle.

Introduction:

Bitter apricot seeds are by-products of the apricot processing industry. The seeds have a high protein content and could be used as a food or feed ingredient. However, they contain approximately 50 nmol of the potentially toxic cyanogenic glycosides amygdalin and prunasin per mg. We reported earlier (Tunçel et al. 1990) on prospects for their detoxification by biotechnological means, i.e. by using the endogenous enzymes combined with microbial fermentation. The tempe fermentation process using the fungus *Rhizopus oligosporus* as inoculant enabled about 70% of total cyanide potential to be removed. As we considered this inadequate from toxicological point of view, we developed a simple method based on the picrate test (Guignard 1906), as used by Brimer and Moelgaard (1986), to screen microbial strains for their ability to degrade amygdalin by hydrolysis. This paper describes the method and gives selected preliminary data on amygdalin degradation by lactic acid bacteria, yeasts and filamentous fungi.

Experimental methods:

Materials: D-Amygdalin (Sigma St.Louis USA, A6005) was used for standard as a standard as a 1mM solution in distilled

water. Pectinase (EC 3.2.1.15) from *Rhizopus* sp. (Sigma St.Louis USA, P2401) was used as a source of hydrolytic enzymes (Brimer and Rosling 1993), for positive controls, as a 0.2% w/v solution in distilled water. Picrate reagent sheets were prepared according to Brimer et al. (1983) as follows: pre-coated ion-exchange sheets (Polygram ionex 25-SB-Ac, Machery-Nagel, Düren, Germany) were cut to size (dimensions of microtiter plate), and were impregnated by immersion (plastic side up) in saturated picric acid aqueous solution, followed by blotting on absorbent paper, drying with hot air (hairdryer), immersion in 1 M Na₂CO₃ solution (plastic side up), and drying with hairdryer. Microbial cultures from to be tested were suspended (10 mg wet weight/mL) in sterile saline (8.5 g NaCl/L).

Procedure: In a microtiter plate, the following wells are prepared: (a) positive control (4 wells/plate): 20 µL amygdalin + 20 µL pectinase + 180 µL saline; (b) negative control (4 wells/plate): 20 µL amygdalin + 200 µL saline; (c) test (8 cultures in duplicate/plate): 20 µL amygdalin + 200 µL microbial suspension. Place a picrate reagent sheet (plastic side up) on top of the microtiter plate, weigh it down gently with a flat object of approx. 100 g, and incubate at 25°C overnight.

Assessment: The red-brown spots on yellow background, typical of positive reactions, were assessed either qualitatively by visually estimating their colour intensity as 0,1,2,3; or semi-quantitatively by measuring the extinction of reflected light at 550 nm using a microtitre plate reader. The extent of amygdalin degradation is calculated as a fraction % of the extinction produced by the positive control.

Results and Discussion:

Table 1 presents the results of the qualitative assessment of a number of lactic acid bacteria, yeasts and filamentous fungi. In Table 2 the semi-quantitative assessment of amygdalin degradation by selected food-grade micro-organisms is presented.

From Table 1 it can be seen that approximately 45% of the tested lactic acid bacteria (mainly *L.plantarum* strains) have a weak to medium ability to degrade amygdalin, the rest being negative. On the other hand, about 71% of the tested yeasts and 96% of the filamentous fungi have a medium to strong ability to degrade amygdalin.

The data of Table 2 indicate firstly that not all strains having a score "3" in Table 1 give a high percentage of degradation. Besides variations concerning cell numbers etc., this is caused mainly by the difficulty of making visual distinctions between -especially strong- colour intensities. All showing a score of 2 or 3 do, however, as anticipated show

degradation in the second experiment. Table 2 also indicates that some of the cultures of yeasts and filamentous fungi are much more powerful amygdalin degraders compared with lactic acid bacteria.

Table 1. Ability of lactic acid bacteria, yeasts and filamentous fungi to degrade amygdalin.

<u>Lactic acid bacteria:</u>	<u>Code*:</u>	<u>Score**:</u>
<i>Enterococcus faecalis</i>	9790	0
<i>Enterococcus faecalis</i>	8043	0
<i>Lactobacillus acidophilus</i>	SB002	0
<i>Lactobacillus acidophilus</i>	SB003	0
<i>Lactobacillus acidophilus</i> 1	SB004	0
<i>Lactobacillus acidophilus</i> 1	SB006	0
<i>Lactobacillus acidophilus</i> 1	SB17	1
<i>Lactobacillus acidophilus</i>	SB023	1
<i>Lactobacillus acidophilus</i>	SB026	1
<i>Lactobacillus acidophilus</i>	SB044	0
<i>Lactobacillus brevis</i>	SB001	1
<i>Lactobacillus casei</i>	-	0
<i>Lactobacillus casei</i> var. <i>casei</i>	-	0
<i>Lactobacillus casei</i> var. <i>tolerans</i>	-	0
<i>Lactobacillus coprophilus</i>	SB019	0
<i>Lactobacillus delbrückii</i>	SB024	1
<i>Lactobacillus fermentum</i>	20049	0
<i>Lactobacillus plantarum</i>	SB035	0
<i>Lactobacillus plantarum</i>	SG1	2
<i>Lactobacillus plantarum</i>	845	2
<i>Lactobacillus plantarum</i>	P743	1
<i>Lactobacillus plantarum</i>	111	2
<i>Lactobacillus plantarum</i>	MS7	1
<i>Lactobacillus plantarum</i>	C31	1
<i>Lactobacillus plantarum</i>	832	2
<i>Lactococcus lactis</i>	PC1	0
<i>Pediococcus acidilactici</i>	M301	0
<i>Pediococcus acidilactici</i>	-	2
<i>Pediococcus acidilactici</i>	M324	0
 <u>Yeasts:</u>		
<i>Candida guilliermondii</i>	132	1
<i>Candida intermedia</i>	121	2
<i>Candida lusitanae</i>	123	3
<i>Candida maltosa</i> komagata	124	2
<i>Hanseniaspora valbyensis</i>	263	3
<i>Hansenula anomala</i>	261	1
<i>Hansenula fabianii</i>	262	1
<i>Kluyveromyces marxianus</i>	321	2
<i>Pichia anomala</i>	530	2
<i>Pichia guilliermondii</i>	502	0
<i>Rhodotorula mucilaginosa</i>	586	0
<i>Rhodotorula rubra</i>	596	0

<u>Yeasts (continued)</u>	<u>Code</u>	<u>Score</u>
<i>Saccharomyces cerevisiae</i>	672	0
<i>Saccharomyces cerevisiae</i>	671	0
<i>Saccharomycopsis fibuligera</i>	677	3
<i>Saccharomycopsis fibuligera</i>	676	2
<i>Schizosaccharomyces pombe</i>	685	1
<i>Schizosaccharomyces pombe</i>	686	0
<i>Trichosporon beigelii</i>	692	2
<i>Yarrowia lipolytica</i>	771	1
<i>Zygosaccharomyces bailii</i>	781	1

Filamentous fungi:

<i>Aspergillus oryzae</i>	007	2
<i>Aspergillus oryzae</i> var. <i>brunneus</i>	008	2
<i>Aspergillus oryzae</i> var. <i>effusus</i>	009	1
<i>Aspergillus tamarii</i>	015	2
<i>Botrytis cinerea</i>	101	1
<i>Geotrichum candidum</i>	241	1
<i>Geotrichum candidum</i>	242	0
<i>Mucor hiemalis</i>	363	2
<i>Mucor circinelloides</i>	M40	3
<i>Mucor circinelloides</i>	361	3
<i>Mucor circinelloides</i>	362	3
<i>Mucor indicus</i>	365	2
<i>Neosartorya fischeri</i>	431	2
<i>Neurospora</i> sp.	-	3
<i>Penicillium brevicompactum</i>	514	1
<i>Penicillium camemberti</i>	504	1
<i>Penicillium nalgiovense</i>	520	3
<i>Pleurotus sajor-caju</i>	503	2
<i>Rhizopus microsporus</i>	573	2
<i>Rhizopus oligosporus</i> NRRL5905	575	2
<i>Rhizopus oligosporus</i>	578	2
<i>Rhizopus oryzae</i>	580	3
<i>Rhizopus oryzae</i>	581	2
<i>Rhizopus oryzae</i>	582	1
<i>Syncephalastrum racemosum</i>	681	3

*= from the collection of the Department of Food Science, Agricultural University, Wageningen;

**= score according to colour intensity of picrate test (qualitative).

Table 2. Semi-quantitative assessment of ability to degrade amygdalin by selected food-grade micro-organisms.

culture	Code*	Score**	%degradation
<u>Bacteria:</u>			
<i>Lactobacillus plantarum</i>	832	2	13
<i>Lactobacillus plantarum</i>	SG1	2	27
<i>Lactobacillus plantarum</i>	845	2	26
<i>Lactobacillus plantarum</i>	111	2	24
<i>Pediococcus acidilactici</i>	-	2	26
<u>Yeasts:</u>			
<i>Candida lusitaniae</i>	123	3	5
<i>Hanseniaspora valbyensis</i>	263	3	95
<i>Saccharomycopsis fibuligera</i>	677	3	98
<u>Filamentous fungi:</u>			
<i>Mucor circinelloides</i>	361	3	15
<i>Mucor circinelloides</i>	362	3	99
<i>Mucor circinelloides</i>	M40	3	95
<i>Neurospora sp.</i>	-	3	4
<i>Penicillium nalgiovense</i>	520	3	95
<i>Rhizopus oligosporus</i>	575	2	9
<i>Rhizopus oryzae</i>	580	3	40
<i>Syncephalastrum racemosum</i>	681	3	6

*, **: as in Table 1.

Acknowledgement:

The financial support by NATO Collaborative Research Grant 920009 is gratefully acknowledged.

References:

- Brimer, L., Brøgger Christensen, S., Moelgaard, P. and Nartey, F. (1983). *J.Agric.Food Chem.* 31, 789-793.
- Brimer, L. and Molgaard, P. (1986). *Biochem. Systematics and Ecol.* 14, 97-103.
- Brimer, L. and Rosling, H. (1993). *Food Chem. Toxicol.* in press.
- Guignard, M.L. (1906). *C.R. Hebd. Seances Acad. Sci.* 142, 545-553.
- Tunçel, G., Nout, M.J.R., Brimer, L. and Göktan, D. (1990). *Int. J. Food Microbiol.* 11, 337-344.