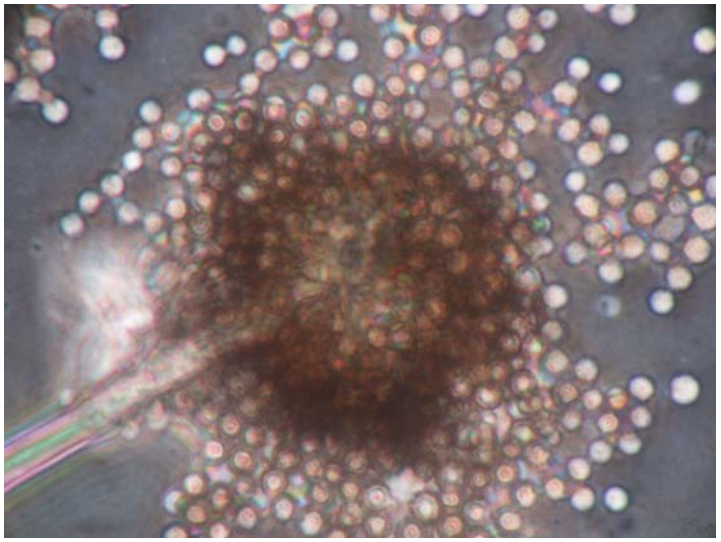
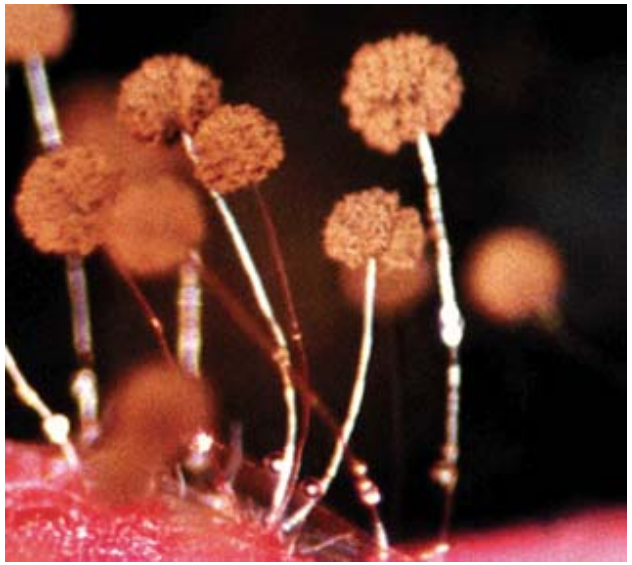
REGULATORY ASPECTS OF ASPERGILLUS NOMENCLATURE





Piet W.M. van Dijck

Aspergillus Systematics in the Genomic Era – an International Workshop

Utrecht, April 12-14 2007

DSM Food Specialties

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REGULATORY ASPECTS OF ASPERGILLUS NOMENCLATURE

Piet W. M. van Dijck
DSM Nutritional Products, Delft, The Netherlands

Aspergillus species, in particular *A. niger* and *A. oryzae*, have since long been used in biotechnology for the production of citric acid and enzymes for the food and feed industry. Commercial production of citric acid started in 1919 at Citrique Belge (Tienen) by surface fermentation of *A. niger* on beet molasses. Although still in use, citric acid and other organic acids today are primarily produced by submerged fermentations. Production of enzymes also started more than half a century ago, first also as surface fermentations but now also predominantly as submerged fermentations.
Regulatory approvals for microbial products are given for the combination of the product with the production organism. For instance in the US the UD FDA has approved several enzymes produced with *A. niger* as GRAS (Generally regarded as safe) on a history of safe use. This means that the product e.g. pectinase was on the market for a given application (e.g. fruit processing) before 1958, the year when the US adoption the GRAS legislative system for food products. So if a producer wants to introduce a new pectinase enzyme in the US market and he can demonstrate that it is produced with a strain of *A. niger* he can legally do this without having to submit a new dossier.
Therefore when due to progress in taxonomy name changes are proposed regulatory authorities (mostly layman with no feeling for taxonomy) will become concerned because the combination of product and producing organism is not present on the positive lists of approved combinations.
A second problem which may arise is that as a consequence of a strain improvement program specific characteristics of a strain are lost to such an extent that proper identification of a production strain becomes difficult.

Aspergillus niger and A. oryzae

Micro-organisms

Current overview of the micro-organisms used by DSM and their applications
2007

I: classical micro-organism; II: homologous or self-cloned GMM (genetically modified micro-organism); III heterologous GMM

Production Micro-organism	I	II	III	Product	Food	Feed	Industrial (Pharma & Fine Chemicals)
Aspergilus niger	●	●		Acid amylase	●	-	-
Aspergilus niger		●		Asparaginase	●	-	-
Aspergilus niger	●			Arabanase	●	-	-
Aspergilus niger	●	●		Arabinofuranosidase	●	-	-
Aspergilus niger	●			Beta-galactosidase	●	-	-
Aspergilus niger	●			Citric acid	●	●	●
Aspergilus niger		●		Fungal lipase	●	-	-
Aspergilus niger	●			Glucoamylase	●	-	-
Aspergilus niger	●			Glucose oxidase	●	-	-
Aspergilus niger	●			Glycosidase	●		
Aspergilus niger	●			Hemicellulase	●	-	-
Aspergilus niger	●			Pectinase	●	-	-
Aspergilus niger	●	●		Pectin methylesterase	●	-	-
Aspergilus niger			●	Phospholipase A2	●	-	-
Aspergilus niger		●		Phytase	●	●	-
Aspergilus niger		●		Polygalacturonase	●		
Aspergilus niger	●	●		Protease, endo-	●	-	-
Aspergilus niger	●	●		Xylanase	●	●	-
Aspergilus niger			●	Xylanase, thermostable	●	●	-
Aspergilus niger var. *awamori*			●	Lactoferrin			●
Aspergillus oryzae	●			Fungal amylase	●	-	-
Aspergillus oryzae		●		Acid lactase	●	-	-

§173.120	Carbohydrase and cellulase derived from *Aspergillus niger* for use in clam and shrimp processing
§173.130	Carbohydrase derived from *Rhizopus oryzae* for use in the production of dextrose from starch
§173.135	Catalase derived from *Micrococcus lysodeikticus* for use in the manufacture of cheese
§173.140	Esterase-lipase derived from *Mucor miehei* var. Cooney *et* Emerson as a flavor enhancer in cheeses, fats and oils, and milk products
§173.145	*Alpha*-galactosidase derived from *Morteirella vinaceae* var. *raffinoseutilizer* for use in the production of sucrose from sugar beets
§173.150	Milk-clotting enzymes, microbial for use in the production of cheese (Milk-clotting enzymes are derived from *Endothia parasitica*, *Baci. cereus*, *Mucor pusillus Lindt* and *Mucor miehei* and *Aspergillus oryzae* modified to contain the gene for aspartic proteinase from *Rhizomucor miehei* var *Cooney et Emerson*
§173.160	*Candida guilliermondii* as the organism for fermentation production of citric acid
§173.165	*Candida lipolytica* for fermentation production of citric acid.
§173.280	A solvent extraction process for recovery of citric acid from *Aspergillus niger* fermentation liquor

Many enzymes made by *A. niger* or *A. oryzae* are GRAS

90	Carbohydrase enzyme preparation from *Aspergillus oryzae*, protease enzyme preparation from *Aspergillus oryzae*, and carbohydrase enzyme preparation from *Rhizopus oryzae*	FDA has no questions
		Additional correspondence
89	Five enzyme preparations from *Aspergillus niger*: Carbohydrase enzyme preparation, catalase enzyme preparation, glucose oxidase enzyme preparation, pectinase enzyme preparation, and protease enzyme preparation	FDA has no questions
		Additional correspondence
113	Lipase enzyme preparation from *Aspergillus oryzae*	FDA has no questions
112	Phytosterols	FDA has no questions
111	Lipase enzyme preparation from *Aspergillus niger*	FDA has no questions
132	Lactase enzyme preparation from *Aspergillus niger*	FDA has no questions

Also from recombinant strains

GRAS Notices Received in 2005

Details about Notices Received in 2005 (GRN No. 163-188)		
GRN No.	**Substance**	**FDA's Letter**
188	Carbon monoxide	Pending
187	L(+) Tartaric acid (alternative method of manufacture)	FDA has no questions
186	Soy lecithin enzymatically modified to have increased phosphatidylserine	FDA has no questions
185	Concentrated tomato lycopene extract	FDA has no questions
184	Isomaltulose	FDA has no questions
183	Phospholipase A2 enzyme preparation from *Aspergillus niger* expressing a gene encoding a porcine phospholipase A2	FDA has no questions
182	Hydrolyzed wheat gluten isolate; pea protein isolate	FDA has no questions

GRAS Notices Received in 2003

Details about Notices Received in 2003 (GRN Nos. 120 - 142)		
GRN No.	**Substance**	**FDA's Letter**
142	Phospholipase enzyme preparation from *Aspergillus oryzae* expressing the gene encoding a phospholipase A1 from *Fusarium venenatum*	FDA has no questions

GRAS Notices Received in 2006

Details about Notices Received in 2006 (GRN No. 189-218)

GRN No.	Substance	FDA's Letter
218	Bacteriophage P100 preparation from *Listeria innocua*	Pending
217	Tailored tryglycerides containing approximately 12 percent medium-chain fatty acids	Pending
216	Lipase enzyme preparation from *Rhizopus oryzae*	Pending
215	*Actinidia arguta* extract	Pending
214	Asparaginase enzyme preparation from *Aspergillus niger*	Pending
213	Hydroxypropyl methylcellulose	Pending

201	Asparaginase enzyme preparation from *Aspergillus oryzae* expressing the asparaginase gene from *A. oryzae*	FDA has no questions

Postive list BRASIL

ENZYMES OF MICROBIAL ORIGIN

Name of Enzyme or Complex	Source(s)
Alpha-acetoacetate decarboxylase	*Bacillus brevis* expressed in *Bacillus subtilis*
Alpha-amylase	*Aspergillus niger* *Aspergillus oryzae* *Bacillus licheniformis* *Bacillus licheniformis* expressed in *Bacillus licheniformis* *Bacillus licheniformis* e *Bacillus amyloliquefaciens* expressed in *Bacillus licheniformis* *Bacillus megaterium* expressed in *Bacillus subtilis* *Bacillus stearothermophilus* *Bacillus stearothermophilus* expressed in *Bacillus licheniformis* *Bacillus stearothermophilus* expressed in *Bacillus subtilis* *Bacillus subtilis* *Pseudomonas fluorescens* *Rhizopus delemar* *Rhizopus oryzae*
Alpha-galactosidase	*Aspergillus niger* *Mortierella vinacea* *Saccharomyces carlsbergensis*

Positive list MEXICO

QUINTO. En la elaboración de alimentos, bebidas y suplementos alimenticios, sólo se podrán emplear la enzimas que se señalan a continuación:

Nombre común	Fuente	Número EC	Nombres químicos y sinónimos
Alfa acetato descarboxilasa	- *Bacillus subtilis*, con un gen de *Bacillus brevis*	4.1.1.5	- (S)-2-hidroxi-2-metil-oxobutenato carboxilasa
Alfa amilasa	- *Aspergillus niger* - *Aspergillus oryzae* - *Bacillus amyloliquefaciens* - *Bacillus licheniformis* - *Bacillus licheniformis* con un gen de *Bacillus licheniformis* - *Bacillus licheniformis* con un gen modificado de *Bacillus licheniformis* - *Bacillus licheniformis* con un gen modificado de *Bacillus stearothermophilus* - *Bacillus subtilis* - *Rhizopus oryzae* - Malta de cebada	3.2.1.1	- 1,4-alfa-D-glucano glucanohidrolasa - Diastasa - Glicogenasa - Ptialina
Alfa galactosidasa	- *Morteirella vinacea*, var. *Raffino ceutilizer*	3.2.1.22	
Amiloglucosidasa	- *Aspergillus niger* - *Aspergillus niger* con un gen de *Aspergillus niger*	3.2.1.3	- 1,4-alfa-D-glucano glucohidrasa - Glucoamilasa - Maltasa ácida

Positive list AUSTRALIA / NEW ZEALAND

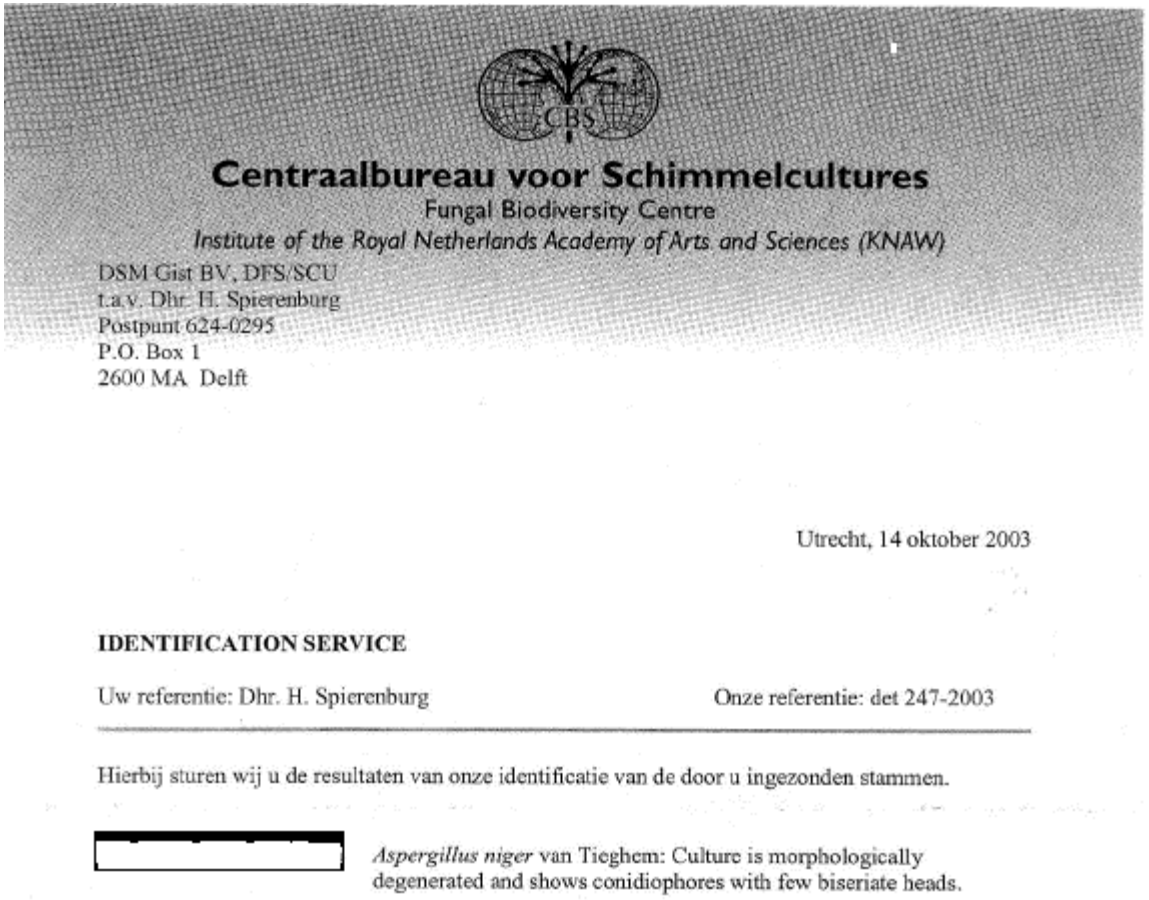
Enzyme	Source
α–Acetolactate decarboxylase EC [4.1.1.5]	*Bacillus subtilis* *Bacillus subtilis*, containing the gene for α–Acetolactate decarboxylase isolated from *Bacillus brevis*
Aminopeptidase EC [3.4.11.1]	*Lactococcocus lactis* *Aspergillus oryzae*
α–Amylase EC [3.2.1.1]	*Aspergillus niger* *Aspergillus oryzae* *Bacillus licheniformis* *Bacillus licheniformis*, containing the gene for α-Amylase isolated from *Bacillus stearothermophilus* *Bacillus stearothermophilus* *Bacillus subtilis* *Bacillus subtilis*, containing the gene for α-Amylase isolated from *Bacillus stearothermophilus*
β-Amylase EC [3.2.1.2]	*Bacillus subtilis*
Arabinase EC [3.2.1.99]	*Aspergillus niger*
Arabino-furanosidase EC [3.2.1.55]	*Aspergillus niger*
Carboxyl proteinase EC [3.4.23.6]	*Aspergillus melleus* *Aspergillus niger* *Aspergillus oryzae* *Rhizomucor miehei*
Catalase EC [1.11.1.6]	*Aspergillus niger* *Micrococcus luteus*
Cellulase EC [3.2.1.4]	*Aspergillus niger* *Trichoderma reesei* *Trichoderma viride*
Chymosin EC [3.4.23.4]	*Aspergillus niger* *Escherichia coli* K-12 strain GE81 *Kluyveromyces lactis*

A. Niger is not always *A. niger*

Editorial note:

Bacillus subtilis covers the strain known under the name *Bacillus amyloliquefaciens*.
The *Aspergillus niger* group covers strains known under the names *Aspergillus aculeatus, A. awamori, A. ficuum, A. foetidus, A. japonicus, A. phoenicis, A. saitor* and *A. usamii*.
Trichoderma reesei is also known as *Trichoderma longibrachiatum*.
Saccharomyces fragilis is also known as *Kluyveromyces fragilis* and *Kluyveromyces marxianus var. marxianus*.
Saccharomyces lactis is also known as *Kluyveromyces lactis*.
Mucor miehei is the former name for *Rhizomucor miehei*.
Micrococcus lysodeikticus is the former name for *Microccocus luteus*.
Bacillus macerans is the former name for *Paenibacillus macerans*.
Penicillium emersonii is the former name for *Talaromyces emersonii*.
Klebsiella aerogenes is the former name for *Klebsiella pneumoniae*.
Streptoverticillium mobaraense is the former name for *Streptomyces mobaraense*.
Humicola lanuginosa is also known as *Thermomyces lanuginosus*.
Mucor javanicus is also known as *Mucor circinelloides* f. *circinelloides*.
Penicillium roquefortii is also known as *Penicillium roqueforti*
Hansenula polymorpha is also known as *Pichia angusta*.

Improved strains sometimes are difficult to identify



Centraalbureau voor Schimmelcultures
Fungal Biodiversity Centre
Institute of the Royal Netherlands Academy of Arts and Sciences (KNAW)

DSM Gist BV, DFS/SCU
t.a.v. Dhr. H. Spierenburg
Postpunt 624-0295
P.O. Box 1
2600 MA Delft

Utrecht, 14 oktober 2003

IDENTIFICATION SERVICE

Uw referentie: Dhr. H. Spierenburg Onze referentie: det 247-2003

Hierbij sturen wij u de resultaten van onze identificatie van de door u ingezonden stammen.

[redacted] *Aspergillus niger* van Tieghem: Culture is morphologically degenerated and shows conidiophores with few biseriate heads.

REGULATORS TEND TO ASK FOR IDENTIFICATION USING GENETIC TOOLS

Mycological methods to be used for identification of moulds:

Filamentous fungi are traditionally identified to genus level by phenotypic characters, such as morphological and cultural characteristics. Unfortunately, there is not one universal mycological textbook or reference compendium which is used for identification of moulds, which makes identification to genus level a highly subjective task. This is further complicated by the necessity to identify fungal strains to the species level as each species within a genus may have very different functional characters, *e.g.* mycotoxin profiles and physiological properties. Again, traditional methods like morphological and cultural characteristics are widely used but also profiles of extrolites have been used within some genera. Phenotypic characteristics do vary according to growth conditions which makes it difficult to construct robust identification keys. No identification key covers all species, so it is recommended seeking advice for identification procedures by contacting specialists in food, feed and industrial mycology – *e.g.* via the International Commission on Food Mycology (ICFM) (http://www.foodmycology.org), which can direct inquiries to recommended specialists.

For filamentous fungi the use of molecular methods for classification and identification is less developed than in the case for bacteria and yeasts. On the other hand, in combination with