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MINI-REVIEW

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On the safety of *Aspergillus niger* – a review

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Abstract *Aspergillus niger* is one of the most important microorganisms used in biotechnology. It has been in use already for many decades to produce extracellular (food) enzymes and citric acid. In fact, citric acid and many *A. niger* enzymes are considered GRAS by the United States Food and Drug Administration. In addition, *A. niger* is used for biotransformations and waste treatment. In the last two decades, *A. niger* has been developed as an important transformation host to over-express food enzymes. Being pre-dated by older names, the name *A. niger* has been conserved for economical and information retrieval reasons and there is a taxonomical consensus based on molecular data that the only other common species closely related to *A. niger* in the *Aspergillus* series Nigri is *A. tubingensis*. *A. niger*, like other filamentous fungi, should be treated carefully to avoid the formation of spore dust. However, compared with other filamentous fungi, it does not stand out as a particular problem concerning allergy or mycopathology. A few medical cases, e.g. lung infections, have been reported, but always in severely immunocompromised patients. In tropical areas, ear infections (otomycosis) do occur due to *A. niger* invasion of the outer ear canal but this may be caused by mechanical damage of the skin barrier. *A. niger* strains produce a series of secondary metabolites, but it is only ochratoxin A that can be regarded as a mycotoxin in the strict sense of the word. Only 3–10% of the strains examined for ochratoxin A production have tested positive under favourable conditions. New and unknown isolates should be checked for ochratoxin A production before they are developed as production organisms. It is concluded, with these restrictions, that *A. niger* is a safe production organism.

Introduction

Aspergillus niger has been the subject of research and industrial use for several decades. It first acquired practical importance in 1919, when its ability to produce citric acid was industrially exploited. Gluconic and fumaric acids have been produced with *A. niger*, although they are of less economic importance. However, since the 1960s, *A. niger* has become a source of a variety of enzymes that are well established as technical aids in fruit processing, baking, and in the starch and food industries. Gene technology has been successfully applied to improve production processes and to make use of *A. niger* as an expression system for foreign proteins. The intense research over the past decade has resulted in a range of new processes and products.

Ecology

Many black *Aspergilli* have been isolated from all over the world. *A. niger* is a filamentous fungus growing aerobically on organic matter. In nature, it is found in soil and litter, in compost and on decaying plant material. Reiss (1986) collected data on the influence of temperature, water activity and pH on the growth of various

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Aspergilli. *A. niger* is able to grow in the wide temperature range of 6–47°C with a relatively high temperature optimum at 35–37°C. The water activity limit for growth is 0.88, which is relatively high compared with other *Aspergillus* species. *A. niger* is able to grow over an extremely wide pH range: 1.4–9.8. These abilities and the profuse production of conidiospores, which are distributed via the air, secure the ubiquitous occurrence of the species, with a higher frequency in warm and humid places (Rippel-Baldes 1955).

Taxonomy

Raper and Fennel (1965) divided the genus *Aspergillus* into groups according to the colour of the conidiospores. *Aspergilli* with brown to black-shaded spores constitute the *A. niger* group. Although the members of this group vary considerably, only a few differ so clearly from the majority that they can easily be classified as separate species (e.g. *A. carbonarius*, *A. japonicus*, *A. ellipticus*, *A. heteromorphus* and *A. aculeatus*). Most of the brown to black *Aspergilli* belong to the other group of species, which are difficult to distinguish: *A. ficuum*, *A. phoenicis*, *A. niger* and *A. awamori* being the most prominent. In practice, this group of species is often together called *A. niger* van Tieghem.

The apparently insignificant differences between members of the *A. niger* group were the decisive reasons for Al-Musallam (1980) to classify some species as varieties of *A. niger*, while Raper and Fennell considered them to be separate species.

Introducing restriction fragment length polymorphism (RFLP) analysis to *Aspergillus* taxonomy, Kusters-van Someren et al. (1990, 1991) analysed the ribosomal banding patterns and the hybridisation patterns of genomic digests from strains in the *A. niger* group, using pectin lyase genes as probes for hybridisation. They proposed a different, but in their opinion more reliable, classification of the *A. niger* group, reducing the number of species from 13 (Raper and Fennel 1965) to 6: *A. carbonarius*, *A. japonicus*, *A. ellipticus*, *A. heteromorphus*, *A. niger* and *A. tubingensis*, the latter of which consists of strains formerly called *A. niger*. The difference between *A. niger* and *A. tubingensis* has been repeatedly confirmed in further studies using RFLP of mitochondrial DNAs and ribosomal repeat units (Varga et al. 1993, 1994; Parenicová et al. 1997), but also in studies using randomly amplified polymorphic DNA (Megnegneau et al. 1993); internal transcribed spacer sequence data (Accensi et al. 1999; Parenicová et al. 2001), and nuclear genes encoding polygalacturonases, arabinoxylan-arabinofuranohydrolase and xylanases (Bussink et al. 1991; de Graaf et al. 1994; Gielkens et al. 1997). Despite this agreement on the molecular separation of *A. niger* and *A. tubingensis*, no phenotypic differences have yet been found between the two species (Varga et al. 2000; Parenicová et al. 2000). This may be the reason that *A. tubingensis* is not yet listed among species of *Aspergillus* in current use (Pitt and Samson 2000; Pitt et al. 2000).

The name *A. niger* is predated by the names *A. phoenicis* and *A. ficuum* and, provided it is accepted that these three taxa are all conspecific, as implied by most molecular studies (Parenicová et al. 2000; Varga et al. 2000), the latter two taxa would have nomenclatural priority. Since these latter two names are nowadays rarely used, it was proposed at the Second International Workshop on *Penicillium* and *Aspergillus* that the name *A. niger* was to be conserved and *A. phoenicis* and *A. ficuum* to be rejected (Frisvad et al. 1990; Kozakiewicz et al. 1992). *A. niger* is a species of major economic importance and the name is now conserved for practical, information retrieval and economical reasons, and in the interest of continuity in legal affairs and approval procedures. Despite this, *A. phoenicis*, *A. awamori* and *A. foetidus* are still mentioned in the list of types of species in current use (Pitt and Samson 2000), whereas the species *A. tubingensis*, *A. acidus*, and *A. citricus* are not listed (Kozakiewicz 1989). A case can be made for keeping the name *A. awamori* for the domesticated form of *A. niger* in parallel with keeping the name *A. oryzae* for the domesticated form of *A. flavus*, *A. sojae* for the domesticated form of *A. parasiticus*, and *Penicillium camemberti* for the domesticated form of *P. commune* (Pitt et al. 2000). The appearance on this list of *A. phoenicis* and *A. foetidus* is dubious, however.

Today's practice, that the designation *A. niger* van Tieghem includes strains which could be named *A. awamori*, *A. ficuum*, *A. foetidus*, *A. phoenicis*, *A. pulverulentus*, *A. tubingensis*, *A. inuii* and *A. usamii* should be continued. If, however, phenotypic differences are found between *A. niger* and *A. tubingensis* this may lead to acceptance of the latter taxon. Some molecular data have also indicated that *A. foetidus* (Parenicová et al. 2000) and a *nomen nudum A. brasiliensis* (Varga et al. 2000) are distinct species, but phenotypic data backing this up are meagre at best. The species currently acceptable in section *Nigri* are listed in Table 1.

The most recent supraspecific scheme for the genus *Aspergillus* was suggested by Gams et al. (1985), placing all species with dark brown to black-shaded conidia into the section *Nigri* of a proposed subgenus *Circumdati*. Their proposal has been accepted by the International Commission on *Penicillium* and *Aspergillus* (Samson 1992).

Industrial use

A. niger became an industrially used organism when citric acid was first produced by fermentation in 1919. Citric acid is widely used in a variety of industries and, by sales volume, greatly exceeds other metabolites such as gluconic acid (Roukas 2000). Citric acid is the primary acidulant in the food and beverage industries. It is used in foods such as soft drinks, fruit juices, desserts, jams, jellies, candy and wine. In the pharmaceutical in-

Table 1 Currently accepted species in *Aspergillus* subgenus *Circumdati* section *Nigri* (Kusters-van Someren et al. 1991; Parenicová et al. 2000, 2001)

Species generally accepted	Ochratoxin A production potential
A. niger	+ (low frequency)
A. carbonarius	++ (high frequency)
A. ellipticus	–
A. heteromorphus	–
A. aculeatus	–
A. japonicus	–
Species distinguishable with molecular data only:	
A. tubingensis (= *A. acidus* = *A. acidus* var. *pallidus*)	–
Species accepted by some authors (but = *A. niger* based on molecular data)	
A. foetidus	–
A. citricus	++ (high frequency)

dustry, iron citrate is used as a source of iron and citric acid as a preservative for stored blood; in the cosmetics and toiletries industries it is used as a buffer, for pH adjustment and as an anti-oxidant. It is also used in industrial applications including detergents, leather tanning, in electroplating and other applications where sequestering agent activity in the neutral to low pH range is required. Citric acid is produced almost exclusively by fermentation of *A. niger* and *A. wentii* because yields of these organisms are economic and formation of undesired side products is minimal. The Food and Drug Administration (FDA) has listed *A. niger* as a source of citric acid (21 Code of Federal Regulations §173.280).

In addition to citric acid, *A. niger* is a rich source of enzymes. Pectinase, protease and amyloglucosidase were the first to be exploited, and were originally produced in surface culture (Frost and Moss 1987). Although it had been shown by Kluyver's group in Delft as early as 1932 that it was possible to cultivate a filamentous fungus like *A. niger* in submerged culture (Kluyver and Perquin 1932), the technology was first applied to the production process of penicillin G by *Penicillium chrysogenum* in 1942. After 1950, production technology for fungal products gradually changed from surface culture to stirred-tank processes, but up until the mid 1960s companies used surface culture processes (Barbesgaard et al. 1992). Several additional enzymes like cellulase and hemicellulase were manufactured using black *Aspergillus* strains in stirred tank processes.

For the manufacture of many products, starch – one of the most abundant carbohydrates – must be hydrolyzed to syrups, which contain glucose, maltose and low molecular weight dextrins. Amyloglucosidase, also referred to as glucoamylase, is an exo-amylase catalysing the release of successive glucose units from the non-reducing ends of starch by hydrolysing α-1,4-D-glucosidic linkages. The glucose syrup and the alcohol industries are the principal users of amyloglucosidase produced by *A. niger*.

Pectin, a heteropolysaccharide, is a principal component in commercially important fruits and vegetables. Several enzymes, including pectin esterases, endo- and exo-polygalacturonidases and pectin lyases, produced from *A. niger* degrade pectin; they are used in wine and fruit juice production to reduce juice viscosity before pressing and improve clarification (Grassin and Fauguenbergue 1999).

It is established practice to improve the baking process by adding hemicellulases from *A. niger* when mixing the dough. The enzymes modify the rheological properties of the dough and give higher loaf volume and better crumb structure of bread and pastry.

A. niger glucose oxidase and catalase are used for determination of glucose mainly in diagnostic enzyme kits, for the removal of either glucose or oxygen from foods and beverages and for the production of gluconic acid from glucose (Berka et al. 1992).

FAO/WHO experts have repeatedly reviewed and accepted enzyme preparations from *A. niger* including the organism itself (FAO/WHO 1972, 1978, 1981, 1987, 1990), listing them with an Acceptable Daily Intake of 'not specified'. The FDA in the United States has accepted numerous enzymes for food use: in the early 1960s the FDA issued opinion letters recognizing that α-amylase, cellulase, amyloglucosidase, catalase, glucose oxidase, lipase and pectinase from *A. niger* can be 'generally regarded as safe' (GRAS) under the condition that non-pathogenic and non-toxigenic strains and current good manufacturing practices be used in production. In addition to these enzymes, Godfrey and Reichelt (1983) claimed GRAS status for β-galactosidase and protease from *A. niger*. Carbohydrase and cellulase from *A. niger* are also approved as a secondary direct food additive by the FDA as an aid in clam and shrimp processing (21 Code of Federal Regulations §173.120).

Until the 1980s, *A. niger* industrial production strains were isolated through the use of classical mutagenesis followed by screening and/or selection. Parasexual crossing has also been used in strain improvement efforts in *Aspergilli*, which lack a sexual cycle. For example, Das and Roy (1978) have reported improved production of citric acid by a diploid strain of *A. niger* generated by parasexual crossing.

With the development of DNA-mediated transformation of *Aspergilli*, initially in *A. nidulans* (Ballance et al. 1983; Tilburn et al. 1983), and subsequently in *A. niger*

(Buxton et al. 1985; Kelly and Hynes 1985; Van Hartingsveldt et al. 1987; Ward et al. 1988; Campbell et al. 1989), this very useful technology was applied to using *A. niger* as a host for gene expression. For example, the production of native *A. niger* catalase has been increased using recombinant techniques (Berka et al. 1994a, b), whereas a 1,000-fold improvement in the expression level for *A. niger* phytase was achieved by using recombinant technology (Van Gorcom et al. 1991; Van Hartingsveldt et al. 1993; Selten 1994).

The long history of safe use on an industrial scale makes *A. niger* exceptionally well suited to be used as a host for heterologous expression. A number of genes of commercial importance and their regulatory sequences that could be used as components in industrial expression systems have been cloned (Nunberg et al. 1983, 1984; Bussink et al. 1990; Harmsen et al. 1990; Nguyen et al. 1991).

The strategy of employing the promoter of a highly expressed fungal gene for the expression of a heterologous gene (Cullen et al. 1987) proved successful when Dunn-Coleman et al. (1991) obtained expression by *A. niger* var. *awamori* of commercially viable levels of calf chymosin under the control of the glucoamylase promoter. This enzyme has been accorded GRAS status by the FDA (Federal Register 1993).

After the cloning of the phytase gene (Mullaney et al. 1991; Van Gorcom et al. 1991; Van Hartingsveldt et al. 1993; Piddington et al. 1993) the gene cloned from an *A. niger* strain was inserted in an expression cassette under the control of the strong glucoamylase promoter. This expression cassette was randomly integrated in multiple copies in the genome of an industrial *A. niger* glucoamylase production strain (Selten 1994; Van Dijck 1999). One of the reasons for the high production level of glucoamylase in this particular strain is the multiplication of a region in the DNA containing, among other things, the promoter and coding sequence of the glucoamylase gene, the *gla*A-locus. Using advanced proprietary genetic modification techniques (Selten et al. 1995, 1998), this locus was "emptied" and subsequently "filled" with "genes of interest" in expression cassettes under the control of the host *gla*-promoter. Compared to the original glucoamylase overproducing strain they are completely identical except for the fact that the "gene of interest" replaces the glucoamylase gene (Groot et al. 2000). This has several advantages. New production strains can now be designed and built in a predictable manner. In addition, and this is important from a regulatory point of view, this technique of targeted integration by definition cannot cause any pleiotropic efforts by perturbing the rest of the genome. This is often raised as a (hypothetical) possibility by regulatory bodies in case of product approvals where the production strain was obtained by random integration of genes.

To date, many enzyme products are available on the market from recombinant strains of *A. niger.* In a recent listing prepared by the Enzyme Technical Association, the enzymes α-amylase, arabinofuranosidase, catalase, chymosin, glucoamylase, glucose oxidase, pectin esterase, phospholipase A2, phytase, and xylanase are mentioned as being produced by recombinant strains of *A. niger* (Pariza and Johnson 2001).

Safety aspects

A. niger is generally regarded as a safe organism. This is documented in lists of the organisations responsible for occupational health and safety [e.g. Berufsgenossenschaft der Chemischen Industrie (1998)]. In rare cases when persons are exposed to intense spore dust, hypersensitivity reactions have been observed.

Pathogenicity

A. niger is generally regarded as a non-pathogenic fungus widely distributed in nature. Humans are exposed to its spores every day without disease becoming apparent. Only in few cases has *A. niger* been able to colonise the human body as an opportunistic invader and in almost all these cases the patients have a history of severe illness or immunosuppressive treatment.

Animal studies

Several experimental studies to demonstrate the pathogenic potential of *A. niger* have come to the conclusion that neither ingestion of large doses of spores (Nyiredy et al. 1975) nor inhalation of spores (Bhatia and Mohapatra 1969) induces mycosis in experimental animals. One day after ingestion, *A. niger* was no longer detected in the digestive tract, although ingested *A. nidulans* was isolated from the intestine of the animals. In contrast to *A. fumigatus*, which is known to be pathogenic, *A. niger* showed no significant effect on the animals in the inhalation study.

Compromising the immune system by steroid hormones seems to promote the spreading of the fungus in the body after an infection. Jacob et al. (1984) conducted a study with mice infected intravenously with high doses of *A. niger* isolated from sputum. They found evidence of pathogenic action only in groups that had been treated with a hydrocortisone drug. Addition of Decadron, a steroid hormone, to the culture medium of *A. niger* induced more vigorous corneal ulceration in rabbit eyes infected with spores compared to animals inoculated with spores from medium without the steroid (Hasany et al. 1973). The authors conclude that exposure of the ordinarily harmless fungus to the steroid made them behave like a pathogen.

Medical case reports

Washburn et al. (1986) found in their immunological study using human sera that *A. fumigatus* produces substances inhibiting complement, which induces phagocy-

tosis of fungal cells by leukocytes. This defence mechanism against infection was not impaired by liquid from an *A. niger* culture.

Few cases of primary cutaneous aspergillosis caused by *A. niger* are given in the literature. Cahill et al. (1967) reported a severe infection, which had long been wrongly been diagnosed as leprosy, and its successful treatment with nystatin.

Mycosis of the ear is one of the frequent health problems in the tropics. *A. niger* has been isolated from 5% of cases of chronic otitis media in Nigeria (Ibekwe and Okafor 1983). However, the authors consider the fungus to be a secondary invader rather than the causative organism because in most cases the patients had been treated with antibiotics before *A. niger* was isolated from their ears. Paldrok (1965) identified 61 fungal isolates from ear lesions in Sweden, 22 of which were of the *A. niger* group. The high incidence of *A. niger* in the outer ear, they speculate, could possibly be due to the fact that *Aspergilli* are resistant to the fungistatic action of ear wax (cerumen).

These ear infections, called otomycosis, cause local inflammation and mycelial growth on cerumen on the skin of the external ear canal. Whilst relatively common in the tropics, this is not a serious condition and can be treated easily with topical antifungal ointment (Mugliston and O'Donoghue 1985; Paulose et al. 1989). Loh et al. (1998) point to self-cleaning of the ears, leading to mechanical damage of the skin barrier, as an important factor in the occurrence of otomycosis.

A. niger can cause pulmonary infection (Binder et al. 1982; Denning 1998). In rare cases it will invade existing pulmonary cavities and create a ball of matted hyphae known as aspergilloma. This aspergilloma may be present for years and may produce oxalic acid in situ, which may lead to renal problems caused by oxalosis (Nime and Hutchins 1973; Severo et al. 1997).

A number of reports on secondary aspergillosis (reviewed in Abramson et al. 1986; Rippon 1982; Saravia-Gomez 1979), caused by often unidentified *Aspergilli*, describe infection in patients suffering from diabetes, drug abuse, alcoholism, severe diseases such as pneumonia, tuberculosis, enterocolitis or patients receiving antibiotic, steroid, cytotoxin or radiation therapy. These groups of debilitated patients, whose immune systems are in many cases weakened, are characterised by an increased susceptibility to opportunistic micro-organisms that do not pose any risk to healthy people.

While abnormal ports of entry like wounds, burns and lesions of the mucosa can facilitate infection in patients, the only entrances in the healthy person are the digestive and respiratory tracts. Thus, reports on health problems of people extensively exposed to *Aspergillus* spores are of special relevance for risk assessment.

Hypersensitivity

Tomsikova et al. (1981) investigated the hypersensitivity pneumonitis of workers in a citric acid plant. They were exposed to spore dust from the production organism *A. niger* and from contaminating fungi. Although both *Aspergillus* and *Penicillium* have been isolated from the respiratory tracts of this group, the concentration of antibodies against *Penicillium* was significantly higher and more frequent than that against *Aspergillus*. This led the authors to the conclusion that hypersensitivity pneumonitis has developed mainly as a result of inhaled *Penicillium* spores and not *Aspergillus* spores.

In another citric acid plant, Topping et al. (1985) showed that only one-half of the workers suffering from bronchospasm were sensitive to *A. niger* spores, while the other half was sensitive neither to spores nor to other substances collected by filtration from the air inside the plant. Now that the spores have been recognised as the most frequent source of hypersensitivity, their dispersal is minimised by technical means and by turning from surface culture methods to submerged production processes which reduce sporulation of *A. niger*. In an 8-year follow-up study, Seaton and Wales (1994) conclude that *A. niger* is a weak antigen and that simple hygiene measures effectively protect the workforce. If such measures are taken, exclusion of recruits with positive skin tests is then not necessary.

Toxins

Despite the long history and intensive nature of *Aspergillus* research, only few cases of toxin formation by *A. niger* have been reported. However, in no case has *A. niger* been proven to produce aflatoxins or trichotecenes.

Two reports (Kulik and Holaday 1966; Hanssen 1969) that *A. niger* cultures produced aflatoxin B1 have been disproved. The evidence was mainly based on an assay by thin layer chromatography. Chances are that fluorescing substances with similar mobilities (Murakami et al. 1967) have been erroneously interpreted as aflatoxin B1. Later on, more detailed studies (Parrish et al. 1966), including those strains which Kulik and Holaday classified as positive (Mislivec et al. 1968; Wilson et al. 1968), clearly showed that none of the *A. niger* strains produced any aflatoxin. Bullerman and Ayres (1968) were also unable to demonstrate aflatoxin production in *A. niger* they had isolated from cured meats. From the numerous investigations it becomes very clear that *A. niger* does not have the ability to produce aflatoxins.

Several more incidental findings indicate that metabolic products may be toxic (Moreau 1979; Cole and Cox 1981; Reiss 1981). Reports on poisoning of animals after they were fed mouldy feed (Moreau 1979, page 178) are difficult to interpret because toxin formation took place under uncontrolled conditions in storage where various contaminating organisms grow as a mixture. The author suspects oxalic acid, a metabolite of *A. niger*, to be the compound responsible for the toxic effect. Jahn (1977) presents results showing coincidence of the toxic effect of fodder and the presence of an *A. niger* strain producing unusually high amounts of oxalic acid.

The nephrotoxic and carcinogenic mycotoxin ochratoxin A was first reported for the black *Aspergillus* species by Ueno et al. (1991) in the species *A. foetidus*. This was later confirmed by Téren et al. (1996) for another isolate of *A. foetidus*. Abarca et al. (1994) first reported ochratoxin A production in *A. niger* (var. *niger*) and this was later confirmed by Téren et al. (1996), Nakajima et al. (1997), Téren et al. (1997), Heenan et al. (1998) and Taniwaki et al. (1999). Téren et al. (1996) also reported that *A. awamori* produced ochratoxin A, and 1 year previously Ono et al. (1995) reported ochratoxin A production in *A. awamori* var. *fumeus*, *A. amamori* var. *minimus*, *A. usamii* and *A. usamii* mut. shiro-usamii. As all these names are synonyms of *A. niger*, it seems to be confirmed by several authors that some isolates of *A. niger* produces ochratoxin A. However, as mentioned by Varga et al. (2000) only about 6% (1.7% to 18.5% as listed by Abarca et al. 2001) of *A. niger* isolates appear to produce ochratoxin A. More research is needed in order to find out which conditions are optimal for ochratoxin A production by *A. niger* (Frisvad and Samson 2000).

Strain CBS 618.78, listed as *A. foetidus*, when studied under optimal laboratory conditions, has the potential to produce ochratoxin A. CBS 618.78 was regarded as an *A. niger* by Kusters-van Someren et al. (1991). From comparing the several isolates in the culture collections it appears that CBS 618.78 is related to strain CBS 126.48, listed by CBS as *A. niger*, available also as ATCC 10254, NRRL 337, IMI 015954, DSM 734, and IFO 6428. When checked, both strains *A. niger* CBS 126.48 and *A. foetidus* IMI 041871 – identical to *A. foetidus* CBS 618.78 – also produce large amounts of ochratoxin A and B when studied under optimal mycotoxin-inducing conditions. On the other hand, *A. niger* IMI 015954 produced rather small amounts of ochratoxin A. Strain NRRL 337 has been used extensively for production of enzymes (Le Mense et al. 1947; Elmayergi and Scharer 1973; Iwai et al. 1983; Okomura et al. 1983) and organic acids (Shu and Johnson 1947; Bercovitz et al. 1990), and for treatment of baked bean processing wastewater (Hang and Woodams 1979), alcoholic fermentation from alkaline potato peel waste (Bloch et al. 1973), utilisation of brewery spent grain (Hang et al. 1975, 1977) and fungal treatment of beet waste (Hang 1976). In the latter paper the fungus (NRRL 337) is called a "food fungus" and named *A. niger*.

This should be taken as a warning that all *A. niger* van Tieghem isolates, either from nature or obtained from a culture collection should be carefully checked for their potential to produce ochratoxin A at the start of the development of a production process for an enzyme used in the food industry. If a strain exhibits the potential to produce this compound, a control system should be in place to assure that it does not end up in the product at levels that induce a toxic effect. When above-threshold levels are found in a product, the use of the strain in the process should be discontinued (Pariza and Johnson 2001).

A. carbonarius is a more efficient producer of ochratoxin A and a much higher percentage of the isolates tested have been found positive (Horie 1995; Téren et al. 1996; Wicklow et al. 1996; Heenan et al. 1998; Varga et al. 2000). No other species in *Aspergillus* section *Nigri* has been reported to produce ochratoxin A (Varga et al. 2000).

A summary of the ochratoxin A production potential of the species in the *Aspergillus* subgenus *Circumdati* section *Nigri* is given in Table 1.

Kojic acid, though mentioned by Wilson (1971) to be a metabolite of *A. awamori*, is not produced by the *A. niger* strains, as Parrish et al. (1966) clearly stated. This is confirmed by the industrial experience that, under the conditions of enzyme production using *A. niger*, kojic acid has not been demonstrated in the culture liquid.

In a comprehensive screening for toxins from *Aspergilli*, Semeniuk et al. (1971) did not detect any markedly toxigenic strain in the *A. niger* group after they had cultivated 392 *Aspergillus* strains on wheat and soybean feed and fed it to chickens or mice. The authors classified 15 strains of the *A. niger* group (of 34 in the test) as moderately to mildly toxigenic. In *A. awamori*, *A. ellipticus*, *A. heteromorphus* and *A. pulverulentus* they did not find any toxigenicity at all. Unfortunately, the study did not include known toxic substances as controls, which would have been helpful in judging the significance of the results.

In a few cases only, suspected toxins have been purified from cultures and tested in animal studies. Nigragillin has been isolated from cultures of *A. niger* and *A. phoenicis* (Caesar et al. 1969; Cole and Cox 1981, p. 798). The LD_{50} was found to be approximately 250 mg/kg bodyweight when fed to 1-day-old cockerels. Malformins, a group of closely related cyclic peptides, generate deformations in plants and have been isolated from cultures of *A. niger*, *A. ficuum*, *A. awamori* and *A. phoenicis* (Steyn 1977). Two malformins were checked for toxic action on rodents: malformin A1 showed an LD_{50} of 3.1 mg/kg when applied intraperitoneally but there was no evidence of acute toxicity when up to 50 mg/kg were given orally to male mice (Yoshizawa et al. 1975). Anderegg et al. (1976) found the LD_{50} for malformin C in both newborn and 28-day-old rats to be 0.9 mg/kg when it was given intraperitoneally.

A series of naphto-γ-pyrones, produced by some strains of *A. niger*, have been reported to be vertebrate central nervous system toxins (Ghosal et al. 1979; DeLucca et al. 1983; Ehrlich et al. 1984). However, these secondary metabolites cannot be regarded as mycotoxins (Bennett 1989), as they were not shown to be toxic when administered by a natural route but rather after intraperitoneal injection. Furthermore, they accumulate only in the mycelium (Ehrlich et al. 1984). Thus these naphto-γ-pyrones do not appear to be a cause for concern in biotechnological products.

The whole body of knowledge from the literature is carefully taken into consideration when testing industrial strains for any possible risk during the development of a fermentation process. Whenever possible the production

organism is chosen from strains that have been in use for many years and that are examined for their ability to produce known toxins under the fermentation conditions used. Finally, the products are regularly checked to ensure that they meet the requirements of the health authorities as given in the Food Chemical Codex (1996) or in the FAO specifications (FAO/WHO 1992).

Summary

The *A. niger* group is composed of black-spored *Aspergillus* species, several of which have a long history of safe use in the fermentation industry. These species have never been identified to be the primary cause of any disease in man. The risk of allergic hypersensitivity to inhaled spores can be handled in an industrial environment by minimising the exposure of the workers to spore dust. Sporadic toxin formation under undefined conditions has not been observed under controlled fermentation conditions. Thus it is concluded that *A. niger* is a safe production organism for industrial use provided the rules of good manufacturing practice are observed. The relatively new discovery that a low percentage of *A. niger* strains have the potential to produce ochratoxin A under optimal laboratory conditions requires, however, that all *A. niger* isolates, (over)expressing a specific gene of interest, be evaluated for their potential to produce ochratoxin prior to being further developed into a new accepted production strain. The use of strains of established and proven safe industrial strain lineages as hosts to over-express these genes of interest is a good and fast alternative to avoid this.

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