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Utilization of Milk Powder as a Source of Carbon and Nitrogen by Soil Mycoflora of Saudi Arabia

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Abstract

The present study was conducted to identity the soil mycoflora which could utilize the milk as a sole source of nitroger carbon. Soil samples collected from dairy farms were screened for fungi which could utilize milk powder as a sole some of carbon and nitrogen for their growth. *Aspergillus* was the predominant genus with 23 species followed by *Penicillium* with 21 species. Other dominant genera were *Chaetomiurn* (11 species), *Eupenicillium* (8 species), *Cladosporium* (5 species and *Chrysosporium* (5 species). Altogether 131 fungal species belonging to 50 genera were isolated.

Introduction

Milk and other dairy products are nutritious food. These are also a good medium for microbial growth. Studies on microbiology of milk and milk product have mainly concentrated on bacteria (Parry and Pawsey, 1984; Weiser *et al*., 1971). Various molds like, *Aspergillus*, *Penicillium*, *Alternaria*, *chaetomium*, *Neurospora*, *Monilia*, *Mucor*, etc. have been reported to cause deterioration of foods including milk products (Parry and Pawsey, 1984). Fungi such as *Aspergillus*, *Penicillium*, *Rhizopus* and various yeast and actinomycetes are used commercially for the production of milk products, bakery products, chemical and medicines (Casida, 1991; Weiser *et al*., 1971).

No work has been reported, so far, from Saudi Arabia about the mycoflora of milk and milk products. The aim of this study was to identify the soil mycoflora which could utilize the milk as a sole source of nitrogen and carbon.

Materials and Methods

Soil samples were collected from 3 dairy farms (Sanabil farms, Al Salehia farm, Newesir farm) located at on the outskirts of Riyadh City. Fifty replicate soil samples were collected from each dairy farm. All soil samples collected from one farm were mixed together and five gram of this mixed soil was taken at random and poured into 45 ml of pre-sterilized distilled water. Five replicates were prepared in the same way for each type of soil samples.

Fungi were isolated from soil by the dilution plate method (Bokhary and Parvez, 1992 b,c). One ml of this dilution (10-2) was poured in sterile disposable petridishes of 9 cm diameter (25 plates for each samples). Molten fox medium cooled at 45°C (KCl 0.5 g, $MgSO_4$ 0.19 g, KH_2PO_4 1 g, Agar 15 g, $FeSO_4$ 0.01 g), containing one gram of commercially available skinimed milk powder in plebe of sodium nitrate and sucrose was poured into each plate (20 ml per plate). Streptomycin sulphate (0.03 gL^{-1}) was aseptically added in to the sterilized medium just before pouring the agar medium into plates, to inhibit the growth of bacteria and rose bengal (0.033 gL^{-1}) was added before sterilization of medium to restrict colony diameter of growing fungi.

identification of isolated fungi was carried out with the of morphological characteristics described in the follow literature: Ellis (1971, 1976), Gilman (1971), Pitt (1979) Ramirez (1982), Raper and Fennell (1965), Schipper (1978) and Zycha *et al*. (1969).

Results

Total number of colonies isolated per gram of some different temperature (5-45°C) on milk agar medium is given in Table 1. The highest number of fungal colonies per gram of soil was isolated at 35°C . The least number of colony was isolated at 45°C. A total number of 141 fungal spore belonging to 50 genera were isolated on milk medium (Table 2). *Aspergillus* was the predominant represented by 23 species followed by *Penicillium* was represented by 21 species. *Chaetomium* was and dominant genus represented by 11 species following *Eupenicillium* (8 species), *Cladosporium* (5 species) *Chrysosporium* (5 species). The highest number of five species were isolated at 35°C (123 species belonging to genera) followed by at 25°C (93 species/31 genera), at 15°C (26 species/14 genera 0 and at 45°C (10 species genera), the least number of species was isolated artificially (8 species/6 genera).

Aspergilus fumigatus and *Penicillium chrysogenum* were only species which were isolated at all the temperature incubation. *Aspergillus* and *Penicillium* species genera exhibited the highest number of colonies per gram *Aspergillus fumigatus* and *A. favus* exhibited the high number of colonies per gram as compared to *Aspergillus* species. *Penicillium chrysogenum* exhibited (higher number of colonies per gram for *Penicillium* species Most of the fungal species could grow between 15-30. There were several fungal species which were isolated one particular temperature of incubation only. For exact *Aspergillus aculeatus* lizuka was isolated at only 25°C, *A. penicilloides* Speg at 35°C only. There was no fully isolated which could grow at 5°C or at 45°C only i.e it was no true psychrophilic fungi isolated.

Table 1: Total number of fungal colonies per gram of soil isolated on milk agar medium.

Samples	No. of colonies per gram of soil Temperature of incubation (°C)				
	5	15	25	35	45
1	125(12)	180(15)	1118(13)	1200(19)	110(11)
2	93(9)	186(12)	1663(21)	1764(15)	89(8)
3	126(11)	162(09)	1180(12)	1275(12)	39(7)
Mean	114.6	176	1320	1413	79

Each value is a mean of 5 replicates. Values parenthesis are standard deviation

Table 2: Mycoflora per gram of soil isolated on milk agar medium

Samples	No. of colonies per gram of soil isolation temperature (°C)				
	5	15	25	35	45
Aspergillus aculeatus Lizuka	-	-	12(3)	-	-
A. awarnori Nakazawa	-	-	16(3)	19(4)	-
A. candidus Link: Fr.	-	-	-	23(3)	-
A. carbonarius (Bain.) Thom	-	-	31(5)	-	-
A. clavato-nanica Batista	-	-	13(3)	16(4)	-
A. clavatus Desm.	-	-	-	-	-
A. crustosus Raper & Fennell	-	-	19(4)	-	-
A. flavus Link: fr.	9(3)	24(4)	46(6)	59(8)	-
A. fumigatus Fres.	12(3)	32(6)	59(4)	69(9)	21(3)
A. itaconicus Kinoshita	-	-	14(4)	-	-
A. japonicas Saito	-	-	1 9(4)	-	-
A. niger van Tieghem	16(4)	26(4)	36(50	-	-
A. nutans McLennan & Ducker	-	-	14(4)	-	-
A. ochraceus Wilhelm	-	-	16(4)	-	-
A. oryzea (Ahlburg) Cohn	-	-	26(4)	-	-
A. penicilloides Speg.	-	-	-	32(5)	-
A. restrictus G. Smith	-	-	-	8(2)	-
A. subsessilis Raper & Fennell	-	-	19(3)	36(4)	-
A. sydowi/ (Bain. & Sartory) Thom. Church	-	-	39(4)	26(4)	-
A. wentii Wehmer	-	-	16(3)	-	-
A. xerophilus Samson & Mouchacca	-	-	23(4)	39(5)	-
A. zonatus Kwon & Fennell	-	-	16(3)	23(5)	-
Aureobasidium pullulans (de Bary) Arnaud	-	-	39(4)	43(7)	-
Basidiobolus haptosporus Drechsler	-	-	-	35(5)	-
Bjerkandera adusta (Willd. :Fr.) Karst	-	-	16(3)	-	-
Biastobotrys proliferans Marvanova	-	-	33(6)	46(50	-
Botryomyces caespitosus (de Hoog & Rubio	-	-	-	14(4)	-
Botryosphaeria rhodina (Berk. & Curt.) V. Arx	-	-	8(2)	16(3)	-
Cepallophora irregularis Thaxter	-	-	36(40	22(5)	-
Cerrophora sarnala Udagawa & Muroi	-	-	13(3)	-	-
Cactomedium subfimeti Seth	-	-	29(4)	18(3)	-
Chaetomium aureum Chivers	-	-	18(4)	21(4)	-
C. bostrychodes Zofp.	-	-	29(40	36(5)	-
C. brasiliense Batista & Pontual	-	-	14(2)	16(3)	-
C. globosurn Kunze: Fr.	-	-	16(4)	25(4)	-
C. mollicellum Ames	-	29(4)	18(3)	10(2)	-
C. robustum Ames	-		26(3)	36(4)	-
Chryosporum carmichaelii Van Oorschot	-	8(3)	32(4)	26(3)	-
C. indicum (Randhawa & Sandhu) Garg	-	-	17(3)	15(3)	-
C. keratinophilum D. Frey Ex. Carmichael	-	-	-	26(3)	-

C. merdarium (Link: Fr.) Carmichael	-	-	-	-	-
C. queenslandicum Ainis & Rees	-	-	46(60	-	-
Cladophiialophora ajjelloi Borelli	-	-	36(7)	24(5)	-
Cladorrhinum samala (Subram. & Lodha) W. Gams & Mouchacca	12(3)	19(4)	34(5)	-	-
Cladosporium carrionii Trejos	-	-	-	11(3)	-
C. deveriesii Padyhye & Ajello	-	22(4)	29(4)	-	-
C. herbarum (Pers. :Fr.) Link	-	-	32(5)	16(4)	-
C. trichoides Emmons	-	-	8(2)	-	-
Coccidiodes irnmitis Rixford & Glchr ist	7(2)	16(4)	29(5)	-	-
Connersia rilstonii (C. Booth) Gilchrist	-	-	-	18(3)	-
Cunninghamella echinulata (Thaxter)	-	19(4)	39(5)	27(4)	-
C. elegans Lender	-	11(2)	27(5)	41(5)	-
Curvularia geniculata (Tracy & Earle) Boedijn	-	-	15(5)	19(3)	-
C. lunata (Walker) Boedijn	-	13(30	29(4)	34(4)	-
Doratomyces putredinis (Corda) Morton & G. Smith	-	-	11(2)	-	-
Drechslera austraiiensis (Bugnicourt) Subram & Jain ex. M.B. Ellis.	-	14(2)	42(5)	32(40)	-
D. nicotiae Mouchacca	-	-	22(3)	35(4)	-
D. subpapenthpfii Mouchacca	-	-	18(4)	42(6)	-
Emericelia nidulans (Eidam) Vuill	-	-	269(50)	43(6)	-
E. quadrilineata (Thom & Raper) C.R. Benjamin	-	-	-	34(4)	-
E. unguis Malloch & Cain	-	-	14(3)	26(50)	-
Emencellopis glabra (van Beyrna) Backus & Orput	-	-	-	19(3)	-
E. tericola van Beyma	-	-	-	14(3)	-
Emmonsia parva (Emmons & Ashburn) Cif. & Montemartini	-	-	-	32(6)	-
Epidermophyton floccosum (Harz) Langeron & Milochevitch	-	-	13(3)	31(5)	-
Eupenicillium brefeldianum (Dodge) Stolk & Scott.	-	12(2)	29(4)	33(40)	-
E. catenatum Scott,	-	-	15(3)	-	-
E. cinnamopurpureum Scott & Stolk .	-	-	23(4)	-	-
E. crustaceum Ludwig	-	-	-	17(3)	-
E. euglaucurn (van Beyma) Stolk & Scott.	-	-	14(2)	29(4)	-
E. javanicum (van Beyma) Stolk & Scott.	-	-	13(3)	33(50)	-
E. ochrosalmoneum Stolk & Scott	-	-	18(3)	26(40)	-
E. shearii Stolk & Scott	-	-	-	33(5)	-
Eirrotium intermedium Blaser	-	-	-	21(40)	-
Exophiala jeanselmei (Langeron) Mc Ginnis & Padhye	-	-	29(5)	53(7)	-
E. rnansonii (Castell.) de Hoog	-	-	16(3)	36(50)	-
E. moniliae de Hoag	-	-	-	39(4)	-
Fennellia flavipes Wiley & Sim m or) s	-	-	-	36(50)	-
Fusarium chlamydosporum Wollenw. & Reinking	-	-	49(6)	74(70)	-
F. equiseti (Corda) Saco.	-	14(40	39(4)	27(50)	-
F. oxysporum Schiecht.	11(3)	25(3)	41(4)	32(3)	-
F. proliferatum (Matsushima) Nirenberg	-	-	-	17(4)	-
Geomyces pannorum (Link) Sigler & Carmichael	-	-	-	38(4)	-
Geotricum candiclum Link	-	-	16(3)	49(5)	-
G. capitatum (Diddens & Lodder) v. Arx	-	-	19(4)	32(40)	-
G. clavatum de Hoog & al.	-	-	14(3)	25(4)	-
G. fermentans (Diddens & Lodder) v. Arx	-	-	9(2)	9(3)	-
G. fragrans (Berkhout) Morenz	-	-	11(2)	36(5)	-
Gymnoascus petalosporus (Orr. & al.) v. Arx	-	-	-	21(4)	-
Hendersenula torulioiclea Nattrass	-	-	-	26(3)	-
Histoplasma capsulatum Darling	-	-	-	19(3)	-
Hortaea werneckii (Horta) Nishimuca & Miyaji	-	-	-	26(3)	-
Leptodontidum beauverioides de Hoog	-	-	-	23(5)	-
Madurella mycetomi (Laveran) Brumpt	-	-	31(4)	39(4)	-
Malbranchea arwata Sigler & Carmichael	-	-	-	23(5)	-
Microascus cinereus (Emile-Weil & Gaudin) Curzi	-	-	-	32(30)	-

M. manginii (Loubiere) Curzi	-	-	14(2)	43(7)	-
M. senegalensis v. ArX	-	7 2(2)	24(3)	43(6)	-
Monoascus ruder van Tieghem	-	-	-	17(30)	-
Mondiella suaveolens (Lindner) v. Arx	-	-	12(4)	32(6)	-
Pectinotrichum llanense Varsaysky & Orr.	-	-	-	12(4)	-
Penicillium arabicum Baghdadi	-	12(3)	39(4)	16(3)	-
P. atrovenatum G. Smith	-	-	14(2)	24(4)	-
P. capsulatum Raper & Fennell	-	-	-	15(4)	-
P. chrysogenum Thom.	33(5)	44(5)	88(8)	59(6)	11(2)
P. citreonigrum Dierkx	-	-	12(3)	19(5)	-
P. citrinum Thom.	-	-	16(5)	39(7)	-
P. clariforme Bain	-	-	-	13(3)	-
P. decumbens Thom	-	-	-	19(4)	-
P. dierckxii Biourge	-	-	17(3)	27(4)	-
P. diversum Raper & Fennell	-	-	-	31(5)	-
P. expansum Link	-	-	14(3)	39(4)	-
P. funiculosum Thom	-	-	12(3)	22(4)	-
P. glabrum (Wehmer) Westling	-	2(3)	43(5)	31(4)	-
P. griseofulvum Dierckx	-	-	19(4)	38(5)	-
P. inlflatum Stolk & Mille	-	-	-	26(4)	13(3)
P. italicum Wehmer	-	-	26(4)	62(5)	-
P. lagena (Delitsch) Stolk & Sammson	-	-	-	12(4)	-
P. lividum Westling	-	-	-	19(3)	-
P. melanochlorum (Samson & al.) Frisvad	-	-	-	12(3)	-
P. nalgiovense Laxa	-	-	14(5)	27(5)	-
P. oxalicum Crrie & Thom	-	15(3)	39(5)	46(11)	-
P. piceum Raper & Fennell	-	-	12(4)	32(5)	-
P. velutimum van Beyma	-	-	16(3)	35(5)	-
P. verrucosum Dierckx	-	-	-	13(3)	-
Pseudallescheria boydii (Shear) McGinnis & al.	-	-	-	26(4)	-
Rhizopus oxyzae Went & Prinsen Geerlings	-	21(31	39(4)	44(4)	-
Schizophyllum commune Fr.: Fr.	-	-	-	12(2)	-
Scopulariopsis brevicaulis (Sacc.) Bain.	-	-	16(3)	29(5)	-
Scytalidium hyalinum Cambell & Mulder	-	-	15(4)	45(4)	-
Sporothrix cyanescens de Hoag & de Vries	-	-	-	17(3)	-
Thielavia hyalocarpa V, Arx		-	-	15(2)	-

Each value is a mean of 5 replicates. Values parenthesis are standard deviation.

Discussion

Result of present study showed that a much wider range of potential food spoilage fungal flora was present in the soil samples analyzed, with 55 mycoflora genera represented and a total of 141 different species isolated. Previously only some common molds like *Alternaria*, *Aspergillus* (mainly *A. niger* and *A. flavus*), *Mucor*, *Fusarium*, *Mondia*, *Thamnidium*, *Trichothecium*, *Sporotrichum*, *Cladosporium* and *Geotrichum* etc. were reported as common food spoilage mycoflora including spoilage of milk (Weiser *et al*., 1971; Parry and Pawsey, 1984; Casida, 1991), while present study adds several other genera of fungal in the list of food spoilage mycoflora. *Alternaria* was surpassingly not isolated here although this fungus is a saprophytic fungus frequently reported in Saudi Arabia to be able to grow on various substrates studied (Bokhary and Parvez, 1988, 1992a; Bokhary *et al*., 1990) and also a commonly isolated soil soil fungus (Abdel-Hafez, 1982; Abdulla and El-Gindy 1987; Bokhary and Parvez, 1992 b,c). *Aspergillus*, *Fusarium*, *Penicilliurn* and other genera have been reported to produce various toxic chemicals such as mycotoxins during their growth (Bokhary and Naguib, 1983) which could be a health risk to consumers. Also worthy of note that more than 70 percent of fungal species isolated here are medical importance and have been reported earlier as causing various diseases such as aspergillosis, bronchial asthma, eczema, tinea, onychomycosis, ring worn etc. in humans and animals (Frey *et al*., 1981).

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