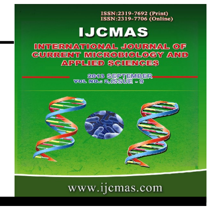

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Original Research Article

Screening and Isolation of organic acid producers from samples of diverse habitats

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A B S T R A C T

Keywords

Fungal cultures; organic acid; acid unitage value; mineral salt agar; dilution plate method.

Fungal cultures are the most preferred ones for production of organic acids as they have the ability to utilise the wide variety of substrates like molasses, starch (wheat, corn, potato), lignocellulose (corn cobs, woody materials). Fungal cultures were isolated from diverse habitats for the present study. Samples were collected from 4 different diverse habitats which include the decaying fruit, vegetables and soil. 47 fungal cultures were isolated on Rose Bengal agar and potato dextrose agar by dilution plate method. The cultures were further checked for their potential to produce organic acid and their acid unitage value was calculated on modified mineral salt agar incorporated bromocresol green. Seven cultures showed the potential to produce organic acid which was concluded from the results of the obtained acid unitage value.

Introduction

An organic acid is an organic compound with acidic properties. The most common organic acids are the carboxylic acids whose acidity is associated with their carboxyl group -COOH. Organic acids have long history of being utilized as food additives and preservatives for preventing food deterioration and extending the shelf life of perishable food ingredient (Cherrington *et al*., 1991). The organic acids produced by various microbes are citric, gluconic, itaconic, lactic, oxalic, fumaric, malic acid. The main organic acids in industrial use are citric, acetic, tartaric, malic, lactic and gluconic acid (Milsonand Meers, 1985; Moeller *et al*., 2007). A large number of microorganisms including bacteria such as *Arthrobacter paraffinens, Bacillus licheniformis, Corynebacterium sp. Lactobacillus casei, L. helveticus, L. paracasei, Streptococcus thermophilus*, fungi such as *Aspergillus niger, A. aculeatus, A. carbonarius, A. awamori, Yarrowia lipolytic*a and related yeast species, may be in use commercially to produce citric acid (Lopez-Garcia, 2002). *A niger* produces gluconic, *A itaconicus* and *A terreus* produce itaconic acid, *Rhizopus oryzae (syn arrhizus*), *Actinomucor* produces lactic acid, *A niger*, Mycorrhizae, plant pathogens produce oxalic acid, *R oryzae, Aspergillus flavus* produces malic acid, *Rhizopus nigricans, Mucor, Cunninghamella, Circinella* species produces fumaric acid (Advances in Fungal Biotechnology for Industry, Agriculture, 2004) *Fusarium* spp. (Foster, 1949), *Aspergillus* spp.(Bercovitz *et al*., 1990), and *Penicillium simplicissimum* (Gallmetzer *et al*., 2002) are known to produce and secrete succinic acid. Fungus can be easily isolated from various habitats like soil, decaying fruit and vegetables or decaying vegetation. Hence samples were obtained from diverse habitats so as to get a wide variety of fungi. Screening and isolation of organic acid producing fungi is being done so as to further carry out the study of lactic acid production by the isolated fungi.

Materials and Methods

Study Area

Samples were aseptically collected from decaying soils of various locations and partially decomposed vegetable and fruit waste from nearby areas of local markets of Aurangabad (Maharashtra), India. It is at an altitude of approximately 513 meters above the sea level. The exact location of the city is 19° 53' 47'' North and 75° 23' 54'' East. All the samples were collected in the month of June i.e. during rainy season so as to get good decomposed samples.

Sample Collection

Samples were collected using aseptic zipped polythene bags previously sterilized by rinsing with 70 % alcohol and were immediately transported to the laboratory and stored at 4^0C in the refrigerator.

Isolation of Fungi

Fungal isolates were made from decaying soil, fruit and vegetable waste samples by serial dilution agar plating method on Rose Bengal agar (Himedia) and Potato dextrose agar (Himedia) plates in triplicates. Based on predominance and distinct morphological properties fungal isolates were selected and purified by repeated subculturing and streak plating.

Screening for Organic Acid Production

Isolated cultures were subjected for screening of organic acid production by determining the acid unitage (AU) values**.** A loopful of fungal spore solution was inoculated on petriplates containing mineral agar acid indicator medium as described (Sunstornsuk *et al*., 1994) with slight modifications and incubated for five days for the formation of yellow zone around the mycelial growth. The medium used was as described by (Park *et al*., 1998) and contained (g/l) : Glucose 120,$(NH_4)_2SO_4$ 3.02, $MgSO_4.7H_2O$ 0.25, $ZnSO_4.7H_2O$ 0.04,KH_2PO_4 0.15, Agar 20, bromocresol green 0.2, Triton X-100 1.5 ml/l in distilled water (pH 5.5). Acid unitage (AU) value of the colonies were determined by dividing the diameter of the yellow zone by the diameter of the colonies. The colonies having notable acid unitage values were picked up and stored at 4^0 C on PDA slants.

Identification of Fungal Isolates

The fungal isolate with significant levels of organic acid production was streaked aseptically on Potato dextrose agar plates,

Table.1 Colony characteristics of cultures on potato dextrose agar after five (5) days of incubation at 30°C.

Sr. No.	Sample Location	Source of Microorganism	Obverse Side	Reverse Side
1	Local Market-1 Shahgunj	Lady's finger	Compact, greyish- white, fluffy	Black
		Green Chillies	Dark green, compact, white margin	White
		Capsicum	Small white, compact, green tinge	Brown
		Tomato	Dull green with brown centre, Compact, white margin	Centre brown, Margin white
		Pomegranate	Small, compact, dirty green with Brown centre	Dark brown
		Beans	Faint green, fluffy, loose	Light brown
2	Local Market-2 Aurangpura	Lady's finger	Confluent faint green, fluffy	Light brown
		Green Chillies	Black	White
		Capsicum	White, fluffy	Black
		Tomato	White cottony with compact centre	White with Brown centre
		Pomegranate	White cottony	White
		Beans	White cottony	White
3	Himayat Bagh	Soil	Greyish black with dull green Centre, fluffy, white margin	Black
			Confluent white cottony growth	White
			White cottony, fluffy	White
			Yellow loose hyphae	White
			White cottony, black spores	White
			White cottony	White
4	Salim Ali Lake	Decaying Soil-1	White cottony	White
			White cottony	White
			Greyish blue, slightly raised	White
			Dirty green, flat	Black
			White, flat, white spores	White
			Greyish green, central part fluffy	Black
			White cottony	Light Brown
			Dirty white, raised and dense central part, filamentous mycelia at periphery	Black
			Rusty brown spores	Black
			Yellow	Black
			Central green, surrounded by yellow and green circular arrangement	Black

			White cottony	Light Brown
			Green mat	Black
			Greyish brown, wrinkled, raised in the centre	Black
			Green mat	Black
			Black	Black
			White, wrinkled central part raised with filamentous periphery	Black
		Decaying Soil-2	White cottony	White
			White cottony	Black
			Faint brown	Faint brown
			Fluorescent yellow	Dirty black
			Green cottony	Black
			Yellow	White with Black centre
			White cottony	Black
			White cottony	Black
			Faint coffee brown	Black
			Very small, white cottony	Light brown
			Greenish grey	Black
			Black	Light black

Table.2 Acid unitage values of isolated fungal strains after five (5) days of incubation on mineral salt medium

Cultures Used	Zone diameter (mm) of fungal colony	Zone diameter (mm) of yellow halo	Acid Unitage Value (AU)
1	15	46	03.06
2	09	31	03.44
3	13	42	03.23
4	18	59	03.27
5	06	23	03.83
6	08	35	04.37
7	09	42	04.66

Figure.1 isolation of fungal cultures on potato dextrose agar plates

Figure.2 isolation of fungal cultures on potato dextrose agar plates

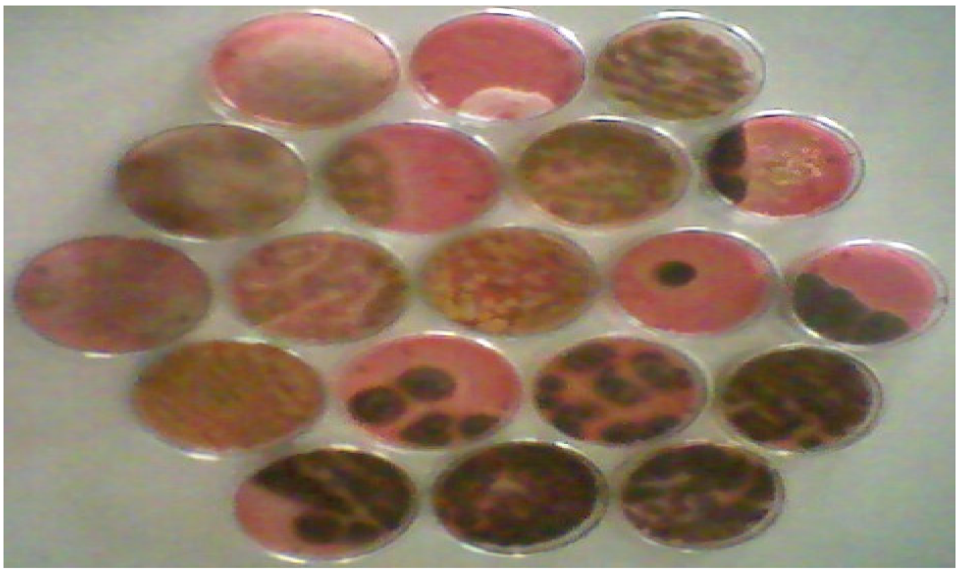
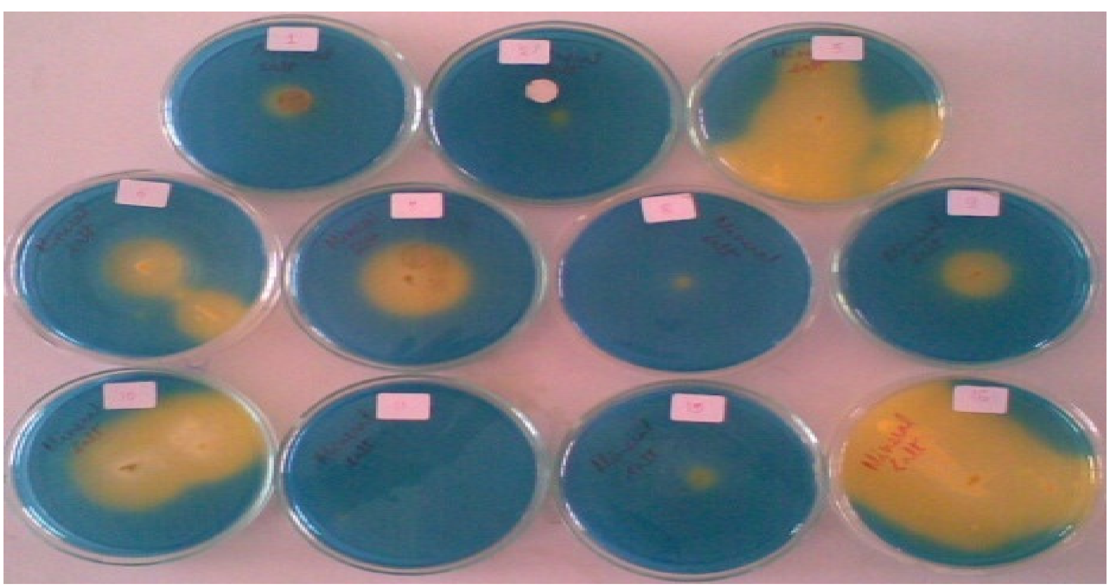


Figure.3 Production of organic acid on modified mineral salt medium



incubated at room temperature for five days and assessed its morphological properties. Further it was characterized by lacto phenol cotton blue staining**.**

Result and Discussion

Figure.1 and 2 represents the isolation of fungal cultures on potato dextrose agar plates and Rose Bengal agar plates respectively. Table 1 describes the morphological features of the 47 fungal isolates on potato dextrose agar plates. Thus, it is concludes the presence of *Aspergillus species, Rhizopus species, Mucor* and some other unidentified fungi. Hence some of the fungi are partially characterized to the genera level by the morphological characteristics. Figure 3 shows the production of organic acid on modified mineral salt medium a yellow halo is produced around the colonies which produce organic acid. Acid unitage values have been determined by measuring the diameter (mm) of colony and yellow halo around the colony. Table 2 represents the zone diameters and acid unitage values. Out of the 47 fungal isolates, 7 cultures showed significant acid production. Cultures 1, 2, 3 and 4 were found to be average, whereas 5 were more than average and 6 and 7 were the best. These cultures will be further checked for its potential to produce lactic acid.

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