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Original Research Paper

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ISOLATION, SCREENING AND CHARACTERIZATION OF ORGANIC ACIDS PRODUCING BACTERIA FROM NOVEL SOURCE

Mili Sandip Shah*

Department of Microbiology, Khyati Institute of Science, Palodia, Ahmedabad 380058*Corresponding Author

ABSTRACT

Microbial production of organic acids is a promising approach for obtaining building-block chemicals from renewable carbon sources. Although some acids have been produced for some time and in-depth knowledge of these microbial production processes has been gained, further microbial production processes seem to be feasible, but large-scale production has not yet been possible. The study aim involves isolation, screening, purification and characterization of organic acids from bacterial origin. For increased yield, various and varying sources of carbon, nitrogen including physical parameters influencing the growth and yield viz. temperature, pH were studied for optimizations of medium ingredients. Maximum production in terms of zone of production was obtained when medium was incorporated with Sucrose (3.0g%) and yeast extract (0.7g%) with pH values 6.3 at 42°C. Organic acids from crude extracts were detected using Rf values by performing thin layer chromatography along with standards alongside the sample in a mobile phase, referred to as the eluent, consisting of EtOH:NH4OH:H2O mixture (75.5:12.5:12, v/v/v) resulting in the progressive separation of the organic acids. Organic acids detection reveals spots corresponding to salicylic acid (Rf 0.78), tartaric (Rf 0.17) and succinic (Rf 0.44). Organic acid detection is to spray the plate with 0.4% (w/v) bromocresol green in ethanol, in which were added few drops of 0.1 N NaOH, which result in colored spots. Succinic, tartaric and salicylic acid is the most important in tonnage having extensive applications in food and pharmaceutical industries.

KEYWORDS : Organic acid, medium optimization, AU Value, TLC

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). Microbial production of organic acids has become a fast-moving field due to the increasing role of these compounds as platform chemicals. In recent years, the portfolio of specialty fermentation-derived carboxylic acids has increased considerably, through innovative fermentation strategies. The organic acids produced by various microbes are salicylic, tartaric,citric, gluconic, itaconic, lactic, oxalic, fumaric, malic and succinic acid. The main organic acids in industrial use are citric, acetic, tartaric, salicilic, lactic and gluconic acid (Milson and Meers, 1985; Moeller et al., 2007). A large number of microorganisms including bacteria such as Arthrobacter paraf inens, Bacillus licheniformis, Corynebacterium sp. Lactobacillus casei, L. helveticus, L. paracasei, Streptococcus thermophilus may be in use commercially to produce acid (Lopez-Garcia, 2002).

MATERIALS AND METHODS

Samples were aseptically collected that include decaying soils, partially decomposed vegetable and fruit waste from nearby areas of local markets and discarded biohazard from neonatal hospitals. These sources were selected on the bases of an outcome from waste discarded natural materials. 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 40°C in the refrigerator. 1 gram of each collected sample was kept for 24 hours at room temperature in 10 ml of sterile distilled water. Serial dilutions were performed till to the level of 30 CFU in a standard petri dish using spread plate technique. Screening medium used was Nutrient agar medium fortified with 3g% CaCO3 and 2g% sucrose. The CaCO3 also helps in detection of organic acid produced as clear zone can be observed against white background due to dissolution of CaCO3 by acid. Medium optimization was performed using various and varying sources of carbon, nitrogen, including physical parameters influencing the growth and yield viz. temperature, pH for increased yield (E. A. Pithawala et al. 2013). The isolate that showed zone of utilization and zone of dissolved $CaCO_3$ surround the colony was measured to quantitative analysis. 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 40°C on optimized medium. Qualitative validation of acids was measured using thin layer chromatography (TLC) analysis. Basically, thin layer chromatography (TLC) consists of immobilized solid stationary phase coated thinly onto a glass plate and liquid mobile phase, which flows over the stationary phase. The organic acids are separated by adsorption process, on silica of the order of 0.25 mm thick on glass plate of 20x20 cm (Sigma). The plates are dried in an oven before use at 120°C for 30 min. This serves to activate the adsorbent. The sample was applied as a narrow band to the stationary phase, 2 cm from the edge by means of a microsyringe. The solvent was removed from the band by gentle heating using of an air blower. Separation takes place in a glass tank that contains the developing solvent (mobile phase) to a depth of about 1.5 cm. This is allowed to stand for at least 1 hour with a lid over the top of the tank to ensure that the atmosphere within the tank becomes saturated with solvent vapor. After equilibration, the thin layer is placed vertically in the tank so that it stands in the solvent. The system is kept at constant temperature whilst the development is occurring. Mobile phase, referred to as the eluent, consists of EtOH:NH4OH:H2O mixture (75.5:12.5:12, v/v/v) (Braun and Geenen, 1962), is then allowed to flow continuously over the stationary phase, resulting in the progressive separation of the organic acids. The plate developed and then removed from the tank and allowed to dry. A specific organic acid detection is to spray the plate with 0.4% (w/v) bromocresol green in ethanol, in which were added few drops of 0.1 N NaOH. The movement of compounds on TLC was characterized by specific Rf values, or retardation factor expressed as Rf = dA/dF where dA is the distance moved by the analyte (organic acid) from the origin and dF is the distance moved by the solvent front from the origin. Organic acid identification is made on the basis of comparison of the movement of the organic acids with those of reference compounds chromatographed alongside the sample on the TLC plate.

RESULTS AND DISCUSSION:

Parameter of the environment controls the bacterial activity within a system. Various carbon sources viz. glucose, sucrose, maltose, fructose, dextran, cellulose etc. were used to observe the maximum Acid Unitage Value (AU) as shown in table 2. Large variety of substrates serves as a carbon sources in nature to support the growth of bacterial and microbial cells. Of all carbon sources, sucrose showed maximum acid production. Maximum Acid unitage 4.50 was observed at 3g% sucrose concentrations. NaCl is the most common salt essential for the growth of a cellular entity. Various concentrations of NaCl are known to effect substantially on growth

of cell thereby the consequent efficiency of the cell in acid production is also affected. Maximum Acid unitage was observed with 0.5 g%. Isolate showed efficient pigment production in an optimum temperature range between 35˚C-42˚C. However acid production AU Values are maximum at 35-37˚C of all isolates. Also isolates were known to work efficiently at neutral pH (pH=7). However the growth was not effected much when pH was increased by one number (i.e. at pH=8), towards alkaline condition. The culture is known to grow in the range of pH- 3.0-8.0. Results so obtained at pH=2 is restricted and approximate 50% of the growth and viable cells were killed due to the stress in pH values. This gives a clear cut indication that the crude should be harvested and medium should be continuously replaced to avoid loss in microbial growth.

Table 1: Cultural and Microscopic characteristics on optimized condition

Sample collection site	Source of Microorganism	Obverse side	Reverse side	Gram's reaction
Khyati Institute of science, Palodiya	Decaying soil	Small, off white, irregular, raised opaque	Opaque	Gram positive
Local Market	Cucumber	Small, white, rough, regular	white	Gram positive
	Lemon	Small, compact, dirty white with Brown centre	Dark brown	Gram positive
	Kiwi	Very small, white, translucent	White	Gram negative
	Ambla	White, wrinkled central part raised with filamentous periphery	White	Gram positive
Veterinary hospital	Biohazard	medium, irregular, flat, dry, opaque, off white	White	Gram positive

Six bacterial colonies that produced zone of production of organic acids and utilizing CaCO3 were obtained. The table 1 shows cultural and microscopic characteristics of various isolates. Out of 6 sources from 3 sample collection site, MS3 isolate showed maximum AU values 4.50 and was studied for further qualitative analysis (Table 2).

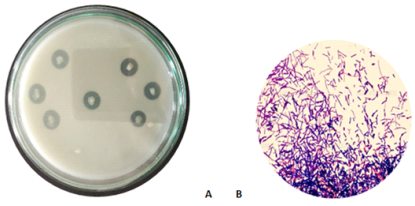

Figure 1: Cultural (A) and Microscopic (B) characteristics of MS3

Table.2 Acid unitage values of isolate on optimized media after 24 hours at 30°C.

Cultures used	Zone diameter (mm) of colony	Zone diameter (mm) of halo	Acid Unitage Value (AU)
Ms_1	4	13	3.25
MS_2	6	11	1.83
MS_3	2	9	4.50
MS_4	5	8	1.60
MS_5	4	12	3.00
MS_6	1	3	3.00
MS_7	3	7	2.33

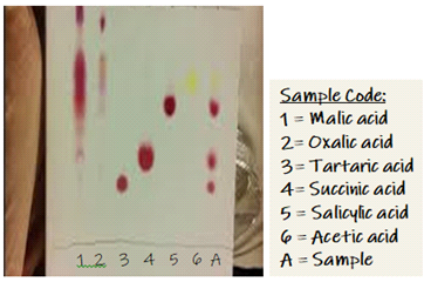

Figure 2: Detection of Organic acids using TLC w.r.t. standards

Crude extracts of MS3 showed the presence of mixture of organic acids viz. Succinic, tartaric and salicylic acid. Organic acids detection reveals spots corresponding to salicylic acid (Rf 0.78), tartaric (Rf 0.17) and succinic (Rf 0.44). Organic acid detection is to spray the plate with 0.4% (w/v) bromocresol green in ethanol, in which were added few drops of 0.1 N NaOH, which result in spots as shown in Figure 2 (Radhouane Chaffai et al. 2006).

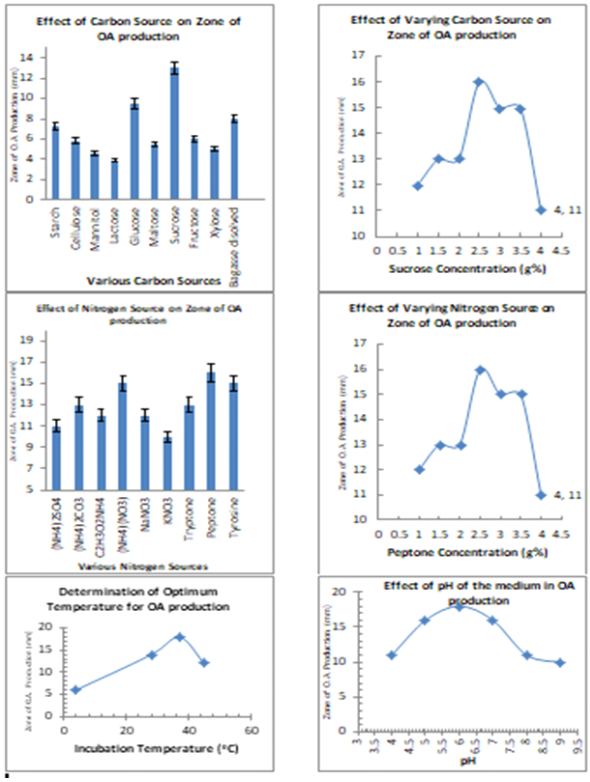

Figure 3: Medium Optimization for increased yeild and effect on OA production

CONCLUSION:

The present study concluded that, to isolate and to screen the organic acid production, bacterial strains from decaying soil, vegetables fruits and biohazard discard wastes of veterinary hospital areas to explore their organic acid production potential and to optimise the cultural conditions for possible future production and application. The factors such as different carbon sources, nitrogen sources, pH, temperature, etc. need to be considered in the cultivation of MS3 since it affects organic acid production. MS3 alone is known to produce total of three organic acids viz. Succinic, tartaric and salicylic acid. Substrate requirements as well as biomass and product yields are some of the basic parameters that need to be

considered in determining the feasibility of the fermentation process.

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