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Short Communication

Effect of carbon source on the antimicrobial activity of *Corynebacterium kutscheri* and *Corynebacterium xerosis*

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In an attempt to screen out new potent antimicrobial substances producing bacteria, *Corynebacterium kutscheri* NB-1 and *Corynebacterium xerosis* NB-2 were isolated and were found antagonistic to bacteria and fungi. Antimicrobial substances production of the bacterial strains was greatly influenced by variation of carbon sources. Galactose and glucose strongly enhanced the antimicrobial activity of *Corynebacterium kutscheri* and *Corynebacterium xerosis*, respectively. But their antimicrobial activity had been repressed by ribose and lactose, respectively.

Key words: Antimicrobial substances, *Corynebacterium kutscheri, Corynebacterium xerosis*, fermentation.

INTRODUCTION

With the increasing misuse of antibiotics, the serious problem of antibiotic resistance is coming up very fast. Therefore, intensive search for new antibiotic is going world wide (Emmert et al., 2004; Leisinger and Margraff, 1979; Katz and Demain, 1977). To make the production of antibiotic feasible, it is necessary to develop the optimum production conditions. Several researchers have contributed considerably in this field (El-Banna and Winkelmann, 1998; Akihiro et al., 1993; El-Banna, 1989). The strains *Corynebacterium kutscheri* NB-1 and *Corynebacterium xerosis* NB-2, isolated from the soil samples from Jerash Private University, Jerash, Jordan, were found to produce several antimicrobial substances that exhibited potent antifungal and antibacterial activity against fungi and bacteria (El-Banna, 2004, 2005).

In the research presented here, the main objective was to determine how carbon source could be manipulated to Enhance the antimicrobial substances produced by corynebacteria.

MATERIALS AND METHODS

Stock cultures

The isolated bacterial strains (*Corynebacterium kutscheri* NB-1 and *Corynebacterium xerosis* NB-2) and the test microorganisms (*Fusarium oxysporium* SQ 11, *Candida albicans* and *Escherichia coli* SQ 22) were obtained from Jerash Culture Collection (JCC). They were stored in nutrient agar slants contained (per liter) 5 g peptic digest of animal, 5 g sodium chloride, 5 g yeast extract and 1.5 g beef extract (HiMedia Laboratories Pvt. Limited, Bombay-400 086, India) at 4ºC and used to seed the production media.

Preculture conditions

Bacteria were transferred by loop from nutrient agar slants to 125-ml flasks containing 50 ml medium as in above without agar. All precultures were incubated at 27ºC on a rotary shaker (Sanyo Gallenhamp PLC, Leicester, LE 3 2uz, UK) at 180 rpm for 24 h. 1 ml of preculture was used to inoculate all experimental cultures.

Flask culture studies

Sources of carbon were varied in 100 ml defined medium, which contained the following ingredients per liter: 35 g carbon source (arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, ribose, starch and sucrose), as independent media, 21.8 g KH_2PO_4, 5.7 g Na_2HPO_4, 0.5 g $MgSO_4$, 0.05 g $ZnSO_4$, 0.5 g $FeSO_4x7H_2O$ and 10 g monosodium glutamate at pH 7, and shaken at 180 rpm for 45-48 h.

Agar diffusion test

Extraction of the active substance from the supernatants and cells grown in liquid cultures (100 ml) was done. Cells were pelleted by centrifugation and extracted with acetone, and the supernatant was

Table 1. Effect of carbon source on the antimicrobial substance production by *Corynebacterium xerosis* NB-2.

Carbon source	Antibiotic yield*	
	Fusarium oxysporium	***Escherichia coli***
Arabinose	11.5	15.1
Fructose	8.5	11.4
Galactose	12.4	16.5
Glucose	11.5	15.0
Glycerol	12.0	16.0
Lactose	9.2	12.1
Maltose	8.8	11.5
Ribose	0.00	0.00
Starch	8.3	11.2
Sucrose	11.9	15.8

*Diameter of inhibition zone (mm). The second organism tested for antimicrobial sensitivity by each strain being examined is the one that proved most sensitive in preliminary work.

Table 2. Effect of carbon source on the antimicrobial substance production by *Corynebacterium xerosis* NB-2.

Carbon source	Antibiotic yield*	
	Fusarium oxysporium	***Candida albicans***
Arabinose	16.5	12.8
Fructose	17.2	13.8
Galactose	17.5	12.5
Glucose	18.5	14.5
Glycerol	16.5	13.5
Lactose	0.00	0.00
Maltose	18.1	14.0
Ribose	17.5	12.6
Starch	0.00	0.00
Sucrose	18.1	14.0

*Diameter of inhibition zone (mm). The second organism tested for antimicrobial sensitivity by each strain being examined is the one that proved most sensitive in preliminary work.

extracted with ethylacetate. Both extracts were evaporated by a rotary evaporator (Heidolph instruments, GmbH and Co KG Vertrieb, Kelheim, Germany) at <50ºC, and the dry substances were dissolved in 0.5 ml methanol. The antimicrobial activity of these extracts was done against test microorganisms by agar diffusion test (El-Banna and Winkelman, 1998).

Biotest plates preparation

Using bacteria and yeasts as test microorganisms, cell suspension of 24 h precultures were prepared ($O.D_{578}$ = 1), and 0.5 ml of this suspension was used to inoculate 250 ml soft agar medium (20 ml per plate, Arab food and Media Applicances Co Ltd. Zarka Industrial Area, Jordan) . In case of spore forming fungi (as test microorganisms), flasks with potato dextrose agar contained (per liter) 200 g potatoe infusion, 20 g dextrose and 15 g agar (HiMedia Laboratories Pvt. Limited, Bombay-400 086, India) were inoculated with fungi and incubated at 27ºC for 10 days. After sporulation, the spores were harvested using 80% saline (0.1% Tween, 0.9% NaCl). The spores were then washed and resuspended in normal saline. 250 ml of test media (soft agar) was inoculated with 1 ml of spore suspension (10^7 spore/ml).

RESULTS AND DISSCUSION

Ten different carbon sources were added to the production media at a concentration of 3.5% (w/v). There was a high degree of variation in the level of antimicrobial activity when the different carbon sources were tested in the medium. The antimicrobial activity of bacterial strains using substrates such as arabinose, fructose, galactose, glucose, glycerol, lactose, maltose, ribose, starch and sucrose, is presented in Tables 1 and 2. The antimicrobial substance production of *C. kutscheri* NB-1 was greatly influenced by addition of galactose reaching the highest antimicrobial activity, followed by glycerol, sucrose, arabinose, glucose, lactose, maltose, fructose and starch, whereas ribose had repressed the production of the antimicrobial substance (Table 1) . The antimicrobial activity of C. *xerosis* NB- 2 was highly induced by glucose, and less activity was observed with maltose, sucrose, galactose, ribose, fructose, arabinose and glycerol. No antimicrobial activity was detected using lactose and starch (Table 2). Variations in the fermentation environment often result in an alteration in antibiotic production. The alteration involves changes both in yields and in the composition of the substances. Roitman et al. (1990) reported that by varying the conditions under which *Burkholderia cepacia* is grown, the yields and the composition of the antibiotic could be changed.

Glucose, usually an excellent carbon source for growth, interferes with the biosynthesis of many antibiotics such as bacitracin (Haavik, 1974) and actinomycin (Gallo and Katz, 1972). During studies on fermentation medium development, polysaccharides or oligosaccharides are often found to be better than glucose as carbon sources for antibiotic production (Martin and Demain, 1980). In medium containing glucose plus a more slowly utilized carbon source, glucose is usually used first in the absence of antibiotic production. After glucose is depleted, the second carbon source is then used for antibiotic biosynthesis (Gallo and Katz, 1972).

Antibiotic production of *Burkholderia cepacia* NB-1 was greatly influenced by nutritional and environmental factors. Glycerol at a concentration of 3.5 g/100 ml strongly enhanced the antifungal activity, whereas glucose, mannose and fructose decreased the antifungal activity. Galactose, lactose, rhamnose and starch had repressed the production of pyrrolnitrin (El-Banna and Winkelmann, 1998).

The carbon source needed for maximal yield of the antibiotic production seems to be different among bacte-

rial strains. Glycerol supported better antibiotic production by *Streptomyces hygroscopicus* D 1.5 (Bhattachryya et al., 1998). Glucose was also reported as the most suitable carbon source for maximum phenazine production by *Pseudomonas fluorescens* 2-79 (Slininger and Shea-wilbur, 1995).

The current nutritional conditions that regulate antimicrobial substances metabolism allows us to choose fermentation process designs that minimize, moderate or maximize antimicrobial substances productivity.

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