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CHARACTERIZATION AND DOCUMENTATION OF BACTERIAL DIVERSITY COLLECTED FROM VARIOUS LOCAL HABITATS-1. DIVERSITY IN *ESCHERICHIA COLI*

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Abstract

Escherichia coli strains have been collected from different local habitats including soil, water, plants, animals and human faces. These strains were characterized for different penicillin acylases (PA), which hydrolyse penicillin G and/or V to yield 6-aminopenicillanic acid (6-APA): a key intermediate of semi synthetic penicillin. The study was conducted with the objectives to describe i) parameters used to differentiate different isolates, ii) to report variation in strains to produce various acylases, iii) to describe the importance and utilization of various strains and iv) to introduce to other microbiologists the *E. coli* strains collected and maintained by bacterial diversity collection section (BDCS) of NIAB. Our ultimate aim is to maintain a collection of locally isolated *E. coli* to supplement the on-going efforts of isolating new PA producing bacteria. About 98 % of the collected *E. coli* strains can produce penicillin G and amplicillin acylases while penicillin V acylase was exhibited by 57% strains. Co-existence of penicillin acylase and β-lactamase was observed in all the strains. Some of the strains also possessed co-existence of more than three acylases. Based on the variability in the production of various acylases, 79 *E. coli* strains were differentiated, which are being described with the hope that in the post WTO era these strains would be able to provide the help to local industry and researchers.

Introduction

During the last decade, awareness of collecting cultures of microorganisms with respect to conservation of genetic resources and biodiversity has tremendously increased (Colwell, 1997; Davison *et al.*, 1998). Similarly, the increasing significance of the genus *Escherichia* (Hunter-Cevera, 1996) with special reference to industrially important enzymes comprising penicillin acylases and β-lactamases (Liu *et al.*, 2000) and its biotechnological application (Chou *et al.*, 1999, 2000) has led to an increased interest in the diversity, abundance and eco-physiological potential of this bacterial group. Penicillin G acylases (PGA) can be produced by a variety of bacteria, molds and yeast; however, *E. coli* is preferentially used (Sobotkova *et al.*, 1995). *E. coli* penicillin acylase (PA) is the most common source for industrial application and is the most frequently used prokaryotic expression system for high-level expression of homologous and heterologous proteins (Ignatova *et al*., 2003). β-lactamases are microbial enzymes which convert β-lactam antibiotics into biologically inactive metabolites such as penicilloic acid and penicic acid (Ghuysen, 1991). The co-existence of penicillin acylase and β-lactamase in wild bacterial strains has been reported in the literature (Baker, 1992; Arshad & Saba, 2001) and is generally considered responsible for rendering the antibiotics ineffective.

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Due to immense importance of penicillin acylases in industry, these enzymes were used to characterize *E. coli* diversity collected from various ecological niches. Our objectives were to describe i) parameters used to differentiate different isolates, ii) to report variation in strains to produce various acylases, iii) to describe the importance and utilization of various strains and iv) to introduce to other microbiologists the *E. coli* strains collected and maintained by BDCS, NIAB, Pakistan.

Materials and Methods

Bacterial cultures were isolated from different habitats comprising soil, water, plant and animal faeces. Different cost-effective media and methods were used to identify *E. coli* strains. Screening and characterization for penicillin acylase and β-lactamase activity was made by different bioassay methods (Arshad & Saba, 2001). Media formulations, biochemical performances, optimum growth conditions (medium, incubation, temperature etc), microbiological assay and enzyme production were achieved as described previously (Arshad & Ahmad, 2000). Three *E. coli* strains ATCC 11105, ATCC 9637 and ATCC 10798, one strain of *Bacillus megaterium* ATCC 14945 and one strain of *Kluyvera cryocrescens* ATCC 21285, were acquired from American Type Culture Collection (ATCC). These foreign strains were revived on media described by supplier and used for comparison with local strains for PA production. Bacterial strains were maintained on nutrient agar slants and sub-cultured monthly. For long-term preservation, strains were maintained in liquid form. For this purpose, a fresh overnight culture, grown in LB broth, was used to which 20% glycerol was added before storing the cultures in ultra cold freezer at – 80ºC.

Results

Of the 79 *E. coli* strains used in the present study, almost all exhibited varying range of high penicillin G acylase (PGA), low β-lactamase and considerably high ampicillin activity nevertheless, 43% of the 79 strains were devoid of any penicillin V acylase (PVA) activity. The enzymes activities were categorized as fair (≤ 0.1 mg 6-APA $hr^{-1}mg^{-1}$ protein), moderate (0.2-0.4 mg 6-APA $hr^{-1}mg^{-1}$ protein), good (0.5-0.6 mg 6-APA $hr^{-1}mg^{-1}$ protein) and excellent (≥ 0.7 mg 6-APA $hr^{-1}mg^{-1}$ protein). In addition to PGA, various levels of PVA and AA (Ampicillin acylase) activities were also detected. Frequency distribution of these strains along-with various expression categories of the strains is being presented in Table 1.

Table 1. Frequency distribution (%) of different *Escherichia coli* strains exhibiting various levels of penicillin acylases.

Enzymes	Excellent	Good	Moderate	Fair	Nil
PGA	31.6	32.9	32.9	02.5	00.0
PVA	00.0	03.8	22.8	30.4	43.0
AA	00.0	15.2	54.4	30.4	00.0
β-lact	01.3	27.8	49.4	21.5	00.0

PGA (penicillin G acylase), PVA (penicillin V acylase), AA (ampicillin acylase), β-lact (β-lactamase). Excellent (≥ 0.7 mg 6-APA $hr^{-1}mg^{-1}$ protein), Good (0.5-0.6 mg 6-APA $hr^{-1}mg^{-1}$ protein), Moderate (0.2-0.4 mg 6-APA $hr^{-1}mg^{-1}$ protein) and Fair (≤ 0.1 mg 6-APA $hr^{-1}mg^{-1}$ protein).

Table 2a. Enzymatic characterization of *Escherichia coli* strains collected from water, soil, and human fAeces.

Sr. #	Habitat	Strain #	Enzyme activity			
			PGA	PVA	AA	β-lact
	Water					
1.		BDCS-N-W5	++++	++	+++	++
2.		BDCS-N-W26	+++	-	+	++
3.		BDCS-N-W50	++++	++	+++	+
4.		BDCS-N-W97	++++	++	++	++
5.		BDCS-N-W113	+++	+	+++	+
	Soil					
6.		BDCS-N-S9	+++	+	+	+
7.		BDCS-N-S21	++++	+++	++	+
8.		BDCS-N-S28	+++	++	++	++
9.		BDCS-N-S40	+++	-	+	++
10.		BDCS-N-S47	+++	++	++	+
11.		BDCS-N-S95	+++	+	++	++
	Human faeces					
12.		BDCS-N-HF9	++++	+	+++	+++
13.		BDCS-N-HF10	++++	++	++	+++
14.		BDCS-N-HF11	++++	++	+++	++
15.		BDCS-N-HF12	+++	_	++	+++
16.		BDCS-N-HF13	++++	++	++	+++

- nil, + fair, ++moderate, +++ good, ++++ excellent
PGA (penicillin G acylase), PVA (penicillin V acylase), AA (ampicillin acylase), β-lact (β-lactamase).

Almost all the strains showed PGA activity that falls in the range of excellent (31.6%), good (32.9%), moderate (32.9%) and fair (2.5%). None of the strains was PGA negative. Similarly, about 30.4 % of the strains exhibited PVA activity in the range of fair category, 22.8 % in the range of moderate and only 3.8% were in the range of good categories. Of the 79 total strains, about 43% were found PVA negative. The highest frequency (54.4%) of ampicilin acylase (AA) producers falls in moderate category, followed by 30.4 % under fair and 15.2% under good category. β-lactamase activity was also detected in all the strains with 49.4% falling under moderate category, 27.8% under good, 21.5% under fair and only 1.3% under excellent category. The details of individual strain along-with differential expression of PGA, PVA, AA and β-lactamase (used for individual strain identification) are being described in Table 2 (a, b and c) under three groups constructed on apparent relationships between the habitats. Table 2a shows characterization of *E. coli* collected from water, soil and faeces of human being. In this group, PGA activity was the highest and ranges between 60%, 17% and 80% for water, soil and human feces, respectively in category of excellent. The second highest activity was of AA followed by β-lactamase and PVA. Table 2b shows enzymatic characteristics of *E. coli* group collected from faeces of buffalo, cow and goat (milk producing animals). In this group, PGA activity was the lowest as 64% of the strains exhibited activity in moderate category, 29% in good and 6% in fair category. PVA activity was also the lowest as 68% strains did not show any activity, 6% showed good and 13% each showed fair and moderate activity, respectively. Table 2c comprises strains collected from faeces

of donkey, horse, and mule. About 67% of strains in mule exhibited PGA activity in excellent category with a comparatively low situation observed in donkey (56%) while in horse, 43% of strains exhibited excellent category of PGA. In this group, β-lactamase activity was the highest as 37% of strains exhibited activity in good category, compared to 19% in the group comprising milk producing animals, and 25% in water, soil and human group. Each strain that produces penicillin acylases displays its own properties with respect to growth pattern and enzyme production.

Table 2b. Enzymatic characterization of *Escherichia coli* strains collected from faeces of buffalo, cow and goat.

Sr. #	Habitat	Strain #	Enzyme activity			
			PGA	PVA	AA	β-lact
	Faeces buffalo					
1.		BDCS-N-FB13	++	-	+	++
2.		BDCS-N-FB16	++	-	++	++
3.		BDCS-N-FB18	+	-	+	++
4.		BDCS-N-FB19	+++	-	++	++
5.		BDCS-N-FB20	++	-	++	++
6.		BDCS-N-FB21	+++	+	++	++
7.		BDCS-N-FB22	+++	-	++	++
8.		BDCS-N-FB23	++	-	+	++
9.		BDCS-N-FB24	+++	-	++	++
10.		BDCS-N-FB25	++	++	+	+
11.		BDCS-N-FB26	+++	-	++	+
	Faeces cow					
12.		BDCS-N-FC1	+++	+++	++	+++
13.		BDCS-N-FC2	++	++	+	++
14.		BDCS-N-FC4	++	+	+	+++
15.		BDCS-N-FC6	+++	+++	++	++
16.		BDCS-N-FC7	+	-	+	+++
17.		BDCS-N-FC8	++	-	++	+++
18.		BDCS-N-FC9	++	-	++	++
19.		BDCS-N-FC10	++	-	++	++++
20.		BDCS-N-FC13	++	++	+	+++
21.		BDCS-N-FC14	++	+	++	+++
	Faeces goat					
22.		BDCS-N-FG5	++	-	+	+
23.		BDCS-N-FG6	+++	-	++	+
24.		BDCS-N-FG7	++	-	+	++
25.		BDCS-N-FG8	++	-	+	++
26.		BDCS-N-FG9	++	++	+	++
27.		BDCS-N-FG10	++	-	+	++
28.		BDCS-N-FG11	+++	-	++	++
29.		BDCS-N-FG1 3	++	-	+	+
30.		BDCS-N-FG14	++	+	+	+
31.		BDCS-N-FG15	++	-	++	+

- nil, + fair, ++moderate, +++ good, ++++ excellent

PGA (penicillin G acylase), PVA (penicillin V acylase), AA (ampicillin acylase), β-lact (β-lactamase).

Table 2c. Enzymatic characterization of *Escherichia coli* strains collected from faeces of Donkey, Horse and Mule.

Sr. #	Habitat	Strain #	Enzyme activity			
			PGA	PVA	AA	β-lact
	Faeces donkey					
1.		BDCS-N-FD1	++++	+	+++	+
2.		BDCS-N-FD2	++++	++	++	+
3.		BDCS-N-FD10	+++	+	+	+
4.		BDCS-N-FD16	++++	+	++	++
5.		BDCS-N-FD20	++++	+	++	+
6.		BDCS-N-FD22	+++	+	++	++
7.		BDCS-N-FD25	++++	-	+	++
8.		BDCS-N-FD26	++	-	++	+++
9.		BDCS-N-FD28	+++	+	++	++
	Faeces horse					
10.		BDCS-N-FH1	++++	++	++	+++
11.		BDCS-N-FH2	++	+	+	+++
12.		BDCS-N-FH3	++	-	++	++
13.		BDCS-N-FH4	+++	+	++	++
14.		BDCS-N-FH5	+++	+	++	+++
15.		BDCS-N-FH6	+++	+	++	+++
16.		BDCS-N-FH7	++	+	+	++
17.		BDCS-N-FH9	++++	-	+++	+++
18.		BDCS-N-FH10	++++	-	++	++
19.		BDCS-N-FH11	+++	+	++	++
20.		BDCS-N-FH12	++++	+	++	++
21.		BDCS-N-FH13	+++	+	+++	++
22.		BDCS-N-FH14	++++	++	++	++
23.		BDCS-N-FH15	++++	++	+++	++
	Faeces mule					
24.		BDCS-N-FMu2	++++	++	+++	+++
25.		BDCS-N-FMu6	++++	-	++	+
26.		BDCS-N-FMu9	++++	-	+	++
27.		BDCS-N-FMu10	++++	++	++	+++
28.		BDCS-N-FMu11	+++	+	+++	+++
29.		BDCS-N-FMu12	++++	+	+++	++
30.		BDCS-N-FMu13	++	-	+	+++
31.		BDCS-N-FMu14	++	-	++	+++
32.		BDCS-N-FMu15	++++	-	++	+++

- nil, + fair, ++moderate, +++ good, ++++ excellent
PGA (penicillin G acylase), PVA (penicillin V acylase), AA (ampicillin acylase), β-lact (β-lactamase).

Of all the 5 foreign (ATCC) strains studied for comparison, PGA, AA and β-lactamase activity which ranges between moderate to good, nil to moderate, and nil to fair, respectively was observed in the four strains (Table 3). None of the strains exhibited PVA activity except *K. cryocrescens*, which exhibited very low activity while *E. coli* ATCC 10798 was completely devoid of PGA, PVA, AA and β-lactamase activity.

Table 3. Characterization of bacterial cultures acquired from foreign culture collection for Penicillin acylase and β-lactamase activity.

Sr. #	Strain name	ATCC No	PGA	PVA	AA	β-lact
1.	*Escherichia coli*	9637	++	-	++	+
2.	*Escherichia coli*	11105	+++	-	++	+
3.	*Escherichia coli*	10798	-	-	-	-
4.	*Kluyvera cryocrescens*	21285	++	+	-	+
5.	*Bacillus megaaterium*	14945	+	-	++	+

- nil, + fair, ++moderate, +++ good, ++++ excellent
PGA (penicillin G acylase), PVA (penicillin V acylase), AA (ampicillin acylase), β-lact (β-lactamase).

Discussion

Culture collections play an important role in many areas including agriculture, food testing, public health, clinical laboratories, veterinary, pharmaceutical industry, biotechnology and scientific research (Nakamura, 1989; Kirsop & Doyle, 1991; Zedan, 1993; Hawksworth, 1996; Hunter-Cevera, 1996; Palleroni, 1996; Colwell, 1997; Davison *et al*., 1998). But their meaningful exploitation is possible only if the properties of cultures are properly documented and the information is easily accessible. In the present study, we are documenting for the first time in the country, a big collection of *E. coli* strains collected from different habitats and characterized on the basis of penicillin acylases. As mentioned earlier penicillin acylase is an industrially important enzyme used primarily for hydrolysis of penicillin to produce 6-aminopenicillanic acid (6-APA), which is a starting compound for several semi-synthetic β-lactam antibiotic (Shewale *et al.*, 1990). β-lactamase which usually co-exist with PGA hydrolyses the amide (C-N) bond of the β-lactam nucleus i.e., 6-APA into biologically inactive metabolites such as penicilloic acid and penicic acid (Ghuysen, 1991). In the present study, we have identified some of the strains, which manifested a broad-substrate profile, hydrolyzing penicillin G, penicillin V and ampicillin and indicated the co-existence of three acylases *i.e*., PGA, PVA and AA. These strains accept penicillin G, penicillin V and ampicillin as substrates and present excellent prospect in the inter-conversion of β-lactam antibiotics. The comparative studies of occurrence of more than one acylase in the same bacterial strain are not very common. Only a few reports are available which indicate the presence of two acylase enzymes in bacteria. The substrate specificity of the penicillin acylase present in *Penicillium chrysogenum* A-9342 and *Cephalosporium* CM1 49137 has been reported (Claridge *et al.*, 1963). *Cephalosporium* acylase mainly hydrolyzes penicillin V, and to a lesser degree carboxy-penicillin, ampicillin and penicillin G as well. However, none of the bacterial strain has so far been reported to have exhibited more than one acylases. The present study is therefore, first of its kind in which not only that *E. coli* strains possessing simultaneously three acylases (PGA, PVA, and AA) are being reported but at the same time, preliminary indication has also been provided on particular habitats of the *E. coli* strains that may possess high PGA compared to β-lactamase activity. The production of these enzymes is of tremendous significance as this may help defining the real role of penicillin acylases in the bioconversion of penicillin into 6-APA.

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