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Effect of Salinity on the Growth and Conjugation of Escherichia Coli

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Abstract

Antibiotics have made indelible contributions to human production and life. Nowadays, antibiotics are widely used in medicine, animal husbandry and aquaculture as a medicine with health care, disease prevention and growth promotion functions. However, the abuse of antibiotics makes the problem of bacterial drug resistance increasingly prominent, which makes it more difficult to prevent and control many diseases. Gene mutation and plasmid conjugation transfer are the main ways for bacteria to obtain antibiotic resistance genes. The frequency of drug resistance mutation is generally low, while the frequency of drug resistance acquired through drug resistance factor conjugation transfer is high, and often multiple drug resistance factors are transferred simultaneously. In the process of prevention and control of Escherichia coli infection, due to the irrational use of antibiotics, the drug resistance of some Escherichia coli increased, and even multi-drug resistant strains appeared, which gradually spread to the environment. In this paper, the effect of salinity on the growth and conjugation of Escherichia coli was analyzed, which provided a new idea for the generation and spread of drug resistance of Escherichia coli.

Keywords

Escherichia coli, Salinity, Joint transfer, antibiotic.

1. Introduction

Since Alexander Fleming discovered penicillin in 1928, antibiotics have made indelible contributions in human production and life [1]. Today, antibiotics are widely used in medicine, animal husbandry and aquaculture as a drug with medical care, disease prevention and growth promotion. Bacterial conjugation is a process of genetic material exchange through close contact between cells, and widely exists in many Gram-negative and positive bacteria [2]. In order to study the genetics of Escherichia coli, it is necessary to establish a genetic information transfer system in Escherichia coli. This can not only promote the study of CO2 fixation and inorganic oxidation mechanism of Escherichia coli, but also introduce some genes of heterotrophic bacteria into Escherichia coli [3]. In animal husbandry production, antibiotics can not only prevent and treat animal diseases, but also promote animal growth as feed additives. However, the long-term use of antibiotics in livestock and poultry is easy to induce bacterial drug resistance and spread between animals and environmental bacteria through movable genetic elements such as plasmids and transposons, which seriously threatens human health and safety [4]. To study the relationship between autotrophy and heterotrophism from the perspective of genetics, it is more important to introduce the gene against heavy metal ions existing in heterotrophic bacteria into ore leaching bacteria and transform ore leaching bacteria to make it more suitable for practical application [5].

The abuse of antibiotics makes the problem of bacterial drug resistance increasingly prominent, which makes it more difficult to prevent and control many diseases. Gene mutation and plasmid

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conjugation transfer are the main ways for bacteria to obtain antibiotic resistance genes. The study of multiple drug resistance of pathogens has important medical significance [6]. The frequency of drug resistance mutation is generally low, while the frequency of drug resistance obtained through drug resistance factor conjugation transfer is high, and multiple drug resistance factors are often transferred at the same time. This transfer can be carried out between species and genera [7]. In the process of preventing and controlling Escherichia coli infection, due to the unreasonable use of antibiotics, the drug resistance of some Escherichia coli is enhanced, and even multi drug resistant strains appear, and gradually spread to the environment [8]. In the environment, the coexistence of sulfa resistant Escherichia coli and sulfa sensitive Escherichia coli is common, but the existing research results mostly study the drug resistance mechanism of sulfa resistant Escherichia coli alone or the mutation mechanism of sulfa sensitive Escherichia coli [9]. A large number of sulfa antibiotics in the environment will not only have toxic effects on environmental organisms, but also induce bacteria to develop drug resistance [10]. Therefore, the study of the effect of sulfonamides on the transmission of antibiotic resistance genes will help us to explore the mechanism of bacterial drug resistance, so as to prevent and slow down the continued spread of args. This paper analyzes the effect of salinity on the growth and conjugation transfer of Escherichia coli, so as to provide a new idea for the generation and transmission of drug resistance of Escherichia coli.

2. Growth count analysis of sulfadiazine resistant Escherichia coli

There are three main ways of antibiotic resistance horizontal gene transfer, namely conjugation, transformation and transduction. Among them, conjugation transfer is the most easy and efficient way for bacterial drug-resistant horizontal gene transfer to occur, and it is also the most important way for bacterial genetic material in nature, especially for drug-resistant genes to spread among the same genus and across species. The conjugation and transfer of drug-resistant genes are mainly mediated by conjugation plasmids with autonomous transfer function, which have genes encoding transfer function, so plasmid transfer can occur without depending on host bacteria [11]. There is no donor bacteria in a single bacterial solution, and sulfa-sensitive Escherichia coli can only obtain drug resistance genes through mutation. When donor bacteria and recipient bacteria were used as control alone, no colonies grew on the zygote selective plate, which indicated that the colonies growing on the plate were zygotes with recipient bacteria as host bacteria. The growth counts of sulfadiazine-resistant Escherichia coli under different antibiotic concentrations and certain salinity are shown in Table 1 to Table 4.

Table 1 Salinity 0‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
512	0	1084	112000	563000	7380000	1140000	620000	107000
1024	1	984	863000	436000	3220000	960000	840000	440000
2048	0	188	22000	14000	450000	1520000	460000	150000
4096	1	77	1134	884	2169	3251	995	756
10000	1	80	96	972	588	512	501	74

Table 2 Salinity 20‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
512	1	376	348000	422000	3320000	5670000	1280000	950000
1024	3	33	610000	512000	1430000	3310000	740000	5170000

2048	1	683	18000	311000	110000	910000	3150000	3110000
4096	2	73	1481	1007	10380	10030	4170	2107
10000	0	60	883	311	203	4120	985	59

Table 3 Salinity 40‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
512	0	36	443	35100	510000	3140000	880000	220000
1024	1	2	223	5600	940000	7870000	310000	310000
2048	0	51	984	4310	22600	230000	860000	100000
4096	0	4	646	791	1044	37400	7710	890
10000	1	7	88	310	514	387	1261	59

Table 4 Salinity 60‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
512	0	42	812	1771	220	4100	358	401
1024	0	18	33	893	430	5100	886	113
2048	0	82	714	228	210	4300	748	67
4096	0	17	23	207	33	75	88	40
10000	1	4	8	33	22	41	7	8

The secretion of glucocorticoids and catecholamine hormones in stressed organism is higher than that in normal state, and the immune response level is obviously decreased, and the susceptibility to bacteria is increased. The change in pH of Escherichia coli growth is shown in Figure 1.

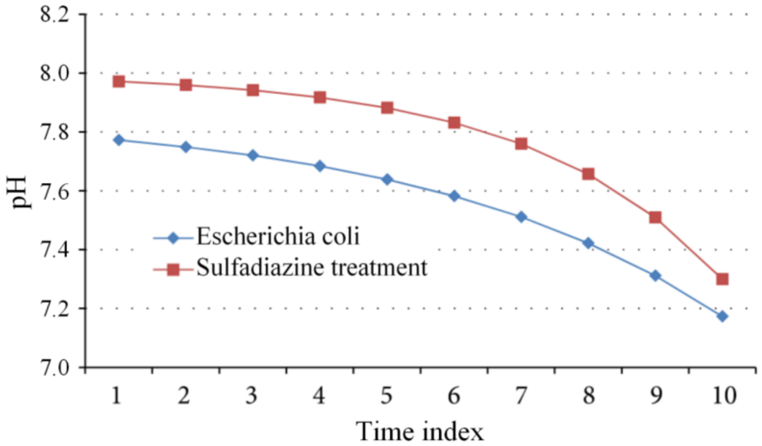

Figure 1 Changes in pH of Escherichia coli growth

RP4 plasmid is a typical IncPa type multi-drug resistant plasmid, which encodes three antibiotic resistance genes, kanamycin, tetracycline and ampicillin, and RP4 is a conjugate plasmid with a wide host, which has been detected in many bacterial species in nature. RP4 plasmid can be transferred to other bacteria by conjugation transfer in liquid phase and solid phase. RK2 plasmid, pCF10 plasmid and RP4 plasmid are all drug-resistant conjugation plasmids which encode antibiotic resistance genes and regulate conjugation transfer genes. RK2 plasmid

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encodes apramycin resistance gene, and pCF10 encodes tetracycline resistance gene. Unlike RP4, RK2 is a plasmid only transferred in Escherichia coli, and its regulatory mechanism is basically the same as RP4. Because of the different conjugation transfer plasmids, their encoded conjugation transfer functions and regulatory mechanisms are different. Therefore, conjugation transfer of plasmids can only occur within specific species or across species. Therefore, it is necessary to establish a suitable and stable plasmid conjugation and transfer model before comprehensively studying the influence of nanomaterials on bacterial drug resistance gene conjugation and transfer.

3. Growth count analysis of tetracycline resistant Escherichia coli

There are three essential factors in the conjugation transfer model of bacterial drug resistance genes, namely, donor bacteria with antibiotic resistance genes, conjugation transfer plasmids with antibiotic resistance genes and recipient bacteria with antibiotic resistance markers. From the discovery of tetracyclines to the 21st century, the drug resistance level of tetracyclines in Escherichia coli isolated from Chinese pig farms remained high, and tetracyclines with high drug resistance rate were also detected in Escherichia coli isolated from some organic farms and healthy pig farms. In addition, there are some differences in drug resistance detected in different regions, such as the relatively low level of drug resistance of Escherichia coli to tetracyclines in inland areas of China. However, in the eastern coastal areas and areas with developed pig industry, the resistance level of Escherichia coli from pigs to tetracycline is relatively high. Establishing a suitable and stable model for conjugation and transfer of bacterial drug resistance genes is an important prerequisite for studying the influence of nanomaterials on conjugation and transfer of bacterial drug resistance. The growth counts of tetracycline-resistant Escherichia coli with different antibiotic concentrations and a certain salinity are shown in Table 5 to Table 8.

Table 5 Salinity 0‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
16	0	432	33000	1200000	3620000	4310000	3100000	120000
32	1	895	843000	2470000	3980000	2120000	5380000	810000
48	0	48	62000	840000	900000	480000	820000	30000
144	1	26	3641	1100000	36000	123000	10000	8106
432	1	8	561	234	54	563	1001	431

Table 6 Salinity 20‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
16	1	33	9600	990000	1170000	4020000	6320000	840000
32	3	106	242000	620000	2250000	770000	5320000	680000
48	1	310	55000	420000	840000	1850000	340000	2260000
144	2	17	363	31000	98400	12800	9100	9980
432	0	2	26	4612	652	8840	891	234

Table 7 Salinity 40‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
16	0	962	1083	12400	130000	1940000	7010000	350000
32	1	134	943	32600	1440000	5170000	960000	940000
48	0	7	3246	8300	4340000	940000	2380000	220000
144	0	44	77	1030	3620	1100	3200	321
432	1	82	310	440	4410	81	723	96

Table 8 Salinity 60‰

Antibiotic concentration (mg/L)	0h	6h	12h	24h	36h	48h	60h	72h
16	0	44	12	44	183	563	88	4
32	0	323	297	358	848	84	3	89
48	0	4	4	44	61	32	22	4
144	0	5	87	19	84	3	6	10
432	1	4	5	5	23	8	3	4

After successful conjugation and transfer between recipient bacteria and donor bacteria, the conjugation showed a significant increase in resistance to quinolone antibiotic enrofloxacin. The growth index of Escherichia coli is shown in Figure 2.

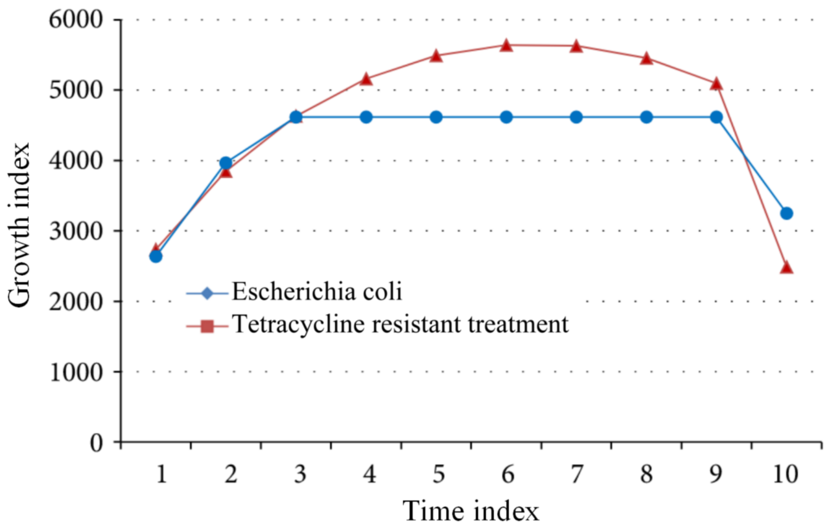

Figure 2 Changes in the growth index of Escherichia coli

The secretion of glucocorticoids and catecholamine hormones in stressed organism is higher than that in normal state, and the immune response level is obviously decreased, and the susceptibility to bacteria is increased. The recipient bacteria in the mixed bacteria solution can obtain the drug resistance gene carried by the donor bacteria containing R388 plasmid by conjugation transfer, and can also obtain the drug resistance gene by mutation and then grow stably. Compared with recipient bacteria, the conjugation showed a different degree of resistance to gentamicin, florfenicol and other antibiotics, and the drug resistance was co-transferred. This phenomenon indicates that conjugation plasmid plays a very important role in the transmission of bacterial drug resistance. Host's physiological stress and psychological stress have important influence on the incidence, duration and severity of host diseases, especially those caused by infection. The horizontal transmission of drug-resistant genes in intestinal tract is promoted by conjugation and transfer, so it is of great significance to strengthen the management of various stress factors in livestock breeding to prevent diseases caused by bacterial infection. The influence mechanism of stress on drug resistance gene horizontal transmission and whether stress hormones are involved in the formation of drug resistance of pathogenic bacteria need further study.

4. Conclusions

By conjugation transfer, plasmid transfer from Gram-negative bacteria such as Escherichia coli to Gram-positive bacteria such as Corynebacterium is convenient and efficient. The establishment of this transfer system is helpful for breeding high-yielding strains by genetic engineering technology in amino acid production. This zygote strain not only acquired the drug resistance gene of the donor strain, but also transmitted the drug resistance phenotype. The secretion of glucocorticoids and catecholamine hormones in stressed organism is higher than that in normal state, and the immune response level is obviously decreased, and the susceptibility to bacteria is increased. If a suitable DNA transfer system can be found between genetic engineering operators such as Escherichia coli and plant cells, it will have great application value in plant genetic engineering. Host stress hormones may participate in the adaptation and evolution of pathogenic bacteria in the host intestinal environment, and promote the horizontal transmission of drug-resistant genes in the intestinal tract by conjugation and transfer. Therefore, strengthening the management of various animal stress factors in animal production and breeding is of great significance to prevent diseases caused by bacterial infection. The influence mechanism of stress on drug resistance gene horizontal transmission and whether stress hormones are involved in the formation of drug resistance of pathogenic bacteria need further study.

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