Study of some genes regulating salt tolerance in bacteria

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thebacterial isolates were identified as salt tolerance; they were Halomonas species, Halococcus salifodinae and Bacillus pasteurii. These species were Gram positive and Gram negative bacteria. The morphological changes associated with salt stress showed a lot of differences such as cell volume, cell length, pigment, and colony shape. The salt stress proteins were demonstrated large differences in the Gram positive and negative isolates. In general, at Halomonas sp.(a) eleven bands (123.7, 112.5, 81.8, 44.2, 39.7, 35.6, 14.9, 12.5, 12.2, 9.6 and 7.9 Kum) were newly synthesised among which three bands (14.9, 9.6 and 7.9 KDa) were common in all different concentrations of NaCI except the control. Two bands (12.5 and 12.2 Koa) appeared at 2.93 and 11.7 % NaCI only. 35.6 KDa band appeared at 5.85, 11.7 and 23.4 % NaCI only. Two bands (81.8 and 39.7 KDa) appeared at 2.93, 5.85 and 11.7 % NaCI only. 44.2 KDa band appeared at 2.93, 11.7 and 23.4 % NaC1 only. 112.5 KDa band appeared at 2.93 % NaCI only. 123.7 KDa band appeared at 11.7 and 23.4 %NaCI only.When cells were subjected from 10 to 23.4% NaCI (high shift), they showed a lot of differences as a response to this shift (long term shift). The general protein synthesis showed twenty one bands ranged from 123.7 to 7.9 KDa at the control (10% NaCI) among which threeten of them (102.1, 77.3, 73.4, 64, 58.3, 53.1, 49, 35.6, 32, 29.9, 22, 14.9, 9.6 and 7.9 KDa) were common in the all different times (1 hr, 2hr and 4hr). Two bands (28.1 and 13.3 KDa) newly synthesised by high shift (10 to 23.4Sunintaly 134% NaCI) compared with the control. There were two novel bands (28.2 and 11.4 KDa) appeared only after at 4hrs., while two other bands (39.7 and 8.3 KDa) disappeared in all different times except the control. Five bands (123.1, 89.4, 81.8, 26.1, and 12.2 KDa) appeared at lhr. and 2hrs. only.When cells were shifted from 23.4 to 10 % NaCI, they showed a lot of differences as a response to this shift (short term shift). The general protein synthesis demonstrated nineteen bands ranged from 132.7 to 7.9 KDa in the control (23.4 % NaCI) among which twelve of them (123.7, 64, 58.3, 53.1, 49, 44.2, 29.9, 28.1, 26.1, 22, 9.6 and 7.9 KDa) were common in all different times. Nine bands (94.7, 81.8, 39.7, 17.4, 14.3, 12.2, 11.3, 10.5 and 8.3 KDa) were newly synthesized and among which three of them (94.7, 81.8 and 10.5 KDa) appeared at 15 min. and 1 hr. only. Three bands (39.7, 17.4 and 12.2 KDa) appeared in all different times, as well as, 14.3 KDa band appeared at 30 min. and 1 hr. only, while 11.3 KDa band appeared at 1hr. only and 8.3 KDa band appeared at 30 min. only. The growth curves illustrated that 2.0 M curve was the best curve since the bands were more intensive and the lag phase was shortcompared with 4.0 M curve.In Halomonas sp. (b) twelve bands (115.9, 89.1, 72.5, 52, 42.4, 27.1, 24, 21.4, 19.5, 16.2, 11.5 and 7.7 KDa) were newly synthesized by NaCI compared with the control, among which three bands of them (89.1, 24 and 16.2 KDa) appeared at all concentrations except the control. Two bands (115.9 and 19.5 KDa) appeared at 5.85, 11.7 and 23.4 % NaCI only. Two bands (72.5 and 42.4 KDa) appeared at 5.85 % NaCI only. Two bands(115.9 and 19.5 KDa) appeared at 5.85, 11.7 and 23.4 % NaCI only, while 27.1 KDa appeared at 23.4 % NaCI only , as well as, 21.4 KDa band appeared at 2.93 % NaCI only, 11.5 KDa band appeared at 11.7, 23.4 % NaC1 only and 7.7 KDa band appeared at 2.93 and 5.85 % NaCI only. 52 KDa band appeared at 11.7 and 23.4 % NaCl.When cells were shifted to a high shift from 10 to 23.4% NaCI , they showed a lot of differences as a response to this shift (long term shift). The general protein synthesis demonstrated twenty four bands ranged from 188.2 to 7.7 KDa at the control (10% NaCI), among

which eleven bands of them (160.2, 102.1, 64.8, 33.8, 24, 19.5, 13.7, 12.4, 10.5, 8.1 and 7.7 KDa) were common in all different times. Five bands (188.2, 131.1, 62.6, 25.9 and 16.2 KDa) appeared at 4hrs. only. Six bands (115.9, 89.1, 52, 18.3, 15.1 and 9.9 KDa) appeared at 2hrs. and 4hrs. only. Two bands (39.6 and 11.5 KDa) appeared at 1 hr. and 4hrs.only.Seven bands (57.1, 27.1, 21.4, 10.5 and 7.7 KDa) were newly synthesized, among which three bands (57.1, 10.5 and 7.7 KDa) appeared at lhr. and 2hrs. only. 27.1 KDa band appeared at 2hrs. only. 21.4 KDa band appeared at l hr. only.When cells were shifted to a low shift from 23.4 to 10 % NaC1, they showed a lot of differences as a response to this shift (short term shift). The general protein synthesis showed twenty two bands ranged from 160.2 to 7.7 KDa at the control (23.4% NaCI), among which eight bands of them (160.2, 89.1, 64.8, 62.6, 57.1, 24, 19.5 and 13.7 KDa) disappeared at all different times, while 115.9 KDa band appeared 30 min.and ihr.only. Fivebands (102.1, 27.1, 18.3, 15.1 and 9.9 KDa) appeared at 15 min. only. Six bands (33.8, 25.9, 16.2, 12.4, 10.5, 8.1 and 7.7 KDa) appeared at 15 min. and 1 hr. only, while 11.5 KDa band appearedat 1 hr. only.Nine bands (131.1, 72.5, 45.6, 42.4, 39.6, 37.3, 31.7, 22.7 and 9.5 KDa) were newly synthesized, among which three (131.1, 22.7 and 9.5 KDa) appeared at 1 hr. only. Two bands (72.5 and 42.4 KDa) appeared at 30 min. only. Three bands (45.6, 39.6 and 37.3 KDa) appeared at 15 min. and 30 min. only. 31.7 KDa band appeared at 15 min. only. The growth curves illustrated that 1.0 M and 2.0 M curves werethe best curves since the bands were more intensive and both curves were very similar.In Halococcus salifodinae Twelve bands (133.3, 87.9, 70.2, 65.9, 40.1, 32.5, 26.8, 24.6, 17.6, 13.8, 12.9 and 10.8 KDa) were newly synthesized, among which three bands of them (133.3, 97.9 and 24.6 KDa) appeared at all concentrations of NaCI except the control (0.0 % NaCI). Three bands (70.2, 40.1 and 12.9 KDa) appeared at 5.85, 11.7 and 23.4% NaCI only, while two bands (65.9 and 10.8 KDa) appeared at 11.7 and 23.4 % NaCI only, 32.5 KDa band appeared at 2.93 and 11.7 % NaCl only. 26.8 KDa band appeared at 2.93, 5.85, 11.7% NaCI only, 17.6 KDa band appeared at 23.4 % NaCI only and 13.8 KDa band appeared at 11.7% NaCI only.When cells were subjected to a high shift from 10 to 23.4% NaC1 , they showed a lot of differences as a response to this shift (short term shift). The general protein synthesis showed twenty three bands ranged from 133.3 to 10.8 KDa in the control (10% NaCI), among which fourteen bands of them (133.3,109.2, 98.2, 87.9, 72.1, 65.9, 40.1, 37.9, 24.6, 22.6, 15.8, 12.9 , 11.8 and 10.8 KDa) were common in all different times. Three bands (132.2, 70.2 and 46.7 KDa) appeared at 4hrs. only. Two bands (114.7 and 26.8 KDa) disappeared at all different times. Three bands (59.2, 32.5 and 13.8 KDa) appeared at lhr. and 2hrs. only and 79.7 KDa band appeared at 2hrs. and 4hrs. only. Two bands (20.2 and 17.6 KDa) were newly synthesized at 4hrs. and(2hrs. and 4hrs.) respectively.The general protein synthesis showed twenty one bands ranged from 133.2 to 10.8 KDa in the control (23.4 % NaC1) and when the cells subjected to low shift (short term shift) from 23.4 to 10 % NaCI, they showed no differences due to long interval cells adaptation to synthesize the protein according to Mojica F.J. M. et al, (1997).In Bacillus pasteurii Nineteen bands (157.4, 145.3, 138.6, 132.7, 116, 105.9, 63.2, 44.4, 38.3, 37.2, 25.4, 24.6, 21.9, 15.7, 13.2, 10.9, 10.3, 8.6 and 7.8 KDa) were newly synthesized, among which three bands (13.2, 10.9 and 10.3 KDa) appeared in all different concentrations of NaC1 except the control (0.0 % NaC1), while two bands (21.9 and 8.6 KDa) band appeared at 5.85, 11.7 and 23.4 % NaCI only. Five bands (145.3, 44.4, 38.3, 15.7 and 7.8 KDa) appeared at 11.7 , 23.4 % NaC1 only, 37.2 KDa appeared at 5.85 and 11.7 % NaCl only, 157.4 KDa band appeared at 11.7 % NaCI only, 25.4 KDa band appeared at 2.93 and 5.85 % NaCI only, two bands (105.9 and 63.2 KDa) appeared at 2.93, 5.85 and 11.7 % NaC1 only, 116 KDa band appeared at 2.93, 11.7 and 23.4 % NaCI only, 132.7 KDa band appeared at2.93 % NaCI only and 138.6 KDa band appeared at 5.85 % NaCI only.When cells were subjected to a high shift from 10 to 23.4% NaCI , they showed a lot of differences as a response to this shift (long term shift). The general protein synthesis showed twenty four bands ranged from 171.1 to 6.8 KDa in the control (10% NaCI), among which thireteen bands of them (171.1, 53.6, 48.5, 44.4, 40.7, 38.3, 21.9, 17.3, 15.7, 14.2, 10.9, 10.3 and 8.6 KDa) were common in all different times, three bands (145.3, 116 and 6.8 KDa) appeared at 4hrs. only, 7.4 KDa band appeared at lhr. and 2hrs. only, three bands (71, 29.9 and 8.1 KDa) appeared at 2hrs. and 4hrs. only, three bands (31.5 , 13.2 and 7.8 KDa) appeared at 1hr. and 4hrs. only, and 63.2 KDa disappeared at all different times. 157.4 KDa band appeared at l hr. only.Six bands (138.6,

37.2, 29.9, 25.4, 20.1 and 12.6 KDa) were newly synthesized, among which two bands (138.6 and 20.1 KDa) appeared at 1hr. and 2hrs. only. Three bands (37.2, 29.9 and 12.6 KDa) appeared at 2hrs. and 4hrs. only. 25.4 KDaband appeared at 4hrs. only.When cells were shifted to a low shift from 23.4 to 10 % NaC1, they showed a lot of differences as a response to this shift (short term shift). The general protein synthesis showed twenty two bands ranged from 171.1 to 6.8 KDa at the control (23.4% NaCI), among which three bands of them (145.3, 44.4 and 38.3 KDa) appeared at lhr. only, seven bands of them (171.1, 116, 53.6, 48.5, 31.5, 15.7 and 13.2 KDa) appeared at 15 min. only, three bands (40.7, 8.6 and 6.8 KDa) appeared at 15 min. and 30 min. only, 14.2 KDa appeared at 30 min. and lhr. only and sevenbands (71, 29.9, 21.9, 17.3, 10.9, 10.3 and 7.8 KDa) disappeared at all different times.Five bands (138.6, 92, 63.2, 24.6, and 7.4 KDa) were newly synthesized, among which 138.6 KDa band appeared at 15 min. and 30 min. only, two bands (92 and 63.2 KDa) appeared at 30 min. and 1 hr. only, 24.6 KDa band appeared at 15 min. only and 7.4 KDa appeared at lhr. only.The growth curves illustrated that 1.0 Mand 2.0 M curves were very similar and have short lag phase while 4.0 M curve had long lag phase andd log phase compared with 1.0 M and 2.0M curves.In Escherchia coli Four bands (155.2, 72.9, 28.8 and 18.5 KDa) were newly synthesized, among which 155.2 KDa band appeared at 11.7 % NaC1 only, 72.9 KDa band appeared at 8.78 % NaC1 only, 28.8 KDa band appeared at 2.93 % NaCI only and 18.5 KDa band appeared at 2.93 and 5.85 % NaCI only.The effect of salinity and sucrose on protein synthesis of three bacterial species were illustrated a lot of differences. Five bands (122.7, 92.3, 67, 49.6 and 37.7 KDa) were common in the three bacterial species, while 171.1 KDa band was newly synthesized at 20 % Sucrose and 23.4 % NaCI only, in the three bacterial species and five bands (72.6, 53.2, 45.2, 29.1 and 25.8 KDa) were newly synthesized at 20 % and 50 % sucrose in the three different bacterial species.Fourteen bands (22.6, 20.2, 17.6, 15.2, 15.8, 14.9, 14.2, 12.9, 10.9, 10.5, 10.3, 9.6, 8.6 and 8.1 KDa) appeared at 20 % sucrose and 23.4 % NaCI in Halomonas sp. (a) only, while fourbands (145.3, 139.1, 112.2 and 80.9 KDa) appeared at 20 % sucrose and 50 % sucrose in Halomonas sp. (a)I and Bacillus pasteurii only.As shown in fig. (17) and table (9), the protein patterns of response to a hyperosmotic shock were quite similar for organic (sucrose) and inorganic solutes on three different species of bacteria (Halococcus salifodinae , Halomonas sp. (a) , and Bacillus pasteurii) after grown the cells on 23.4% NaCI, 23.4% NaCI + 20% sucrose and 50% sucrose respectively. The synthesis of low salt proteins decreased simultaneously in three different concentrations of NaCI and sucrose. While high osmolarity proteins started to be synthesis. The only apparent differences correspond to 42 KDa and 55 K Da proteins, whose synthesis rates were notably, depressed under the high sucrose concentrations.When cured plasmid from Halococcus salifodinae and then the cells were exposured to salt stress found that their tolerated to salt stress still.This result indicates that plasmid do not carry any tolerant genes; However, the tolerant genes should be carried on chromosomal DNA of bacteria cells. On the other hand, curing of plasmid of Bacillus pasteurii did not affect its salt tolerance. This result indicates that the salt tolerant genes are finding on chromosomal DNA of bacteria.The resulted fragment of the ect ABC operon from Bacillus pasteurii by PCR was 3.4 Kb. This operon encoded to glycine betaine pathway. The resulted fragment of the otaC operon Halomonas species by PCR was 1.4 Kb. This operon encoded to ectoine pathway.