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# Effect Of Phosphate- Starvation On phoH In Acinetobacter baumannii

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## Abstract

Acinetobacter baumannii is a Gram-negative bacteria that causes fatal bacterial infections and is resistant to environmental conditions such as lack of nutrients. Inorganic phosphate is considered one of the essential nutrients in bacterial cell metabolism. the period of our study from September 2020 to May 2021, In our study, the effect of phosphate starvation on A.baumannii growth in M9 minimal medium, and on the expression of phoH (psiE) gene was determined. The expression level of phoH increased about 10 folds in the presence of $K_2HPO_4$ and about 5-fold with $KH_2PO_4$ in A. baumannii ATCC17904. Unlikely, in A. baumannii HHR1, phoH gene expression is remarkably induced with $Na_2HPO_4$, where the level of expression is about 25-folds higher than that of global strain. However, phoH increased by 3–fold with $KH_2PO_4$ in comparison to global strain. The growth of A. baumannii isolates in M9 medium exhibits different traits in the presence of inorganic phosphate sources, whereas the highest growth in global strainATCC17904 with $Na_2HPO_4$/$NaH_2PO_4$ together. While the local isolate exhibits the highest growth in the presence of $NaH_2PO_4$, whereas the eight clinical isolates showed growth variation in response to different sources. The clinically isolated A. baumannii L1, and L4 showed a higher growth rate with $Na_2HPO_4$, whereas L3 has the highest growth in $K_2HPO_4$/$KH_2PO_4$. L2, L5, L6, L7 and L8 reached a high growth rate with the combination of $Na_2HPO_4$/ $NaH_2PO_4$ , as for PhoH protein was around 41 kDa, inorganic phosphate salts have been varying effects on PhoH protein in both strains , in stander strain is obvious in all fractions of the standard strain, however, in the presence of $NaH_2PO_4$ and a combination of $Na_2HOP_4$/ $NaH_2PO_4$, expression of PhoH protein in HHR1 is decreased and appeared as a fiant band in SDS-PAG

## Introduction:

*Acinetobacter baumannii* is a genus that is considered as a gram-negative[1] , which belongs to gammaproteobacteria, ubiquitous, and nonmotile organism[2] *A. baumannii* caused a large number of nosocomial infections worldwide, and responsible for intensive care unit( ICU)-acquired infections, and also caused multi-infections such as pneumonia, bacteremia, urinary tract infections, skin and soft tissue infections, meningitis and osteomyelitis *A.baumannii* has ability to survival and developed in hospital environments and mucosal surfaces and resistance to drying, which is an essential factor in their survival and protects them from exertion conditions, such as dry environment, and antibiotics so can biofilm infections continue Inorganic phosphate [3, 4] (Pi) is a crucial nutrient in cell metabolism as it is made up of nucleic acids, phospholipids, teichoic acids, membranes and phosphorylated nucleotides and phosphorylated proteins. It is part of the respiratory chain, and it plays an essential role in cascade signalling[5]. Inorganic phosphate is known to be essential for bacterial development but likely rare in some environments. Therefore, microorganisms should be able to regulate Pi uptake, to adaptation, and to live in phosphate -limiting environments. When phosphate is not available, a special pathway occurs to take and uses other sources of phosphorus by two-component regulatory system (TCR) is re-encoded by the genes within a (Pho) regulon , while the Pho regulon is an important regulator component in biological processes of phosphorus uptake when Pi is found in less concentration in nature[6].

The main aim of our study was to find out the effect of phosphate starvation on the induction of *pho*H gene and *A.baumannii* growth.

## MATERIA AND METHODS

### Bacterial Sample Preparation

A global strain *A.baumannii* ATCC17904 and local strain *A.baumannii* HHR1(accession number MH685112.1) were used in this study, and HHR1 identified by 16s RNA. In addition, , eight *A.baumannii* local isolates were isolated clinically during February 2021 from different sources among urine, blood , burn, and wounds samples, four were isolated from a patients in Baghdad Teaching Hospital and others were from patients of the Health Center in Karbala Governorate, were identified by a Vitek system listed in Table 1,

**Table 1:** A.baumannii isolates used in our study.

| The name | The position | The source |
|---|---|---|
| ATCC17904 | USA | ATCC |
| HHR1 | Baghdad | Urine |
| Local 1 | Baghdad | Blood |
| Local 2 | Baghdad | Urine |
| Local 3 | Baghdad | Wound |
| Local 4 | Baghdad | Sputum |
| Local 5 | Karbala | Urine |
| Local 6 | Karbala | Urine |
| Local 7 | Karbala | Wound |
| Local 8 | Karbala | Wound |

**Effect of inorganic phosphate on *A.baumannii***

In M9 medium, the medium prepared according to (Ng, n.d.) with modification by adding inorganic potassium phosphate: three flasks, the first flask as control ( containing D-glucose 4.0g, $NH_4Cl$ 1.0g, NaCl 0.5g, and Yeast Extract 1.0g), other flasks have the same components of control, but one containing either $Na_2HPO_4$, 46.78g; $NaH_2PO_4$ 43.0g or both together, and the other containing either $K_2HPO_4$, 46.78g or $KH_2PO4$ 43.0g or together. The pH was adjusted to 7.0, and the total volume was 1 L before autoclaving. All local isolates of *A. baumannii* and the standard strain were incubated in M9 medium for 24 hours at 37 ° C in a shaker incubator, and then bacterial growth was measured by spectrophotometer at $OD_{600}$ nm.

**Cytoplasmic PhoH extraction**

Overnight bacterial cultures of *A. baumannii* (ATCC 17904 and HHR1) in M9 minimal media with phosphate salt sources and without phosphate salt (control) were centrifuged at (6000 rpm, 5min). The pellets were re-suspended by 10 mM Tris-HCl (pH8) and centrifuged twice as described before for washing. The ultrasonic disintegrator (SANYO) was used (15 seconds, pulses 4 X at a frequency amplitude of 20) to break down the pellet and separate the soluble and insoluble fractions. The cell free extract (CFE) was obtained by centrifugation as above. The pellets were discarded then the supernatant was stored at -20˚C[7].

**Determination of proteins concentration**

The determination of protein concentration was based on the Bradford assay method with some modifications (Bradford, 1976). Bio-Rad dye Reagent was prepared and 10µl of protein soluble fraction was added to clean cuvette. A 800 µl of deionized distilled water ($dH_2O$) was added to 200µl of Bio-Rad dye Reagent concentrator. The mixture was mixed gently by converting the cuvette up and down and was measured by spectrophotometer at $OD_{595}$nm, the concentration was calculated by a special arithmetic method: Concentration (mg $ml^{-1}$) = $OD_{595}$ ×15/protein volume (µl).

**Extraction of Genomic DNA, Primer design, and PCR reaction**

Based on the ABIOpure protocol, the Genomic DNA of A. baumannii ATCC 17904 and HHR1 were extracted and used in the PCR reaction. The genes of interest were amplified using specific primers listed in table 2. The primers were prepared in nuclease-free water stored at -20°C.

**Table2:** primers used in this study

| name | Primer sequence | References |
|---|---|---|
| *pho*H-F | 5`-CCGCAGTGGACATGTTAGAA-3` | Designed in this study |
| *phoH*-R | 5`-CTGGGTTAAATCACCAGGTAAGA-3` | |
| *rpl*B-F | 5`-GTAGAGCGTATTGAATACGATCCAAACC-3` | [8] |
| *rpl*B-R | 5`-CACCACCACCGTGCGGGTGATC-3` | |

**RNA Extraction and Quantitative PCR Analysis (qPCR)**

According to the protocol of TRIzolTM Reagent, RNA was extracted from *A. baumannii* ATCC and HHR1, The gene *rpl*B was used as a housekeeping gene for the quantification. The

primer sequences for genes are listed in Table 2. The optimization program was done as listed in Table 3

**Table 3:** Program of qPCR optimization.

| Optimization | Temp.°C | Time m: s | No.cycles |
|---|---|---|---|
| Enzyme Activation | 37 | 15:00 | 1cycle |
| Initial denaturation | 95 | 10:00 | |
| Denaturation | 95 | 00:15 | 40cycle |
| Annealing | 50/60 | 00:30 | |
| Extension | 72 | 00:30 | |

## RESULTS

### Effect of various sources of inorganic phosphate on *A.baumannii* in M9minimal medium

In our this study, *A.baumannii* ATCC17904 and *A.baumannii* HHR1 were subcultured in minimal medium (M9) containing $K_2HPO_4$, $KH_2PO_4$, $Na_2HPO_4$, $NaH_2PO_4$ and a combination of either ($K_2HPO_4$, $KH_2PO_4$) or ($Na_2HPO_4$, $NaH_2PO_4$). We noticed that the growth of global strain is disparate and variable according to the type of phosphate source. All sources exhibit significant enhancement in the growth; compared to control, the combination of Na2HPO4/NaH2PO4 exerts the highest growth rate at 0.778 in comparison to control with OD600 of 0.220 (Fig.1).

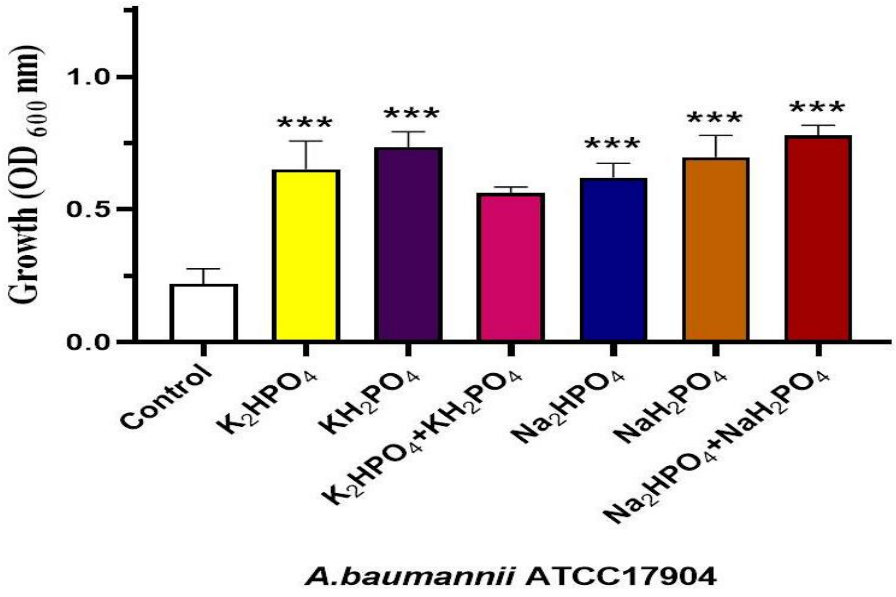



**Fig. 1:** *Growth rate of A.baumannii ATCC17904 with different sources of inorganic phosphate salts. A.baumannii ATCC17904 was cultured in M9 medium containing 30 mM of each phosphate salt. Error bars represent the standard deviation from the mean of three independent biological replicates. The statistical analysis was conducted by student t` test with *** P< 0.001.*

Unlike the ATCC, *A.baumannii* HHR1 exhibits different patterns in response to inorganic phosphate salt (Fig. 2). The growth of HHR1 was enhanced by the addition of $NaH_2PO_4$, where the growth rate was about $OD_{600}$ of 1.340 after 24 hr incubation. In addition,

both $K_2HPO_4$ and $NaH_2PO_4$ significantly increased the growth of A.baumannii HHR1 up to OD600 of 1.168 and 1.152, respectively, in comparison to control with $OD_{600}$ 0.664.

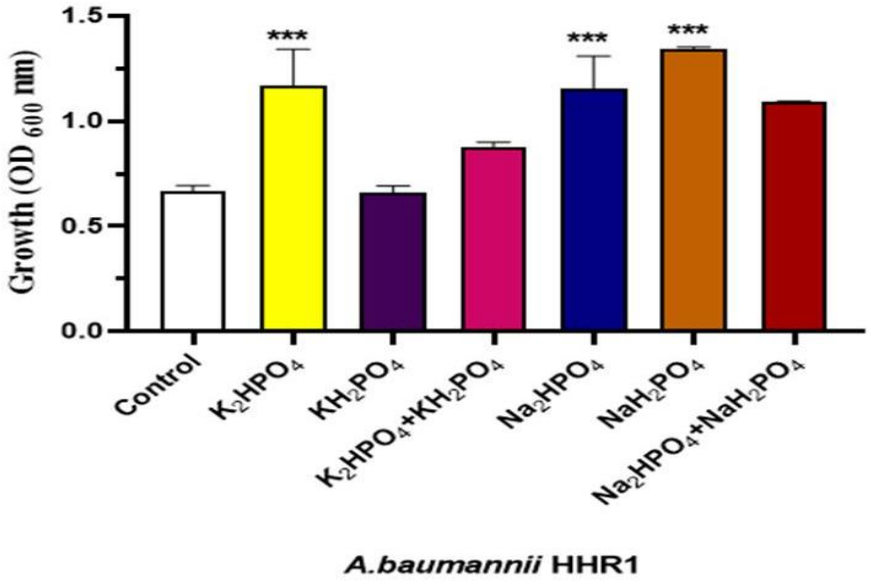



**Fig. 2**:*Effect of inorganic phosphate salts on A.bumannii HHR1 growth A.bumannii HHR1 was cultured in M9 medium containing 30 mM of each phosphate salt. Error bars represent the standard deviation from the mean of three independent biological replicates. The statistical analysis was conducted by student t` test with *** P< 0.001.*

The response of eight clinical isolates in the M9 medium was obvious from Fig.2, which showed that the combination of Na2HPO4/NaH2PO4 predominantly increased the growth of all isolates. However, L1 was able to grow better in the presence of either of $Na_2HPO_4$ or $NaH_2PO_4$, where the growth rate reached to $OD_{600}$ of 1.053 and 1.029 respectively (Fig.2a), in compared to the control (0.536).

In L2 isolate (Fig. 2b), the highest growth was 1.165 at $OD_{600}$ nm with $Na_2HPO_4/NaH_2PO_4$ , compared to control with $OD_{600}$ of 0.643. Notably, the $NaH_2PO_4$ and $KH_2PO_4$ have a moderate effect on boosting the growth of L2 in the M9 medium. However, the combination of K2HPO4 and KH2PO4 beside Na2HPO4 exhibits a high growth rate in comparison to others. Furthermore, L3 isolate was enabled to grow well with most of inorganic salts (Fig. 3 c), in $K_2HPO_4/KH_2PO_4$ complex, as the growth reached $OD_{600}$ of 0.753. Unlike L4 isolate showed to have higher growth rate when it was cultured in medium containing $Na_2HPO_4$, and with the combination of $Na_2HPO_4/NaH_2PO_4$ (Fig. 3 d). In this assay, the growth of L4 was increased significantly ($OD_{600}$ of 1.494) in the presence of $Na_2HPO_4$, and to $OD_{600}$ of 1.299 with $Na_2HPO_4/NaH_2PO_4$ complex. Other phosphate salts slightly increase the growth, compared to control that were enabled to grow up 8to 0.625 at $OD_{600}$ nm. To some extent, L5 isolate showed similar pattern of growth, where it grew better with the combination of $Na_2HPO_4/NaH_2PO_4$ (Fig. 4 e). The rate of viability was 1.096 at $OD_{600}$ nm, whereas, control recorded the lowest growth at $OD_{600}$ (0.655). Similarly, the growth rate of L6, L7, and L8 isolates remarkably increased when only $Na_2HPO_4/NaH_2PO_4$ complex was used as a phosphate source.The complex enhances the growth up to $OD_{600}$ 1.206, 0.875, and 1.052 for L6, L7, and L8 respectively, in comparison to control of 0.561, 0.599, and 0.527 at $OD_{600}$ nm.

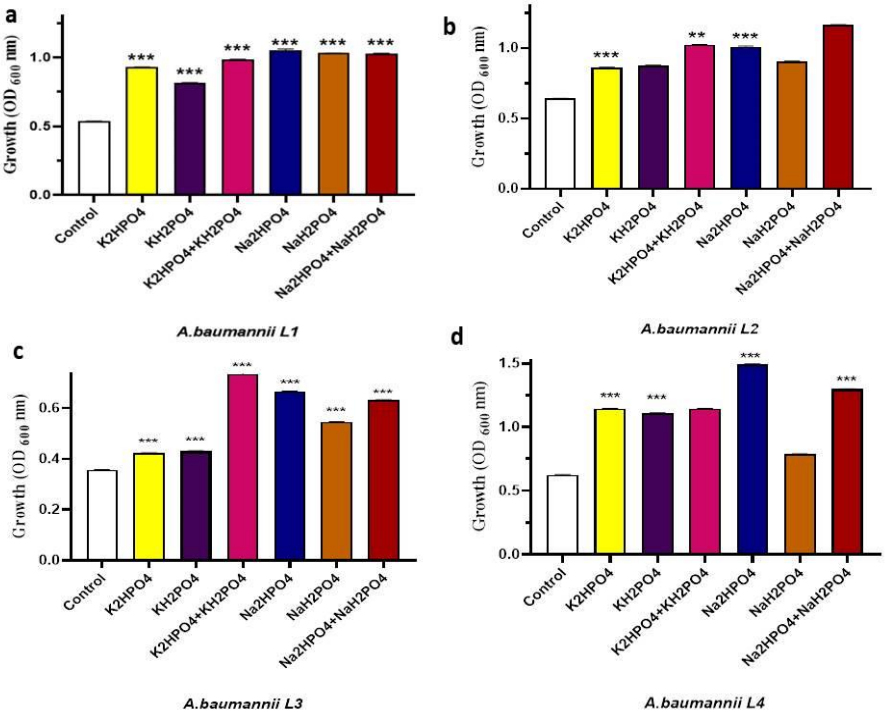



**Fig. 3:** *The response of clinically isolated (Baghdad patients)A.baumannii toward inorganic phosphate salts. isolates were cultured in M9 medium containing 30 mM of each phosphate salt. a) to L1; b) L2; c) L3; d) L4.Error bars represent the standard deviation from the mean of three independent biological replicates. The statistical analysis was conducted by student t` test with *** P< 0.001.*

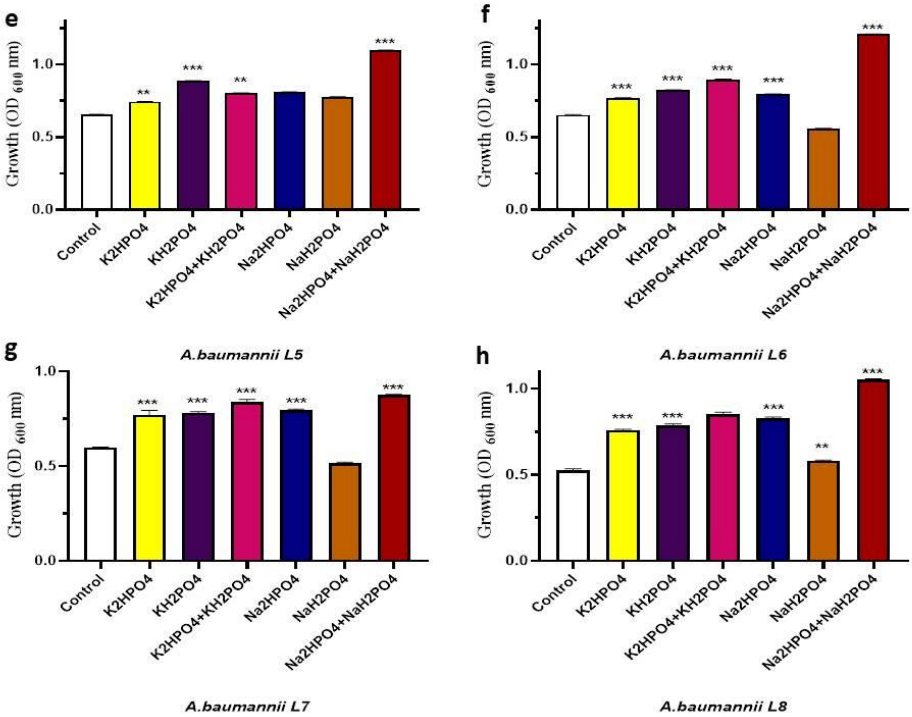


**Fig. 4:** *The response of clinically isolated (karbala patients) A.baumannii toward inorganic phosphate salts. isolates were cultured in M9 medium containing 30 mM of each phosphate salt.* ***e)*** *L5;* ***f)*** *L6;* ***g)*** *L7; and* ***h)*** *L8.Error bars represent the standard deviation from the mean of three independent biological replicates. The statistical analysis was conducted by student t` test with *** P< 0.001.*

**PhoH protein in *A. baumannii***

**Table 4:** Concentration of protein in *A.baumannii*

| Treatments | Protein concentration in ATCC (mg $ml^{-1}$) | Protein concentration in HHR1(mg $ml^{-1}$) |
|---|---|---|
| Control | 0.815 | 0.828 |
| $K_2HPO_4$ | 0.654 | 0.953 |
| $KH_2PO_4$ | 0.878 | 0.868 |
| $K_2HPO_4/KH_2PO_4$ | 0.818 | 0.868 |
| $Na_2HPO_4$ | 0.838 | 0.949 |
| $NaH_2PO_4$ | 0.851 | 0.934 |
| $Na_2HPO_4/NaH_2PO_4$ | 0.849 | 0.919 |

**Characterization of PhoH protein in *A. baumannii***

PhoH protein is a cytoplasmic protein involved in signalling and cellular processes.It is a putative signalling protein with 362 amino acids (a.a); the data provided by the Expasy para tool (https://web.expasy.org/cgi-bin/protparam/protparam), the protein is about 41 kDa with an isoelectric point (pI) about 5.77. PhoH protein is composed of 53 negatively charged residues and 45 positively charged residues. The protein has no signal peptidase (SP) or lipoprotein signal peptidase, which proves that PhoH is a cytoplasmic protein[9] (Fig. 5).

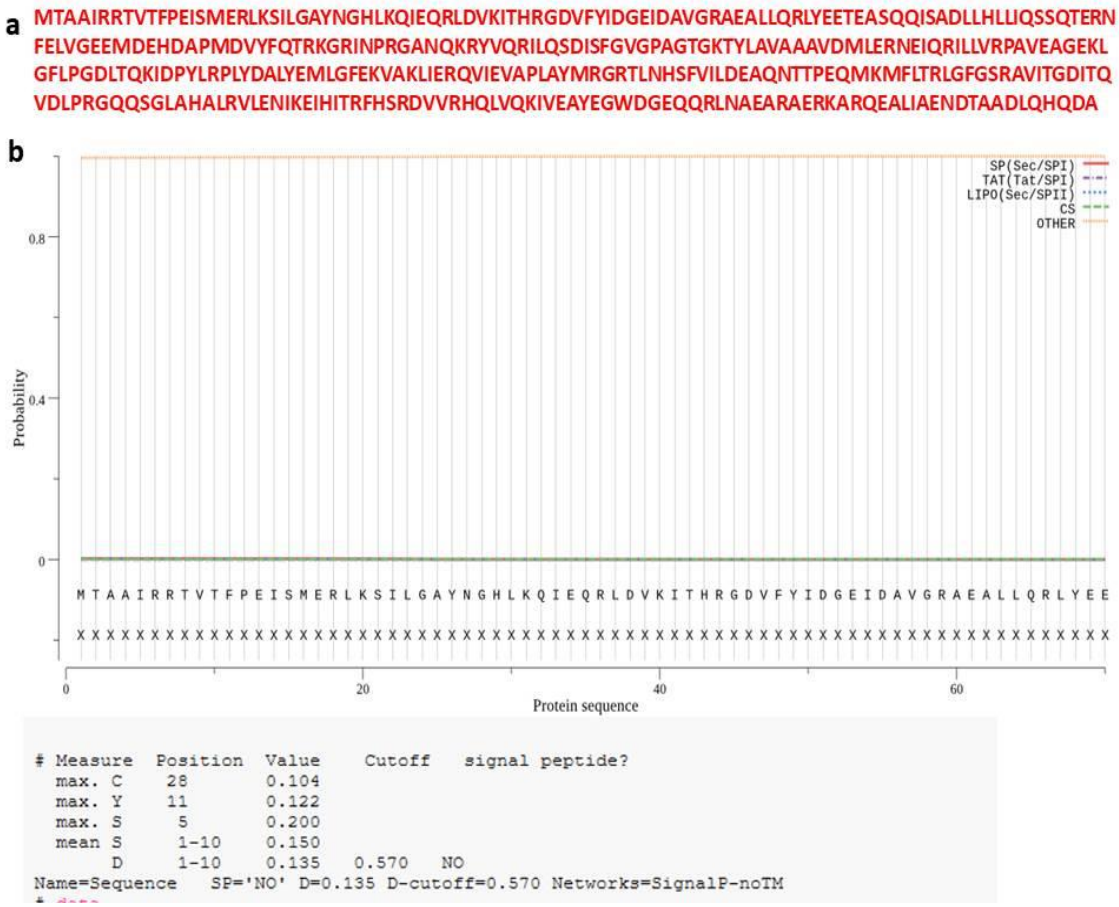



**Fig. 5**: *Protein sequence analysis tool of PhoH proetin. a) The amino acid sequence of PhoH protein showen in red. b) Bioinformtic analysis of signal peptidase of mature mature protein. No signal Sp was detected according to (SignalP v 5.0).*

**Three Dimensional Structure of PhoH protein**

The predicte the structure model of phospshate starvation inducible proetein PhoH was assembled using SWISS-MODEL server (https://swissmodel.expasy.org), using PhoH2 as a template to align both structures, and under the the STML ID 3b85.1.A. The amino acid sequense of PhoH was analysed by Swiss-Model server, and the structure was superimposed with the corresponding template.TheBDP entry 3b85.1.A is available in https://swissmodel.expasy.org/interactive/Nh4aDC/models/. The 3dimensional structure revealed that the mature PhoH protein is a monomer composed of 6 β-strand running antiparalel near the center, and wrapped by 8 α-helices connected by short turns at N-terminal and C-terminal (Fig. 6).The six β-strands are:β1 is located from amino acid isoleucin 139 to prolin 145; β2, form arginin 169 to prolin 175; β3 from isoleucin 223 to prolin 227; β4 from serine 239 to asparagin 244; β5 form arginin 266 to asparagin 272; and β6 from isoleucin 299 to phenalalanine 304 (Fig. 6a).The sequence of PhoH clearly showed that the mature protein is a cytoplasmic protein with no signal sequence and no active binding site (Fig. 6 b), thus suggested that phoH is might be an inducer or regulator for PhoP-PhoQ system in the shortage of phosphate sources[10].

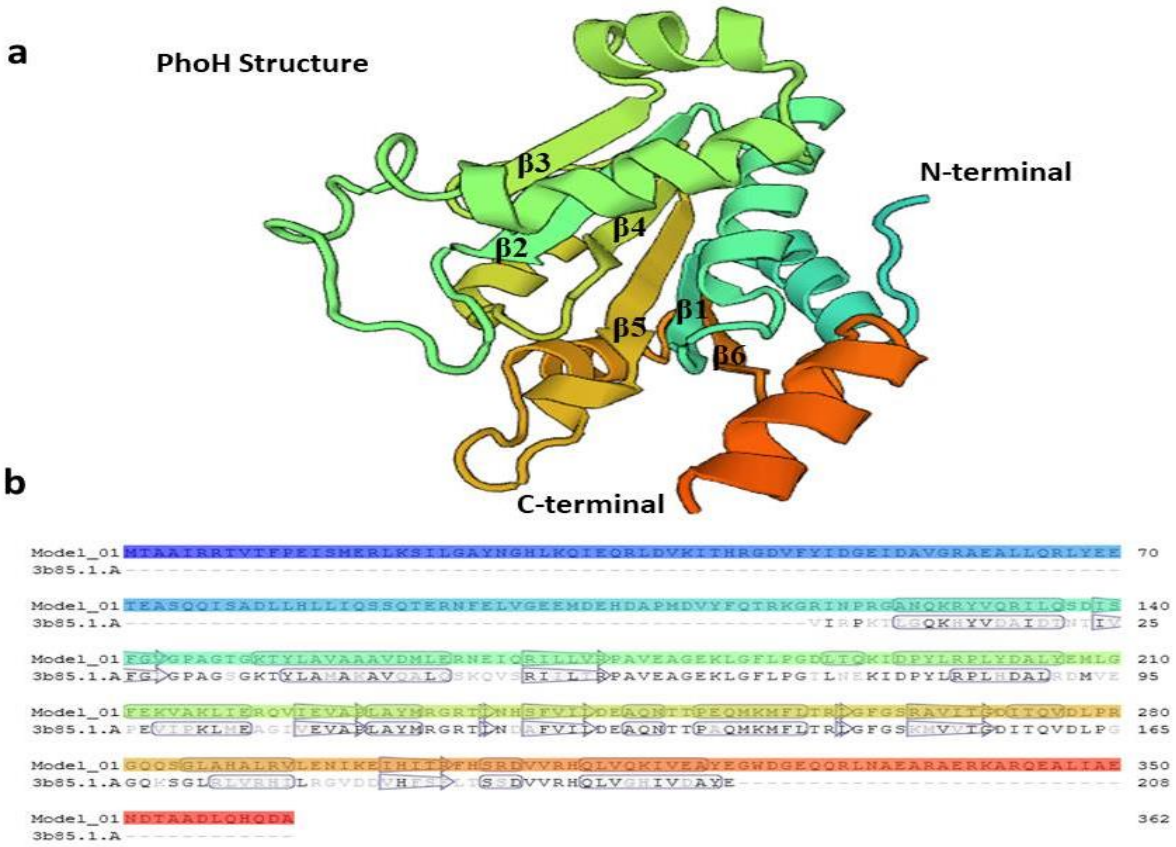
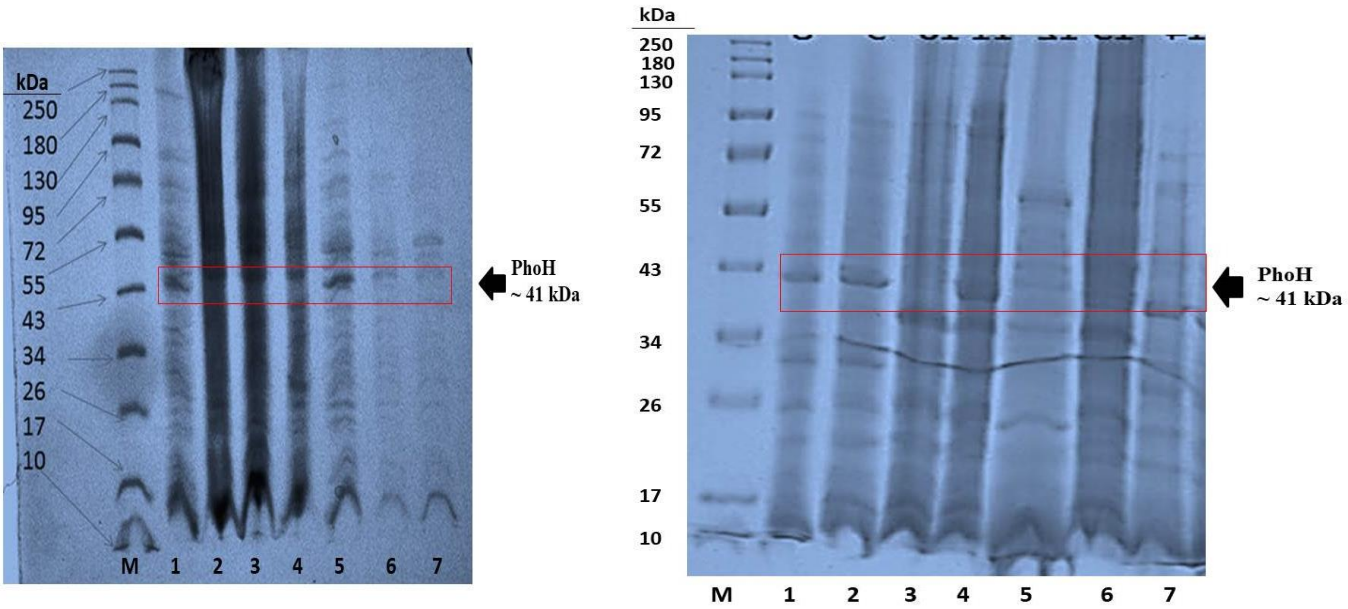



**Fig. 6:** *The Structure modelling of PhoH in A. baumannii ATCC17904 and A. baumannii HHRI and sequence alignment. a) The epredicted structure is built up by a Swiss model superimposed with the PhoH2 (ID: 3b85.1.A). The six β-sheets are indicated by numbers. b) Alignment sequence of PhoH and phoH2. Different colores represent the rainbow structure. The β-strands are indicated by blank arrows, and α-helices by blank boxes. The fig. is adopted by the Swiss-Model server.*

**SDS-PAGE of PhoH protein (One dimensional)**

The SDS-PAGE protocol was prepared based on the Laemmli method by using 4% stacking and12% resolving gels[10]**,** The SDS pattern exhibits that the PhoH protein (MW 41 kDa) is a cytoplasmic protein that is naturally expressed in the cytoplasim of *A.baumannii* in the absence of phosphate as in the control of both isolates. Interestingly, PhoH protein is obvious in all fractions of the global strain. However, in the presence of NaH2PO4 and a combination of $Na_2HOP_4$/ $NaH_2PO_4$, expression of PhoH protein in HHR1 is decreased and appeared as a faint band in SDS-PAGE (Fig. 7a, b)




**(A)** **(B)**

**Fig. 7:** *12 % SDS-PAGE of cytoplasm in (A)A. baumannii ATCC 17904(B) A.baumannii HHR1. Cultured A.baumannii ATCC 17904 and HHR1 in M9 medium with different sources of inorganic phosphate at 37°C for 24hour, and cytoplasmic fraction was prepared and loaded into polyacrylamide gel. M: PageRuler™ Prestained Protein Ladder (Fermentas).Lane 1: refers to control; lane2: $K_2HPO_4$; lane 3: $KH_2PO_4$; lane 4: $K_2HPO_4$ / $KH_2PO_4$; lane 5: $Na_2HPO_4$; lane 6: $NaH_2PO_4$, lane 7: $Na_2HPO_4/NaH_2PO_4$. All fractions were mixed with 4X SDS loading dye and 50 µg protein was loaded into each well.*

## Gene Detection

In order to investigate the presence of *pho*H gene and whether its functions as a regulator or activator in the two main strains *A.baumannii* ATCC 17904 and *A.baumannii* HHR1, the gene of interest was PCR amplified using phoH-FWD/phoH-R primer pairs (Fig. 8a, b). PCR product was electrophoresed on 1.5 % agarose. The PCR result revealed that both stander and local isolates(*A.baumannii* ATCC 17904 and *A.baumannii* HHR1) were harboured phoH gene, and the product of interest was 107 bp (Fig. 8b). The phoH gene is located upstream tRNA-I (a putative modification enzyme tRNA-2-methylthio-N6-dimethylalladenosine synthase), and downstream rRNA maturation factor as shown in (Fig. 8 a).

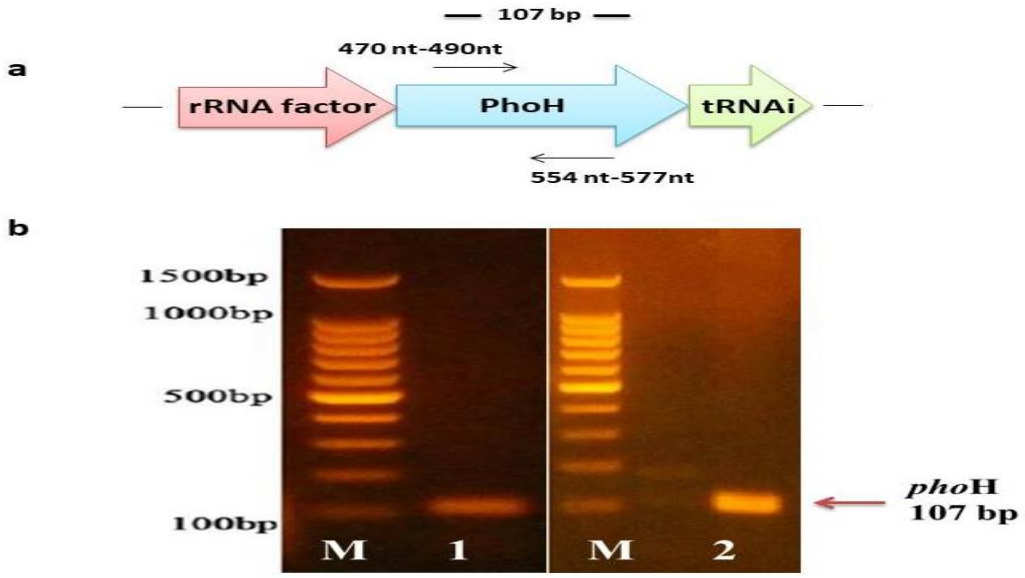



**Fig. 8**: *Gene map organization and PCR of phoH gene in A. baumannii ATCC17904 and A. baumannii HHR1. a) Gene map illustrates the location of phoH within the genome of both isolates. bDNA(10µ) has been loaded directly in the well, then 1.5 % agarose gel of 107 bp PCR product of phoH gene in both isolates. turned on the Electrical power for 75min at 100v/mAmp.M: DNA marker; lane 1 phoH gene in A. baumannii ATCC17904; Lane 2: phoH gene in A. baumannii HHR1. phoH gene is indicated by the red arrow. The gene expression of phoH (RT-PCR).*

In our study, the expression of *the phoH gene is affected by the absence and presence of a phosphate source, whereas the mRNA expression of the phoH gene in A.baumannii ATCC 17904 was clearly increased in the presence of K2HPO4 to about 10-fold*, in comparison to control. Whereas, in the local strain, the gene was down-regulation, in the case of KH2PO4, the phoH gene in the global strain was slightly expressed up to 5-fold, but it was up to 3-fold in the local strain. Most notably that, *pho*H was highly expressed in the presence of $Na_2HPO_4$ in local strain (25- fold), while it exerts a pattern of down expression in standard strain. In addition, neither $NaH_2PO_4$ nor a combination of $K_2HPO_4$ /$KH_2PO_4$ and $Na_2HPO_4$/$NaH_2PO_4$ are affected on the expression of *pho*H gene in both isolates (Fig.9).

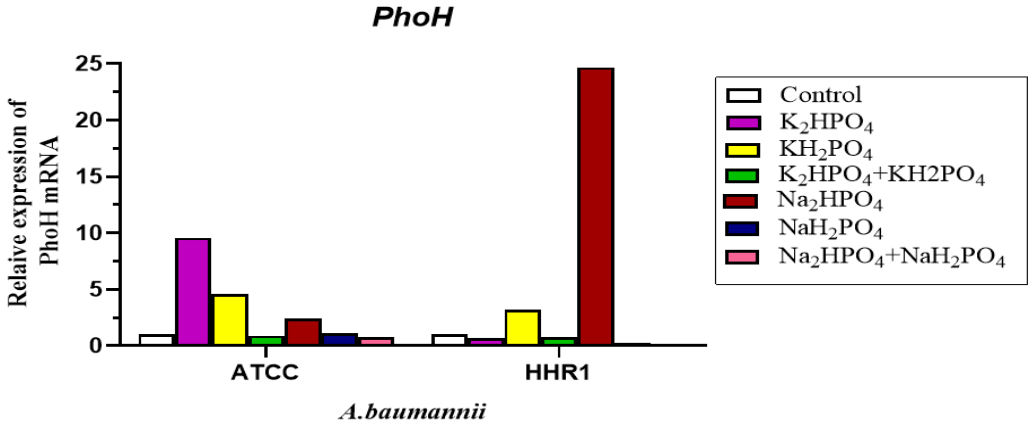



**Fig. 9**: *Effect of inorganic phosphate salts on gene expression of phoH gene in A. baumannii ATCC 17904 & A. baumannii HHR1*

## DISCUSSION

### Effect of inorganic phosphate salts on *A.baumannii* in M9 minimal medium

All *A. baumannii* isolates have variable responses in the M9 medium; this media was selected to study the best phosphate source for each isolate. The highest growth of ATCC was determined with the combination of $Na_2HPO_4$/$NaH_2PO_4$ and for HHR1 in $NaH_2PO_4$, the other isolates L1, L4 with $Na_2HPO_4$, while, L3 in the combination of $K_2HPO_4$/$KH_2PO_4$. The heist growth rate of L5, L6, L7, and L8 was found with a combination of $Na_2HPO_4$/$NaH_2PO_4$. Taking the above results, it is clearly indicated that the bacterial isolates were enabled to grow in media lacking a phosphate source and exhibit the importance of inorganic phosphate for the growth and survival of bacteria for a long time. In our study, the differences in the response of *A. baumannii* to the presence of many phosphate sources may be due to a group of biological activities that include metabolism, gene expression, transport, and signalling to achieve conversion reactions for population growth and development [11].

### PhoH protein in *A. baumannii*

PhoH is a cytoplasmic protein of *A.baumannii* (ATCC, HHR1)induced by phosphate starvation, and plays a role in signalling and cellular processes. The SDS shows that PhoH protein (MW 41 kDa) is a cytoplasmic protein that is naturally expressed in the cytoplasm of *A.baumannii* in the absence of phosphate for both isolates(Fig 7a, b), whereas the Pho proteins present in the cytoplasm, but under certain conditions is released and the response is increased to phosphate starvation. It has been revealed that under phosphate condition starvation, Pho proteins were produced and accumulated[12]. Interestingly, PhoH protein is obvious in all fractions of the standard strain. However, in the presence of $NaH_2PO_4$ and the combination of $Na_2HOP_4$/ $NaH_2PO_4$, expression of PhoH protein in HHR1 is decreased and appears as a faint band in SDS-PAGE, because sometimes under high inorganic phosphate conditions, inorganic phosphate binds to the membrane protein complex leading to dephosphorylation, and lead to inhibition of Pi regulon transcriptional regulatory protein. The inhibition of PhoB leads to decreasing the expression of high-affinity Pi transporters and phosphatases, whereas this Pi regulon system is activated during inorganic phosphate-limiting conditions[13]. The PhoH sequence showed that the mature protein is a cytoplasmic protein with no signal sequence and no active binding site (Fig.5), thus suggesting that phoH might be an inducer or regulator for PhoP-PhoQ system in the depletion of phosphate sources.

### Effect of inorganic phosphate on *pho*H gene expression in *A.baumannii*

The expression of *pho*H gene in *A.baumannii* was variable in two strains ATCC17904 and HHR1 in the presence of different phosphate sources in M9 medium (fig.9) *pho*H gene represent the Pho regulon in *A.baumannii*. Usually the Pho regulon has high gene expression in phosphate starvation or in phosphate limitation, In our study, it was discovered that the Pho expression induced by phosphate deprivation, in addition, stress and other factors may activate or inhibit the expression[14, 15] , but in our study The mRNA expression of *pho*H gene in *A.baumannii* ATCC 17904 has high expression in the presence of $K_2HPO_4$ to about 10-fold, in comparison to control. Whereas, in the local strain, the gene was down regulation, in the case of $KH_2PO_4$, phoH gene in global was slightly expressed up to 5-fold but it was up to 3-fold in the local strain. Most notably that, *pho*H was highly expressed in the presence of $Na_2HPO_4$ in the local strain (25- fold), while it exerts a pattern of down expression in the

standard strain. In addition, neither $NaH_2PO_4$ nor a combination of $K_2HPO_4$ /$KH_2PO_4$ and $Na_2HPO_4$/$NaH_2PO_4$ are affected by the expression of *pho*H gene in both isolates, these the may explain the effect of incubation with inorganic phosphate on pho regulatory system. In control, it was noticed that the level of expression is normal and is not affected due to the absence of Pi, but in presence of Pi, because Pi excess leads to inhibition of Pho regulon or expression of two regulatory system in bacteria so, some inorganic phosphate in our study at which expression levels were elevated can be considered as stimulants for growth and gene expression at the same time. As in some studies, materials were used, including materials that were considered as a source of phosphate, suitable for bacterial growth and at the same time stimulating gene expression[16].

## CONCLUSION

The conclude the inorganic phosphate salts have been effects on *A.baumannii* growth in different isolates , The PhoH protein is induced in the presence some inorganic phosphate with both strains(ATCC, HHR1). The effect of different inorganic phosphate sources on gene expression of phoH gene was apparent in both strains(ATCC, HHR1).

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# تأثير تجويع الفوسفات على phoH في الراكدة البومانية .

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الراكدة البومانية، الفوسفات الغير عضوي، التعبير الجيني لل(*phoH*)

**معلومات المؤلف**

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**الخلاصة:**

*Acinetobacter baumannii* هي بكتيريا سالبة الغرام وهي مقاومة للظروف البيئية مثل نقص العناصر الغذائية. يعتبر الفوسفات غير العضوي أحد العناصر الغذائية الأساسية في أيض الخلايا البكتيرية. تأثير تجويع الفوسفات على نمو *A.baumannii* في الوسط M9 على التعبير الجيني للـ (*psiE*) *phoH*, زاد مستوى تعبير *phoH* بحوالي 10 أضعاف في وجود $K_2HPO_4$ وحوالي 5- أضعاف مع $KH_2PO_4$ في *A. baumannii* ATCC17904، في *A. baumannii* HHR1، يتم تحفيز التعبير الجيني لـ *phoH* بشكل ملحوظ باستخدام $Na_2HPO_4$، حيث يكون مستوى التعبير أعلى بحوالي 25 مرة من مستوىATCC. ،بينما كان 3 -أضعاف مع $KH_2PO_4$ مقارنة بالسلالة العالميةATCC. أظهر نمو عزلات *A. baumannii* في الوسط M9 سمات مختلفة في وجود مصادر الفوسفات غير العضوية، في حين أن أعلى نمو في السلالة العالميةATCC17904 مع $Na_2HPO_4/NaH_2PO_4$ معًا. في حين أظهرت العزلة المحليةHHR1 أعلى نمو في وجود $NaH_2PO_4$، في حين أظهرت العزلات السريرية الثمانية تباين النمو استجابة لمصادر مختلفة. أظهرت *A. baumannii* المعزولة سريريًا L1 وL4 معدل نمو أعلى مع $Na_2HPO_4$، في حين أن L3 لديه أعلى نمو في $K_2HPO_4/KH_2PO_4$. بينما العزلات L2 وL5 وL6 وL7 وL8 كان معدل نمو مرتفع مع مزيج $Na_2HPO_4/ NaH_2PO_4$، حيث كان بروتين PhoH حوالي 41 كيلو دالتون، وظهر عند استخدام $K_2HPO_4$ و$KH_2PO_4$ و$Na_2HPO_4$،كان بروتين PhoH غائبًا في وجود $NaH_2PO_4$ و$Na_2HPO_4/NaH_2PO_4$ في السلالة المحلية HHR1.