Food spoilage by certain species of bacteria is reported to be regulated by quorum sensing (QS). Acinetobacter johnsonii and Pseudomonas fluorescens, the major specific spoilage organisms, are found to be limited in their QS and co-culture interactions. The aim of this study was to determine how QS-regulated proteins affect the spoilage potential of co-cultured A. johnsonii and P. fluorescens obtained from spoiled bigeye tuna (Thunnus obesus) using a proteomics approach. The A. johnsonii, P. fluorescens, and their co-culture tested the N-acyl-homoserine lactone (AHL) activities using reporter Chromobacterium violaceum CV026 and LC-MS/MS in qualitative and quantitative approaches, respectively. These latter showed that, of the 470 proteins and 444 proteins in A. johnsonii (A) and P. fluorescens (P), respectively, 80 were significantly up-regulated and 97 were significantly down-regulated in A vs. AP, whereas 90 were up-regulated and 65 were down-regulated in P vs. AP. The differentially expressed proteins included the AI-2E family transporter OS, 50S ribosomal protein L3, thioredoxin reductase OS, cysteine synthase CysM OS, DNA-binding response regulator, and amino acid ABC transporter ATPase OS. The cellular process (GO:0009987), metabolic process (GO:0008152), and single-organism process (GO:0044699) were classified into the gene ontology (GO) term. In addition, energy production and conversion, amino acid transport and metabolism, translation, ribosomal structure and biogenesis, post-translational modification, protein turnover, and chaperones were distributed into the clusters of orthologous groups of proteins (COG) terms. The KEGG pathways revealed that 84 and 77 differentially expressed proteins were divided into 20 KEGG pathways in A vs. AP and P vs. AP, respectively, and amino acid metabolism, carbohydrate metabolism, energy metabolism, and translation were significantly enriched. Proteins that correlated with the spoilage-related metabolic pathways, including thioredoxin
reductase OS, cysteine synthase OS, and pyridoxal phosphate-dependent enzyme family protein OS, were identified. AI-2E family transporter OS and LuxR family transcriptional regulator OS were identified that related to the QS system. These findings provide a differential proteomic profile of co-culture in A. johnsonii and P. fluorescens, and have potential applications in QS and the regulation of spoilage potential.

**Keywords:** quorum sensing, autoinducers (AHLs), AI-2, proteome, Acinetobacter johnsonii, Pseudomonas fluorescens, spoilage

**INTRODUCTION**

Bigeye tuna (*Thunnus obesus*) is a highly sought after fish species used to prepare sashimi in many countries around the world (Wang and Xie, 2019). However, bigeye tuna is easily spoiled by specific spoilage organisms during refrigerated storage, which leads to a reduced shelf life (Sun et al., 2013; Silbande et al., 2016). Currently, more attention has been paid to convenient methods of refrigeration for storing bigeye tuna than to how spoilage bacteria in refrigerated tuna develop through their interactions with each other (Wang et al., 2017). The microbial spoilage of aquatic products is correlated mainly with Gram-negative bacteria, including *Acinetobacter* spp., *Shewanella* spp., *Pseudomonas* spp., *Aeromonas* spp., lactic acid bacteria, and the Enterobacteriaceae family, when stored under different storage conditions. The main species of bacteria leading to the spoilage of aquatic products during cold storage are *Acinetobacter johnsonii* and *Pseudomonas fluorescens*, which are commonly referred to as specific spoilage organisms (SSOs) (Jia et al., 2018; Pang and Yuk, 2019; Zhu et al., 2019). It is a significant SSOs due to its ability to produce volatile sulfides, amines, trimethylamine extracellular enzymes, trimethylamines, organic acids and some spoilage metabolites.

Quorum sensing (QS) is the mechanism by which cell population-dependent signaling and interactions are recognized by bacteria in order to modulate their collective behaviors, including spoilage activity, enzyme secretion, bioluminescence, biofilm formation, virulence, and several signal molecules that mediate this mechanism have now been reported (Natrah et al., 2012; Zhu S. et al., 2015). There are various QS molecules, such as CAI-1 (cholera autoinducer 1), DKPs (Diketopiperazines), HAQs (4-hydroxy-2-alkylquinolines), DSFs (Diffusible Signal Factors), AI-2 (Autoinducer-2) and indole, etc (Monnet and Gardan, 2015; Papenfort and Bassler, 2016). QS-mediated communication is based on the prevailing interspecies communication in both Gram-negative and Gram-positive bacteria occurring via autoinducer-2 (AI-2) and auto-inducing peptides (AIPs) (Stephens et al., 2019). *P. fluorescens* is a Gram-negative bacteria that has been reported to use N-acyl-homoserine lactone (AHL) signals to monitor its local population through the exchange of extracellular signal molecules (Li et al., 2018). SSOs utilize QS communication of circuits to regulate a diverse array of physiological activities microbial, including eavesdropping, biofilm genesis, and bioluminescence. Recently, several studies have found that QS signal molecules were N-butyryl-DL-homoserine lactone (C4-HSL), N-hexanoyl-DL-homoserine lactone (C6-HSL), octanoyl-L-homoserine lactone (C8-HSL), decanoyl-homoserine lactone (C10-HSL), and N-dodecanoyl-L-homoserine lactone (C12-HSL), which significantly reduced the protease activities and spoilage potential of SSOs. Moreover, QS systems can govern bacterial behavior in food spoilage ecology (Whiteley et al., 2017). Bacterial growth, protease production, spoilage potential, electrochemical tests, and spoilage protein expression were significantly enriched through AHL signal regulation (Zhu et al., 2017). However, few studies have focused on how two species of SSOs regulate their protein function in relation to spoilage potential.

Recently, proteomic analysis has become a beneficial and quantifiable technique for providing relative measurements of proteins from different samples by nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS) without any isotope labeling. In a previous study on QS signaling, an absolute quantitative proteomic experiment was designed to investigate how Cyclo (L-phenylalanine-L-proline) effects protein expression in *Staphylococcus aureus*. Cyclo (L-phenylalanine-L-proline) has focused mainly on LuxR-mediated QS systems in bacteria, while the mechanism of extracellular QS signal molecule remained known (Ai et al., 2019; Wang et al., 2019) identified 1103 acetylated proteins and 2929 acetylation sites in *Shewanella baltica* from aquatic products to evaluate spoilage activity by LC-MS/MS analysis. A label-free quantitative proteomic approach has been applied to analyze the differential protein expression of *Enterococcus faecalis* SK460 to determine potential factors related to enterococcal biofilm formation. The results of this study showed that the related protein in the relevance of LuxS QS and phenome in the biofilm development of *E. faecalis* was due to the Fsr being lacking in QS (Suryaletha et al., 2019). Based on proteomic analysis, a total of 338 vesicular proteins of *Pseudomonas aeruginosa* were identified with high confidence by LC-MS/MS analysis. This proteome profile provides a basis for future studies to illustrate the pathological functions of outer membrane vesicles from *P. aeruginosa* (Choi et al., 2011). However, there are very few studies demonstrating the fact that two SSOs have a possible role in QS for controlling expression of their target proteins and regulating bacterial behavior.

The aim of this study was to examine AHL production and AI-2 activity in *A. johnsonii, P. fluorescens*, and their co-culture, and to examine AHL-based QS systems regulating biofilm formation, protease activity, spoilage potential, and key proteins. Proteomic analysis of *A. johnsonii, P. fluorescens*, and their co-culture was used to elucidate the potential role of QS systems...
and their expression of proteins, interspecies communication, and metabolic pathways, which might be useful for developing effective methods for detecting spoilage capability.

**MATERIALS AND METHODS**

**Preparation of Bacterial Strain Cultures**

Two kinds of SSOs (A. johnsonii and P. fluorescens) were originally isolated from spoiled bigeye tuna muscle (Zhejiang Fenghui Ocean Fishing Co., Ltd., China) by 16s rRNA gene sequences and VITEK® 2 CompactA system (bioMérieux, France). Stock cultures containing 25% glycerine were stored at −80°C. Before use, A. johnsonii and P. fluorescens were pre-cultured individually over two successive periods of 18 h in a brain-heart infusion broth at 30°C and then cultured in tryptic soya broth until the maximal concentration (10⁸ CFU/mL) was attained. The bioreactors were inoculated with single strains overnight culture at a 1% (v/v) inoculation level. The co-culture consisted of a mixture of equal amounts (v/v) of A. johnsonii and P. fluorescens. All bacterial strains were cultured overnight at 30°C, then centrifuged at 10,000 g for 10 min and collected in a 15 mL centrifuge tube to remove the medium. The precipitate was finally collected and washed three times with pre-cooled 10 mL 1 × PBS solution. The precipitate obtained was transferred to a new 2 mL centrifuge tube and immediately stored at −80°C. Chromobacterium violaceum CV026 and Vibrio harveyi BB170 were kindly donated by Dr. Mi (Bohai University, Jinzhou, China).

**Preparation of Reagents**

C4-HSL, C6-HSL, and C8-HSL with purities of over 96% were purchased from Sigma-Aldrich (St. Louis, MO, United States). Other reagents were analytical grade and were purchased from Sangon Biotech Co., Ltd (Shanghai, China) and Aladdin Industrial Corporation (Shanghai, China).

**Detection of AHLs and Al-2 Activity**

**Detection of AHLs by Cross-Feeding Plate Assay**

Detection of AHLs was carried out by the cross-feeding plate assay reported by Chu et al. (2011). Test strains (A. johnsonii, P. fluorescens and co-culture) were cultured with shaking in TSB at 30°C for 24 h. CV026 was cultured with shaking in Luria-Bertani (LB) broth supplemented with kanamycin (20 μg/mL) as the reporter strain at 30°C for 24 h. All strains were grown to approximately 10⁸ CFU/mL (OD600≈0.8). The test strain and CV026 were struck in parallel on the LB nutrient agar plate. The test strain and co-culture) were cultured with shaking in TSB. BB170 was inoculated into the Autoinducer Bioassay (AB) liquid medium (Bodor et al., 2008) and cultured overnight with consistent shaking in AB liquid medium adjusted to 10⁸ CFU/mL (OD600≈0.8). The fresh AB medium and the test bacterial solution were mixed and diluted at a ratio of 1:5000. A volume of 20 μL of culture supernatant and 180 μL of V. harveyi BB170 dilution were mixed at 100 r/min for 4 h at 30°C in a 96-well black microtitr plate. Finally, a light unit at a wavelength of 460 nm was measured every 1 d with a multiplate reader (Synergy 2, BioTek, Winooski, VT, United States). The cell-free culture of V. harveyi BB170 supernatant was defined as a positive control. Sterile AB medium served as a blank control. This test was repeated three times.

**Extraction and Detection of AHLs by HPLC-MS**

To acquire the supernatant, the concentrated bacteria of the test bacterial cultures during the logarithmic phase of growth was centrifuged at 10,000 r/min for 10 min at 4°C. A volume of 50 mL of the supernatant was extracted three times into an equal volume of acidified ethyl acetate (0.5% acetic acid). The AHLs were dissolved in an appropriate volume of methanol and stored at −20°C for further experiments.

The solution of AHLs was drained of solvent, and then 1 mL of the extracted solution (methanol:water = 80:20) was added to an ultrasonic steel ball grinding machine (6 min, 50 Hz) at −20°C for 30 min and centrifuged at 13000 g for 15 min at 4°C to obtain 200 μL of supernatant.

Quantitative experiments involving the AHLs were performed on a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific, Waltham, MA, United States) coupled to an Easy-nLC 1200 nano-flow UHPLC. The chromatographic parameters were as follows: column: BEH C18 (100 × 2.1 mm i.d., 1.7 μm; Waters Corporation, Milford, MA, United States), flow rate: 0.4 mL/min, sample injection volume: 5 μL, gradient mobile phase: water with 0.1% formic acid as solvent A and 0.1% formic acid and methanol as solvent B, column temperature: 40°C, and ion mode: positive ion mode (ESI+). The gradient mobile phase program applied was as follows, t = 0 min, 90% A; t = 4 min, 5% A; t = 9 min, 5% A; t = 7.1 min, 90% A; t = 10 min, 90% A. The conditions of MS/MS included the ion source temperature: 320°C, spray voltage: 3.5kV, sheath gas flow rate: 50 Arb and MS/MS collision energy: 70–1050 V.

**Protease Activity Assay**

The protease activity of the culture supernatant was determined according to the method described by Zhu J. et al. (2015), with a slight modification. A volume of 20 μL of the culture supernatant was added to 2.5 μL of the protease substrate solution. Incubation buffer was added to adjust the final assay volume to 50 μL. The tube was sealed and incubated at 37°C for 0.5 h. Following incubation, 50 μL of precipitation agent were added, and the contents were mixed and incubated again at 37°C for 10 min. The tubes were then centrifuged at 12,000 g for 5 min. All experiments were conducted in triplicate.

**Biofilm Formation Assay**

Biofilm formation was investigated using an alteration of the method reported by Li et al. (2018). Biofilm formation was measured by crystal violet assay. Briefly, the above 96-well polystyrene microtiter plates inoculated A. johnsonii,
**P. fluorescens** and co-culture. Following incubation, the biofilm in the plates in each well was carefully washed thrice with sterile phosphate buffered saline (PBS, pH 7.4) to remove unattached cells. After drying, the wells were added 0.2% (w/v) crystal violet to stain 15 min. The wells were then washed and dried as described above, and the dye attached to the biofilm was re-solubilized with 95% ethanol for 5 min. A volume of 200 µL of the sample solution was measured at 595 nm using a microplate reader.

### Proteome Analysis

**Protein Extraction and Digestion**

The bacterial cells were mixed with lysis solution (8 M urea, protease inhibitor cocktail), and incubated in ice-bath for 30 min, vortex-oscillated for 5 s every 5 min. The mixture was centrifuged at 12,000 g, 4°C for 30 min to obtain supernatant. Protein concentrations were measured using a BCA Assay Kit (Thermo Fisher Scientific, United States). Each sample tube contained 150 µg of protein. The sample solution was added to tris(2-carboxyethyl)phosphine (TCEP) to reach a final concentration of 10 mM and incubated at 37°C for 60 min. Then, an appropriate quantity of iodoacetamide (IAM) was added to achieve a final concentration of 40mM and incubated for 40min in the dark. Finally, 100 mM triethylammonium bicarbonate (TEAB) buffer was added to dilute the concentration of the solution. To each sample tube was then added trypsin solution in the ratio 1:50, and the tubes were then incubated at 37°C overnight.

### Mass Spectrometry Analysis and Protein Identification

A total of nine samples from three groups (A, P, AP groups) were analyzed on a Q Exactive HF-X mass spectrometer coupled to an Easy-nLC 1200 nano-flow UHPLC. Three biological replicates were used per sample. Each peptide sample (0.5 µg/L) was injected for nano-LC-MS/MS analysis. Each sample was loaded onto the C18 reversed phase column (75 µm × 25 cm, Thermo Fisher Scientific, United States) having two solvent systems (buffer A: 2% acetonitrile and 0.1% formic acid; buffer B: 80% acetonitrile and 0.1% formic acid) for 160 min at a flow rate of 300 nL/min. The full scan MS spectra ranged from 350 to 1300 m/z and were acquired with a mass resolution of 70 K. Three biological replicates were used per sample.

MS/MS spectra were screened by Proteome Discoverer™2.2 software (Thermo Fisher Scientific, United States) against the uniprot-Acinetobacter johnsonii-taxonomy_40214-20190813, uniprot-Pseudomonas fluorescens-taxonomy_294-20190813 and the decoy database with following parameters. The highest score for a given peptide mass (best match to that predicted in the database) was used to identify parent proteins. The parameters for protein searching were set as follows: tryptic digestion with up to two missed cleavages, carbamidomethylation of cysteines as a fixed modification, and oxidation of methionines and protein N-terminal acetylation as variable modifications. A 1% false discovery rate (FDR) was used to identify peptide spectral matches based on q-Values.

### Statistical Analysis

All statistical values are expressed as the mean ± standard deviation. The biofilm formation, AHLs, AI-2 expression, and protease activity were analyzed by one-way analysis using SPSS 22.0 software (SPSS Inc., Chicago, IL, United States). The differential expression proteins were assigned to functional analysis according to the gene ontology (GO), clusters of orthologous groups of proteins (COG), and KEGG pathway 3 databases.

### RESULTS AND DISCUSSION

**Identification and Detection of AHLs in A. johnsonii, P. fluorescens, and Co-culture**

The reporter strain of the cross-feeding plate method is the fastest and most direct method to qualitatively detect AHLs in bacteria. CV026 is one of the commonly used reporter strains. CV026 does not produce AHLs by itself; however, CV026 is able to sense some of the AHLs of CivR protein (Yu et al., 2019). CV026 is fully capable of producing violacein in response to its cognate signal molecule. As shown in Figure 1A, *P. fluorescens* and the co-culture were capable of producing AHLs induced CV026 color reaction, while *A. johnsonii* could have low capable of producing AHLs not induce CV026 color reaction. The AHL signals for *A. johnsonii, P. fluorescens*, and their co-culture are shown in Figure 2 and Supplementary Figure S2. The three peaks with retention times of 3.26, 4.29, and 4.84 min were identified as C4-HSL, C6-HSL, and C8-HSL, respectively. The AHL (C4-HSL, C6-HSL, and C8-HSL) concentrations of the co-culture samples were significantly higher than those of *A. johnsonii* and *P. fluorescens*, indicating that the co-culture had pronounced AHL activities. The cooperative behaviors of the *A. johnsonii* and *P. fluorescens* cultures in response to AHLs activities might be to accelerate aquatic products spoilage. These trends are in agreement with the results presented in Table 1. The HPLC-MS chromatograms of the AHLs of the co-culture in C4-HSL, C6-HSL, and C8-HSL, respectively. The AHL (C4-HSL, C6-HSL, and C8-HSL) concentrations of the co-culture samples were significantly higher than those of *A. johnsonii* and *P. fluorescens*, indicating that the co-culture had pronounced AHL activities. The cooperative behaviors of the *A. johnsonii* and *P. fluorescens* cultures in response to AHLs activities might be to accelerate aquatic products spoilage. These trends are in agreement with the results presented in Table 1. The HPLC-MS chromatograms of the AHLs of the co-culture in C4-HSL, C6-HSL, and C8-HSL contained 3.145, 7.359, and 1.560 ng/ml, respectively (Table 1).

**Detection of AI-2 Activity in A. johnsonii, P. fluorescens, and Co-culture**

The activities of AI-2 signal molecules of *A. johnsonii*, *P. fluorescens*, and their co-culture were detected (Figure 1B). There were significant differences in the AI-2 activity of *A. johnsonii*, *P. fluorescens*, and their co-culture at different incubation times (*p* < 0.05). With increased culture time, the AI-2 activity first increased significantly from 0 to 4 days, then decreased, which was caused by increased bacterial growth density of bacteria, and bacteria in logarithmic phase. At the end of culture time, bacteria were in stationary phase and decline phase. Therefore, AI-2 activity decreased. It indicated that the
changes in AI-2 activity were related to the secretion of the spoilage bacteria and environmental changes. Similar studies have shown that the AI-2 of QS is a global regulatory factor in aquatic products and influences the potential for spoilage (Peng et al., 2018; Li S. et al., 2019).

**Protease Activity of A. johnsonii, P. fluorescens, and Co-culture**

Protease activity plays an essential role in food SSOs and is regulated by QS (Moradi et al., 2019; Li T. et al., 2020). Moreover, protease activity decomposes aquatic food proteins into small peptides and amino acids that are metabolized into volatile nitrogenous end products (Li J. et al., 2019). In this study, protease activity exhibited no significant effect after 3 days. The protease activity of A. johnsonii, P. fluorescens, and their co-culture first increased and then decreased ($p < 0.05$) during different culture periods (Figure 1C). The previous study reported that SSOs growth consumed low-molecular-weight compounds, and then protein was degraded by protease which caused SSOs growth and protease activity increase.
### TABLE 1 | Contents of AHLs in *A. johnsonii*, *P. fluorescens* and co-culture.

| AHLs (ng/ml) | *A. johnsonii* | *P. fluorescens* | Co-culture |
|--------------|----------------|-----------------|------------|
| C4-HSL       | 1.204 ± 0.076<sup>a</sup> | 2.043 ± 0.060<sup>b</sup> | 3.145 ± 0.014<sup>a</sup> |
| C6-HSL       | 7.325 ± 0.299<sup>a</sup>  | 6.802 ± 0.344<sup>b</sup> | 7.359 ± 0.379<sup>a</sup> |
| C8-HSL       | 1.543 ± 0.110<sup>a</sup> | 1.334 ± 0.308<sup>b</sup> | 1.560 ± 0.554<sup>a</sup> |

<sup>a,b,c</sup>Means in the same line with different superscripts are significantly different (*p* < 0.05).

(Moradi et al., 2019). At the end of culture time, molecular-weight compounds were almost depleted. Therefore, protease activity decreased. The protease activities of the co-cultured samples were also higher than those of the single bacteria groups, in accord with the results reported in the literature (Li J. et al., 2019). This result suggests that protease activity as a key spoilage characteristic of co-cultured samples was regulated significantly, and at least partially, by an AHL-based QS system.

### Biofilm Formation by *A. johnsonii*, *P. fluorescens*, and Co-culture

Not only can biofilms influence food spoilage, resulting in reduced shelf life, but they can also adhere to bacteria and colonize surfaces (Zhou et al., 2019). Previous studies have provided some evidence to verify the correlation between biofilm formation and the SSOs of QS systems, by which exogenous bacteria can affect the formation of biofilms (Cui et al., 2020). Figure 1D shows that *A. johnsonii*, *P. fluorescens*, and their co-culture had the ability to form biofilms, which were greater in the co-cultured samples than those of the single bacteria. After 4d of culture, biofilm production reached maximum levels of 1.22, 1.38, and 1.39 by *A. johnsonii*, *P. fluorescens*, and their co-culture, respectively. Biofilm production increased when the incubation period was extended to 4d, but slowly decreased after 5d. Among the three groups, the presence of the co-cultured samples resulted in a significant increase in biofilm formation. This revealed that the cooperative behaviors of the *A. johnsonii* and *P. fluorescens* cultures in response to various signaling molecules helped to accelerate biofilm production.

### Proteome Analysis of *A. johnsonii*, *P. fluorescens*, and Co-culture

Acinetobacter johnsonii, *P. fluorescens*, and their co-cultured samples were prepared using the label-free technique, which showed that a total of 1,176,121 spectra were identified in *A. johnsonii* and *P. fluorescens* by Proteome Discoverer<sup>TM</sup> 2.2 software with a peptide FDR $\leq$ 0.01. As the fold change was $>1.2$ or $<0.83$, a *p*-value of $<0.05$ was used as the threshold to define the significance of the difference in protein expression. This enabled the quantification of 470 proteins and 444 proteins in *A. johnsonii* and *P. fluorescens*, respectively. As shown in Figure 3A, there were 80 significant up-regulated proteins and 97 down-regulated proteins in the A group, compared with the AP group. In addition, 90 up-regulated and 65 down-regulated proteins were in the P group, compared with the AP group (Figure 3B). The differences in protein expression are given in Tables 2, 3. Among the remarkably up-regulated proteins, the AI-2E family transporter OS, such as those of A0A2S8XHJ7, A0A166PHR0, A0A423M0X2, A0A2N1DUG2, and A0A165YHM6, showed notable up-regulation, which mediated the regulation of QS system expression (Quintieri et al., 2019; Saipriya et al., 2020). The result of AI-2 protein expression obtained above was similar to those shown in Figures 1B, 2. Moreover, some ribosomal proteins were significantly up-regulated, of which
### TABLE 2 | The proteins differentially expression and most abundant proteins uniquely identified in A vs AP

| Accession | Protein name | Regulate | Fold change | p-value   |
|-----------|--------------|----------|-------------|-----------|
| A0A0B7DGZ6 | 60 kDa chaperonin OS | Up       | 1.239       | 0.01994   |
| A0A3S4MXA1 | Chaperone protein HtpG OS | Up       | 1.287       | 0.007272  |
| A0A3M4NO99 | ATP-dependent protease ATPase subunit HslU OS | Up | 1.223       | 0.007429  |
| A0A4L3HY88 | Thioredoxin TxvA OS | Up       | 1.225       | 0.008509  |
| A0A166XT9 | RpRB (Fragment) OS | Up       | 1.309       | 0.02291   |
| A0AIV5UF04 | Molecular chaperone DnaK OS | Up | 8.057       | 0.001808  |
| A0A0D0S76 | Chaperone protein CipB OS | Up       | 2.421       | 0.000832  |
| A0A109KWR2 | Chaperone protein HtpG OS | Up       | 38.01       | 0.001208  |
| A0A3S4MHS8 | ATP-dependent Cip protease ATP-binding subunit CipA OS | Up | 1.205       | 0.036855  |
| A0A2W5E835 | Elongation factor 4 OS | Up       | 1.213       | 0.01939   |
| J2Y774    | Thioredoxin reductase OS | Up       | 1.994       | 0.000966  |
| A0A109KSE5 | Arginine deiminase OS | Up       | 3.039       | 0.000112  |
| A0A3M4FUT4 | Carbamoyl-phosphate synthase large chain OS | Up | 1.249       | 0.001582  |
| A0A4P3X4W6 | UDP-N-acetylgalactosamine 2-epimerase OS | Up | 1.269       | 0.019     |
| A0A0D0SKK3 | ATP synthase subunit alpha OS | Up | 1.611       | 0.02238   |
| A0A3M4FNE4 | Elongation factor Tu OS | Up       | 69.56       | 0.000101  |
| I4KE62    | Ornithine carbamoyltransferase OS | Up | 6.397       | 0.002375  |
| A0A0D0PLG1 | 50S ribosomal protein L14 OS | Up | 1.621       | 0.000769  |
| Q3K4A0    | Chaperone protein DnaK OS | Up       | 1.931       | 0.002845  |
| A0A3S9DI7 | ABC transporter ATP-binding protein OS | Up | 1.4         | 0.3852    |
| A0A3S9HY5 | Transcriptional regulator MvaT, P16 subunit OS | Up | 7.616       | 0.01267   |
| A0A3M4G573 | Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS | Up | 2.525       | 0.03714   |
| A0A0D0PNN7 | Aldehyde dehydrogenase OS | Up       | 5.861       | 0.00719   |
| U1TQN4    | 60 kDa chaperonin OS | Up       | 139.12      | 0         |
| A0A4V5UP6 | Chaperonin GroEL OS | Up       | 139.12      | 0         |
| A0A0B7DGY7 | Elongation factor Tu OS | Up       | 139.12      | 0         |
| A0A0W0HJ9 | Alkyl hydroperoxide reductase C OS | Up | 139.12      | 0         |
| I4K6X1    | Putative lipoprotein OS | Up       | 139.12      | 0         |
| I4K3GJ8   | 50S ribosomal protein L1 OS | Up | 139.12      | 0         |
| A0A109KXR2 | Heat-shock protein OS | Up       | 139.12      | 0         |
| A0A125QDK7 | Elongation factor Ts OS | Up       | 139.12      | 0         |
| A0A075PA24 | 10 kDa chaperonin OS | Up       | 139.12      | 0         |
| A0A109KX60 | Electron transfer flavoprotein subunit alpha OS | Up | 139.12      | 0         |
| A0A07C4B3 | Dipicolinate synthase OS | Up       | 139.12      | 0         |
| A0A015RMN0 | Succinate–CoA ligase [ADP-forming] subunit alpha OS | Up | 139.12      | 0         |
| A0A4L3G4I4 | Nucleotide exchange factor GrpE OS | Up | 139.12      | 0         |
| A0A0C2A4F3 | Nucleoside diphosphate kinase OS | Up | 139.12      | 0         |
| A0A0A1YUR8 | Membrane protein OS | Up       | 139.12      | 0         |
| A0A1QGKX17 | Cold-shock protein OS | Up       | 139.12      | 0         |
| A0A0D0T7C5 | Succinate–CoA ligase [ADP-forming] subunit beta OS | Up | 139.12      | 0         |
| A0A0B7D1Y4 | 50S ribosomal protein L2 OS | Up       | 139.12      | 0         |
| A0A3M3XNL5 | Ferritin domain-containing protein OS | Up | 139.12      | 0         |
| A0A0A1Z5I2 | 50S ribosomal protein L7/L12 OS | Up | 139.12      | 0         |
| A0A1T2YCAS | Ornithine aminotransferase OS | Up | 139.12      | 0         |
| A0A010SEN6 | 50S ribosomal protein L6 OS | Up       | 139.12      | 0         |
| A0A010RGK5 | Endoribonuclease OS | Up       | 139.12      | 0         |
| A0A0A1Y247 | Transcriptional regulator HU subunit alpha OS | Up | 139.12      | 0         |
| A0A075P8C2 | 30S ribosomal protein S4 OS | Up       | 139.12      | 0         |
| A0A010S5L6 | 50S ribosomal protein L9 OS | Up       | 139.12      | 0         |

(Continued)
### TABLE 2 | Continued

| Accession | Protein name | Regulate | Fold change | p-value |
|-----------|--------------|----------|-------------|---------|
| A0A0W0HKK7 | 50S ribosomal protein L15 OS | Up | 139.12 | 0 |
| A0A075PC10 | 50S ribosomal protein L11 OS | Up | 139.12 | 0 |
| A0A0C1ZX7 | Lipoprotein OS | Up | 139.12 | 0 |
| A0A010RRM4 | 30S ribosomal protein S10 OS | Up | 139.12 | 0 |
| C3K2S4 | Osmotically inducible protein Y OS | Up | 139.12 | 0 |
| A0A0A1Z8J0 | 50S ribosomal protein L4 OS | Up | 139.12 | 0 |
| A0A3M6J63 | Fatty acid oxidation complex subunit alpha OS | Up | 139.12 | 0 |
| A0A0N7H007 | Urocarnate hydratase OS | Up | 139.12 | 0 |
| E2XZ08 | 50S ribosomal protein L29 OS | Up | 139.12 | 0 |
| A0A0W0HLG0 | 50S ribosomal protein L10 OS | Up | 139.12 | 0 |
| A0A3M3Y045 | Aspartate ammonia-lyase OS | Up | 139.12 | 0 |
| A0A448WBD3 | Spermidine/putrescine import ATP-binding protein PotA OS | Up | 139.12 | 0 |
| A0A3M3XD24 | Nucleoid-associated protein ALQ35_00435 OS | Up | 139.12 | 0 |
| A0A0W0HH67 | Chromosome partitioning protein ParA OS | Up | 139.12 | 0 |
| A0A1010RSX6 | Adenosylhomocysteinase OS | Up | 139.12 | 0 |
| A0A1200SR9 | DUF2383 domain-containing protein OS | Up | 139.12 | 0 |
| A0A109KM78 | 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS | Up | 139.12 | 0 |
| A0A03EG5Q2 | Single-stranded DNA-binding protein OS | Up | 139.12 | 0 |
| A0A0K1QL49 | Protein-export protein SecB OS | Up | 139.12 | 0 |
| A0A1099KQ7 | Uncharacterized protein OS | Up | 139.12 | 0 |
| A0A3M3XR7 | 50S ribosomal protein L3 OS | Up | 139.12 | 0 |
| A0A387BYY7 | 50S ribosomal protein L25 OS | Up | 139.12 | 0 |
| U1TYF4 | 30S ribosomal protein S17 OS | Up | 139.12 | 0 |
| A0A3M3X13 | 50S ribosomal protein L23 OS | Up | 139.12 | 0 |
| A0A3M5N9X5 | 30S ribosomal protein S2 OS | Up | 139.12 | 0 |
| A0A3M4G1L6 | Aldehd domain-containing protein OS | Up | 139.12 | 0 |
| A0A0W0H1J6 | Elongation factor P OS | Up | 139.12 | 0 |
| A0A3M5N1D0 | Superoxide dismutase OS | Up | 139.12 | 0 |
| A0A0D0TBX7 | CmpW protein OS | Up | 139.12 | 0 |
| A0A109LG0 | Cysteine synthase B OS | Up | 139.12 | 0 |
| A0A28XU67 | Al-2E family transporter OS | Up | 139.12 | 0 |
| A0A109LMS3 | Cupin domain protein OS | Down | 0.7153 | 0.000434 |
| A0A0B7G9C9 | Serine hydroxymethyltransferase OS | Down | 0.78 | 0.000137 |
| A0A3S4N2A1 | DNA-binding transcriptional dual regulator Crp OS | Down | 0.7301 | 0.000463 |
| A0A3M4MV0 | ATP-dependent Clp protease ATP-binding subunit ClpX OS | Down | 0.82897 | 0.00003 |
| A0A3M4GP41 | Threonine-tRNA ligase OS | Down | 0.7967 | 0.001523 |
| A0A0C1X1G2 | Ribosomal RNA large subunit methyltransferase J OS | Down | 0.8124 | 0.02763 |
| A0A010SG6 | Aconitate hydratase OS | Down | 0.6839 | 0.000558 |
| A0A3S4M37 | 2-dehydro-3-deoxypyruvocinatic aldolase OS | Down | 0.7282 | 0.02122 |
| A0A263S7G8 | Elongation factor Tu [Fragment] OS | Down | 0.2323 | 0.0226 |
| A0A448BG48 | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) OS | Down | 0.5716 | 0.000746 |
| A0A3S4D68 | Glycerol kinase OS | Down | 0.7652 | 0.01836 |
| A0A44BGUH7 | Transcription termination factor Rho OS | Down | 0.7681 | 0.04901 |
| A0A109LM0 | DUF8 domain-containing protein OS | Down | 0.7598 | 0.000255 |
| A0A3S4NH0 | Pyruvate kinase OS | Down | 0.5626 | 0.01609 |
| A0A3M3X2N23 | Adenylosuccinylate lyase OS | Down | 0.8096 | 0.01257 |
| A0A612S47 | Glycerol-3-phosphate dehydrogenase OS | Down | 0.5523 | 0.000078 |
| A0A448BM26 | Elongation factor G OS | Down | 0.679 | 0.03014 |
| A0A4P3X0P0 | Integration host factor subunit alpha OS | Down | 0.5545 | 0.001699 |

(Continued)
| Accession | Protein name | Regulate | Fold change | p-value |
|-----------|--------------|----------|-------------|---------|
| A0A345XK6 | Transcription termination/antitermination protein NusG OS | Down | 0.7081 | 0.001008 |
| A0A0K1X87 | Glycerol kinase OS | Down | 0.7005 | 0.0138 |
| A0A345VJU2 | dTDP-4-dehydrorhamnose reductase OS | Down | 0.5598 | 0.000511 |
| Q8QT2 | Pyruvate dehydrogenase E1 component OS | Down | 0.781 | 0.00092 |
| E2XK43 | Transcriptional regulator, Crp/Fnr family OS | Down | 0.7111 | 0.001972 |
| A0A10LJK0 | Nucleotidyltransferase domain protein OS | Down | 0.627 | 0.002719 |
| A0A1T2YJK3 | Glycerol kinase OS | Down | 0.4863 | 0.006571 |
| A0A1R3X2E2 | Uncharacterized protein OS | Down | 0.7417 | 0.00254 |
| A0A01RT21 | Site-determining protein OS | Down | 0.5787 | 0.002727 |
| A0A0D9AUP5 | 60 kDa chaperonin OS | Down | 0.2131 | 0.000078 |
| A0A448BVJ5 | ATP synthase subunit alpha OS | Down | 0.6884 | 0.003857 |
| A0A0B7D324 | Translation initiation factor IF-2 OS | Down | 0.7328 | 0.02568 |
| A0A01OS216 | GTP-binding protein TypA OS | Down | 0.4553 | 0.0148 |
| A0A34MJU8 | Succinate-CoA ligase [ADP-forming] subunit beta OS | Down | 0.717 | 0.01519 |
| A0A10LLO2 | Uncharacterized protein OS | Down | 0.4868 | 0.000044 |
| A0A34MBG5 | NAD-dependent malic enzyme OS | Down | 0.826 | 0.002371 |
| A0A0FIVK8 | Quinone oxidoreductase OS | Down | 0.6851 | 0.000545 |
| A0A35MBJ2 | DNA topoisomerase 1 OS | Down | 0.826 | 0.01052 |
| A0A34MGX7 | Alanine–fRNA ligase OS | Down | 0.8054 | 0.01262 |
| A0A448BVI9 | Glycine dehydrogenase (decarboxylating) OS | Down | 0.6628 | 0.0003 |
| A0A33XUGU9 | Glucans biosynthesis glucosyltransferase H OS | Down | 0.43 | 0.000429 |
| A0A370XT9 | G/U mismatch-specific DNA glycosylase OS | Down | 0.7478 | 0.003514 |
| A0A10KB2 | Glutathione peroxidase OS | Down | 0.6022 | 0.003997 |
| A0A0F4TL9 | Sulfate adenylyltransferase subunit 2 OS | Down | 0.5883 | 0.000132 |
| A0A448BV68 | Protease OS | Down | 0.7601 | 0.01423 |
| A0A33XL87 | Carbonic anhydrase OS | Down | 0.5356 | 0.000698 |
| A0A3S4MT4 | 2Fe-2S ferredoxin OS | Down | 0.6699 | 0.000918 |
| A0A1OS2A6 | UDP-N-acetylglucosamine 2-epimerase OS | Down | 0.7554 | 0.03542 |
| A0A0D7T17 | NAD/NADP-dependent betaine aldehyde dehydrogenase OS | Down | 0.7868 | 0.02845 |
| A0A3S4PY8 | Succinate-CoA ligase [ADP-forming] subunit alpha OS | Down | 0.753 | 0.0247 |
| A0A1T2Y0Q8 | Oxidoreductase OS | Down | 0.7698 | 0.00724 |
| E2XYQ5 | UDP-N-acetylglucosamine 2-epimerase OS | Down | 0.7967 | 0.01584 |
| A0A34G016 | Lipoam synthase OS | Down | 0.7383 | 0.01066 |
| A0A0D9B4C3 | NADH-quinone oxidoreductase subunit I OS | Down | 0.7234 | 0.01535 |
| A0A0F4K66 | Diene lactone hydrolase OS | Down | 0.6862 | 0.003742 |
| A0A10FR6 | Multifunctional fusion protein OS | Down | 0.4251 | 0.000001 |
| A0A35PCY6 | NADH-quinone oxidoreductase subunit F OS | Down | 0.8283 | 0.004383 |
| A0A3S4B87 | UDP-glucose 6-dehydrogenase (Fragment) OS | Down | 0.7358 | 0.008413 |
| A0A10TH05 | Glucarate dehydratase OS | Down | 0.7564 | 0.003172 |
| B3PL47 | Thioredoxin reductase OS | Down | 0.3985 | 0.006945 |
| A0A10TBW6 | Sulfinate dehydrogenase flavoprotein subunit OS | Down | 0.7114 | 0.008916 |
| A0A37WUW9 | Alkyl hydroperoxide reductase OS | Down | 0.4814 | 0.000853 |
| A0A0X78K2 | Alkene reductase OS | Down | 0.7332 | 0.004101 |
| L7H6Z5 | 1,4-alpha-glucan branching enzyme GlgB OS | Down | 0.7367 | 0.005334 |
| A0A0B7D9X9 | 3OS ribosomal protein S7 OS | Down | 0.3928 | 0.001471 |
| A0A34F5P5 | Bifunctional protein PutA OS | Down | 0.5788 | 0.000048 |
| A0A4A1Y8 | Peroxiredoxin OsmC OS | Down | 0.6682 | 0.000699 |
| A0A0D7N2K9 | NADH-quinone oxidoreductase subunit C/D OS | Down | 0.5453 | 0.009922 |

(Continued)
### TABLE 2 | Continued

| Accession | Protein name                          | Regulate | Fold change | p-value |
|-----------|---------------------------------------|----------|-------------|---------|
| A0A04T6P4 | Amino acid ABC transporter ATPase OS  | Down     | 0.2658      | 0.01775 |
| A0A423MU06 | Micronin ABC transporter ATP-binding protein OS | Down     | 0.8686      | 0.005146 |
| A0A379A35 | Cysteine synthase OS                  | Down     | 0.5603      | 0.00057 |
| A0A120G9E8 | DNA-invertase hin OS                | Down     | 0.5688      | 0.0114  |
| A0A3S4RL56 | 50S ribosomal protein L17 OS        | Down     | 0.5046      | 0.00556 |
| A0A2A5REX4 | AI-2E family transporter            | Down     | 0.7659      | 0.006788|
| A0A3M3CL9 | Phosphoenolpyruvate synthase OS     | Down     | 0.2506      | 0.000621|
| A0A105RH4 | Phosphoribosylglycinamide formyltransferase OS | Down  | 0.7039      | 0.000056|
| 1K120 | Malonate decarboxylase, gamma subunit OS | Down     | 0.7353      | 0.02236 |
| A0A0C1WLI8 | 30S ribosomal protein S13 OS        | Down     | 0.3449      | 0.001954|
| A0A3M3XP35 | Alkali domain-containing protein OS  | Down     | 0.3826      | 0.000729|
| A0A1B3D5W7 | AI-2E family transporter OS         | Down     | 0.7473      | 0.03427 |
| A0A21DZ50 | Type VI secretion system tube protein Hcp OS | Down | 0.3845      | 0.006341|
| A0A4P77D8 | AAA family ATPase OS                | Down     | 0.4335      | 0.004957|
| A0A3M4GSW6 | DNA polymerase III subunit alpha OS | Down     | 0.6754      | 0.004196|
| A0A0P8XQZ8 | Succinate dehydrogenase and fumarate reductase iron-sulfur family protein OS | Down | 0.2746      | 0.02616 |
| A0A1T2CZ1 | Alkyl hydroperoxide reductase C OS  | Down     | 0.000001    | 0       |
| A0A109KFA5 | Peptide methionine sulfoxide reductase MsrA OS | Down  | 0.000001    | 0       |
| A0A04WKH3 | Electron transfer flavoprotein subunit beta OS | Down     | 0.000001    | 0       |
| A0A423N111 | TetR family transcriptional regulator OS | Down  | 0.000001    | 0       |
| A0A255EAC0 | Citrate synthase OS                 | Down     | 0.000001    | 0       |
| A0A109F8R | Ketol-acid reductoisomerase (NADP(+)) OS | Down     | 0.000001    | 0       |
| A0A423MKX5 | DNA-binding response regulator OS    | Down     | 0.000001    | 0       |
| A0A0873911 | 1-(5-phosphoribosyl)-5-[(5-phosphoribosylamino)methylideneamino] imidazole-4-carboxamide isomerase OS | Down | 0.000001    | 0       |
| A0A0B7DDH5 | Iron-sulfur cluster assembly scaffold protein IscU OS | Down | 0.000001    | 0       |
| A0A327MN2A | AI-2E family transporter OS         | Down     | 0.000001    | 0       |
| A0A327ML2 | AI-2E family transporter OS         | Down     | 0.000001    | 0       |
| A0A2K9MA22 | Thioredoxin reductase OS            | Down     | 0.000001    | 0       |
| A0A506RJ5 | Cysteine synthase CysM OS           | Down     | 0.000001    | 0       |
| A0A423LS5V9 | AI-2E family transporter OS        | Down     | 0.000001    | 0       |
| A0A2ArR442 | AI-2E family transporter OS         | Down     | 0.000001    | 0       |

50S ribosomal protein L3 OS, 50S ribosomal protein L25 OS, 30S ribosomal protein S17 OS, 50S ribosomal protein L23 OS, 30S ribosomal protein S2 OS, 50S ribosomal protein L29 OS, 50S ribosomal protein L10 OS, 50S ribosomal protein L4 OS, 30S ribosomal protein S10 OS, 50S ribosomal protein S4 OS, 50S ribosomal protein L9 OS, 50S ribosomal protein L15 OS, 50S ribosomal protein L11 OS, 50S ribosomal protein L6 OS, 50S ribosomal protein L7/L12 OS, 50S ribosomal protein L2 OS, and 50S ribosomal protein L1 OS were expressed at much higher levels, and their fold changes were all greater than 139.12. A similar result was tested for the progression of the ribosome through a regulatory open reading frame (ORF), which controlled the protein synthesis expression of many genes in *P. fluorescens* ITEM 17298 and influenced the expression of the downstream gene (Vazquez-Laslop et al., 2011; Gupta et al., 2013; Quintieri et al., 2019). The results showed that a majority of up-regulated proteins could accelerate protein expression and biological activity. There were some down-regulated proteins, such as thioredoxin reductase OS (A0A2K9M4Z2), cysteine synthase CysM OS (A0A506RJ5), DNA-binding response regulator (A0A423MKX5), amino acid ABC transporter ATPase OS (A0A0F4T6P4), that were expressed at a higher level in A vs. AP and P vs. AP. In the present study, bacteria played a critical role in transporting some molecules, including sugars, amino acids, vitamins, peptides, polysaccharides, lipids, thioredoxin, and ABC transporters (Rees et al., 2009; Zhong et al., 2019). Interestingly, more down-regulated proteins of the AI-2 family transporter OS were obtained in A vs. AP than in P vs. AP, which demonstrated more down-regulated proteins affecting AI-2 expression in *A. johnsonii*. Further investigations of AI-2 activity in *A. johnsonii* revealed it to be at a lower level than in the co-culture, suggesting that the latter might more easily contribute to AI-2 protein expression.
| Accession   | Protein name                                                                 | Regulate | Fold change | p-value  |
|------------|-------------------------------------------------------------------------------|----------|-------------|----------|
| A0A448DVQ8 | Thioredoxin reductase OS                                                      | Up       | 1.226       | 0.005186 |
| A0A010SSP5 | Electron transfer flavoprotein subunit beta OS                               | Up       | 1.519       | 0.03911  |
| A0A109KZR3 | 30S ribosomal protein S1 OS                                                   | Up       | 1.22        | 0.000809 |
| A0A3S4NMX4 | Cysteine synthase OS                                                          | Up       | 1.447       | 0.01686  |
| A0A010T165 | Cysteine desulfurase IscS OS                                                  | Up       | 1.316       | 0.004729 |
| A0A166PHR0 | AI-2E family transporter OS                                                   | Up       | 1.441       | 0.01166  |
| A0A3S4NMM4 | Proline-tRNA ligase OS                                                        | Up       | 1.213       | 0.03312  |
| A0A423M0X2 | AI-2E family transporter OS                                                   | Up       | 1.21        | 0.03034  |
| A0A423LFX7 | Glycine betaine ABC transporter substrate-binding protein OS                 | Up       | 1.929       | 0.006274 |
| A0A3M4HEE6 | Fn3_like domain-containing protein OS                                         | Up       | 1.361       | 0.01977  |
| A0A109L3N2 | Elongation factor G OS                                                        | Up       | 1.288       | 0.001337 |
| A0A0D0SI76 | Chaperone protein ClpB OS                                                     | Up       | 2.025       | 0.0105   |
| A0A4V5UF04 | Molecular chaperone DnaK OS                                                   | Up       | 6.781       | 0.000406 |
| A0A0B7DHH8 | S-adenosylmethionine:tRNA ribosyltransferase-isomerase OS                    | Up       | 1.319       | 0.001532 |
| A0A109KSE5 | Arginine deiminase OS                                                         | Up       | 2.538       | 0.000025 |
| A0A109KWR2 | Chaperone protein HtpG OS                                                     | Up       | 26.25       | 0.000119 |
| A0A3M4FNE4 | Elongation factor Tu OS                                                        | Up       | 61.14       | 0.000005 |
| I4KE62     | Ornithine carbamoyltransferase OS                                             | Up       | 5.734       | 0.002111 |
| A0A0X7K650 | Alkyl hydroperoxide reductase OS                                              | Up       | 2.631       | 0.000265 |
| I4K7P9     | ATP synthase subunit beta OS                                                  | Up       | 4.626       | 0.03624  |
| A0A0C1WL18 | 30S ribosomal protein S13 OS                                                   | Up       | 2.938       | 0.04752  |
| A0A3M4GZQ4 | Ribose-phosphate pyrophosphokinase OS                                         | Up       | 1.725       | 0.00228  |
| A0A0D0PLG1 | 50S ribosomal protein L14 OS                                                   | Up       | 1.668       | 0.000929 |
| A0A010RPF8 | 16S rRNA methyltransferase OS                                                 | Up       | 1.548       | 0.01886  |
| A0A3M4G573 | Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex OS | Up       | 4.699       | 0.01403  |
| A0A379DI7  | ABC transporter ATP-binding protein OS                                        | Up       | 1.278       | 0.01057  |
| A0A2K9MAZ2 | Thioredoxin reductase OS                                                      | Up       | 1.908       | 0.04652  |
| A0A2N1DUG2 | AI-2E family transporter OS                                                   | Up       | 2.147       | 0.000219 |

(Continued)
TABLE 3 | Continued

| Accession | Protein name | Regulate | Fold change | p-value |
|-----------|--------------|----------|-------------|---------|
| A0A379HY5 | Transcriptional regulator MvaT, P16 subunit OS | Up | 6.47 | 0.000263 |
| A0A165YHM6 | AI-2E family transporter OS | Up | 1.881 | 0.00109 |
| A0A0D0PNN7 | Aldehyde dehydrogenase OS | Up | 3.797 | 0.001845 |
| U1TQN4 | 60 kDa chaperonin OS | Up | 122.28 | 0 |
| A0A4V5UF6 | Chaperonin GroEL OS | Up | 122.28 | 0 |
| A0A0W0HIU9 | Alkyl hydroperoxide reductase C OS | Up | 122.28 | 0 |
| I4K6X1 | Putative lipoprotein OS | Up | 122.28 | 0 |
| I4K318 | 50S ribosomal protein L1 OS | Up | 122.28 | 0 |
| A0A109KXR2 | Heat-shock protein OS | Up | 122.28 | 0 |
| A0A125QDK7 | Elongation factor Ts OS | Up | 122.28 | 0 |
| A0A075PA24 | 10 kDa chaperonin OS | Up | 122.28 | 0 |
| A0A109KX60 | Electron transfer flavoprotein subunit alpha OS | Up | 122.28 | 0 |
| A0A0X7KI3A3 | Dipicolinate synthase OS | Up | 122.28 | 0 |
| A0A010RMN0 | Succinate-CoA ligase [ADP-forming] subunit alpha OS | Up | 122.28 | 0 |
| A0A0C2A4F3 | Nucleotide exchange factor GrpE OS | Up | 122.28 | 0 |
| A0A0A1YUR6 | Nucleoside diphosphate kinase OS | Up | 122.28 | 0 |
| A0A1Q5X417 | Cold-shock protein OS | Up | 122.28 | 0 |
| A0A0D0TC7S | Succinate-CoA ligase [ADP-forming] subunit beta OS | Up | 122.28 | 0 |
| A0A0B7DIY4 | 50S ribosomal protein L2 OS | Up | 122.28 | 0 |
| A0A0D0PUB7 | Enolase OS | Up | 122.28 | 0 |
| A0A0C3XNL5 | Fenitin domain-containing protein OS | Up | 122.28 | 0 |
| A0A0A1ZS2 | 50S ribosomal protein L7L12 OS | Up | 122.28 | 0 |
| A0A1T2YYC5 | Ornithine aminotransferase OS | Up | 122.28 | 0 |
| A0A010SEN5 | 50S ribosomal protein L6 OS | Up | 122.28 | 0 |
| A0A010RGK5 | Endoribonuclease OS | Up | 122.28 | 0 |
| A0A0A1YZ47 | Transcriptional regulator HU subunit alpha OS | Up | 122.28 | 0 |
| A0A075P9Q2 | 30S ribosomal protein S4 OS | Up | 122.28 | 0 |
| A0A010SL5 | 50S ribosomal protein L9 OS | Up | 122.28 | 0 |
| A0A0W0HKK7 | 50S ribosomal protein L15 OS | Up | 122.28 | 0 |
| A0A075PC10 | 50S ribosomal protein L11 OS | Up | 122.28 | 0 |
| A0A0C1ZK7 | Lipoprotein OS | Up | 122.28 | 0 |
| A0A010RRM4 | 30S ribosomal protein S10 OS | Up | 122.28 | 0 |

(Continued)
| Accession | Protein name | Regulate | Fold change | p-value |
|-----------|--------------|----------|-------------|---------|
| C3K254    | Osmotically inducible protein Y OS | Up       | 122.28      | 0       |
| A0A0A1Z8J0| 50S ribosomal protein L4 OS | Up       | 122.28      | 0       |
| A0A3M5MJ63| Fatty acid oxidation complex subunit alpha OS | Up | 122.28 | 0 |
| A0A0N7HX07| Urocanate hydratase OS | Up | 122.28 | 0 |
| E2XZ08    | 50S ribosomal protein L29 OS | Up | 122.28 | 0 |
| A0A0W0HLG0| 50S ribosomal protein L10 OS | Up | 122.28 | 0 |
| A0A448BQ1 | Phosphoribosylformylglycinamidine cyclo-ligase OS | Up | 122.28 | 0 |
| A0A3M3Y045| Aspartate ammonia-lyase OS | Up | 122.28 | 0 |
| A0A448BWD3| Spermidine/putrescine import ATP-binding protein PotA OS | Up | 122.28 | 0 |
| A0A0W0QVH5| Porin OS | Up | 122.28 | 0 |
| A0A3M3XD24| Nucleoid-associated protein ALQ35_00435 OS | Up | 122.28 | 0 |
| A0A3M4GI8 | Fumarate hydratase class I OS | Up | 122.28 | 0 |
| A0A0W0HH67| Chromosome partitioning protein ParA OS | Up | 122.28 | 0 |
| A0A010RSX6| Adenosylhomocysteinase OS | Up | 122.28 | 0 |
| A0A0K1QHC1| 50S ribosomal protein L18 OS | Up | 122.28 | 0 |
| A0A109KM78| 2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase OS | Up | 122.28 | 0 |
| A0A0G4E5Q2| Single-stranded DNA-binding protein OS | Up | 122.28 | 0 |
| A0A109KQK7| Uncharacterized protein OS | Up | 122.28 | 0 |
| A0A3M3XER7| 50S ribosomal protein L3 OS | Up | 122.28 | 0 |
| A0A387BBY7| 50S ribosomal protein L25 OS | Up | 122.28 | 0 |
| U1TYF4    | 30S ribosomal protein S17 OS | Up | 122.28 | 0 |
| A0A3M3XF13| 50S ribosomal protein L23 OS | Up | 122.28 | 0 |
| A0A3M5NX5 | 30S ribosomal protein S2 OS | Up | 122.28 | 0 |
| A0A34RR46 | Nitrogen regulatory protein Pil OS | Up | 122.28 | 0 |
| A0A3M5MC6 | S1 motif domain-containing protein OS | Up | 122.28 | 0 |
| A0A3M4GL6 | Aldehyde domain-containing protein OS | Up | 122.28 | 0 |
| A0A0W0H1J8| Elongation factor P OS | Up | 122.28 | 0 |
| A0A3M5N1D0| Superoxide dismutase OS | Up | 122.28 | 0 |
| A0A238XHJ7| Al-2E family transporter OS | Up | 122.28 | 0 |

(Continued)
| Accession   | Protein name                                      | Regulate | Fold change | p-value   |
|-------------|--------------------------------------------------|----------|-------------|-----------|
| A0A109LMS3  | Cupin domain protein OS                         | Down     | 0.7207      | 0.0038665 |
| A0A0B7DGC9  | Serine hydroxymethyltransferase OS              | Down     | 0.706       | 0.001258  |
| A0A3M5MW10  | ATP-dependent Clp protease ATP-binding subunit ClpX OS | Down     | 0.798       | 0.00304   |
| A0A010T1X2  | 50S ribosomal protein L5 OS                     | Down     | 0.7538      | 0.005059  |
| A0A448BG48  | 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (flavodoxin) OS | Down     | 0.6935      | 0.005875  |
| A0A109LM37  | DUF86 domain-containing protein OS              | Down     | 0.7715      | 0.007694  |
| A0A3S4N8H0  | Pyruvate kinase OS                              | Down     | 0.816       | 0.03967   |
| A0A012AAA7  | Glucose-1-phosphate thymidyltransferase OS      | Down     | 0.7914      | 0.02799   |
| A0A161Z4S7  | Glycerol-3-phosphate dehydrogenase OS           | Down     | 0.7438      | 0.003224  |
| A0A3M3XN23  | Adenylosuccinate lyase OS                       | Down     | 0.8242      | 0.005162  |
| A0A448BGH7  | Phosphoenolpyruvate carboxylase OS              | Down     | 0.7643      | 0.01978   |
| A0A3S4SXK6  | Transcription termination/antitermination protein NusG OS | Down     | 0.7793      | 0.03637   |
| A0A345VUJ2  | dTDP-4-dehydromannose reductase OS              | Down     | 0.6827      | 0.002461  |
| E2XZ43      | Transcriptional regulator, Crp/Fnr family OS    | Down     | 0.8076      | 0.01046   |
| A0A010RT21  | Site-determining protein OS                     | Down     | 0.7867      | 0.01215   |
| A0A109LMK0  | Nucleotidyltransferase domain protein OS        | Down     | 0.7134      | 0.003733  |
| A0A4P3XCE2  | Uncharacterized protein OS                      | Down     | 0.8129      | 0.0114    |
| A0A0N9VZ3   | Phosphoserine aminotransferase OS               | Down     | 0.8173      | 0.04239   |
| A0A3S4N6L6  | Isocitrate dehydrogenase [NADP] OS              | Down     | 0.78        | 0.02703   |
| A0A370J8N3  | Thioredoxin reductase OS                        | Down     | 0.7602      | 0.02114   |
| A0A4Y4JCO4  | Cysteine synthase B OS                          | Down     | 0.8055      | 0.007142  |
| A0A3S4MUG8  | Succinate–CoA ligase [ADP-forming] subunit beta OS | Down     | 0.7899      | 0.0351    |
| A0A3M5MSW3  | UvrABC system protein B OS                      | Down     | 0.8096      | 0.002896  |
| A0A109LLO2  | Uncharacterized protein OS                      | Down     | 0.6859      | 0.003862  |
| A0A370J4U0  | Cysteine synthase OS                            | Down     | 0.7646      | 0.003665  |
| A0A0A1YZP1  | Histidine–tRNA ligase OS                        | Down     | 0.7848      | 0.00437   |
| A0A3M4G5X7  | Alanine–tRNA ligase OS                         | Down     | 0.7532      | 0.01493   |
| A0A0K1QD3   | LysR family transcriptional regulator OS        | Down     | 0.8259      | 0.04135   |
| A0A370XHT9  | G/U mismatch-specific DNA glycosylase OS        | Down     | 0.6979      | 0.01167   |
| A0A3M3XL78  | Carbonic anhydrase OS                           | Down     | 0.68        | 0.009262  |
| A0A448VB88  | Protease OS                                    | Down     | 0.8028      | 0.01425   |
| A0A109KNB3  | Glutathione peroxidase OS                       | Down     | 0.4815      | 0.002483  |
| A0A3S4MT74  | 2Fe-2S ferredoxin OS                           | Down     | 0.7981      | 0.01977   |
| A0A010RGL6  | Phosphoglucomutase OS                          | Down     | 0.7412      | 0.000758  |

(Continued)
| Accession | Protein name | Regulate | Fold change | p-value |
|-----------|--------------|----------|-------------|---------|
| Q4K9V2    | Thioredoxin reductase OS | Down | 0.7618 | 0.03801 |
| A0A3M4G016 | Lipoyl synthase OS | Down | 0.7969 | 0.03549 |
| A0A010RC6 | Multifunctional fusion protein OS | Down | 0.5799 | 0.000021 |
| A0A0D9B4C3 | NADH-quinone oxidoreductase subunit I OS | Down | 0.64 | 0.00074 |
| A0A010RSQ6 | L-cystine transporter tcyP OS | Down | 0.5553 | 0.01979 |
| A0A0F4T9U8 | Diaminopimelate decarboxylase OS | Down | 0.8255 | 0.002003 |
| B3PL47 | Thioredoxin reductase OS | Down | 0.5017 | 0.002077 |
| A0A010T8W6 | Succinate dehydrogenase flavoprotein subunit OS | Down | 0.8224 | 0.04234 |
| A0A0B7DI73 | Isoleucine-tRNA ligase OS | Down | 0.7257 | 0.00215 |
| A0A3M4GK93 | Phosphoribosylaminomidazole-succinocarboxamide synthase OS | Down | 0.686 | 0.01121 |
| A0A3M5MVH0 | Glucans biosynthesis protein D OS | Down | 0.6468 | 0.03983 |
| A0A0X7K8K2 | Alkene reductase OS | Down | 0.8053 | 0.00967 |
| A0A3M4FP51 | Bifunctional protein PutA OS | Down | 0.6509 | 0.000256 |
| A0A0A1YRY8 | Peroxiredoxin OsmC OS | Down | 0.7267 | 0.003565 |
| A0A3S4N1Z0 | Cytosine deaminase OS | Down | 0.7876 | 0.007186 |
| A0A0N8W7I2 | Catalase OS | Down | 0.6428 | 0.001721 |
| A0A0F4T6P4 | Amino acid ABC transporter ATPase OS | Down | 0.474 | 0.02104 |
| A0A0B7D1W3 | Glycine betaine transport ATP-binding protein OpuAA OS | Down | 0.7214 | 0.01965 |
| A0A379A35 | Cysteine synthase OS | Down | 0.7158 | 0.004647 |
| I4K120 | Malonate decarboxylase, gamma subunit OS | Down | 0.8073 | 0.01994 |
| A0A010SRH4 | Phosphoribosylglycinamidine formyltransferase OS | Down | 0.7332 | 0.000756 |
| A0A327MWL2 | Al-2E family transporter OS | Down | 0.6227 | 0.009033 |
| A0A0N8NY49 | Aldo/keto reductase family protein OS | Down | 0.7843 | 0.04723 |
| A0A0B7D6X0 | Glutathione import ATP-binding protein GsiA OS | Down | 0.6386 | 0.003439 |
| A0A0F4TUQ5 | Glucose dehydrogenase OS | Down | 0.454 | 0.02673 |
| A0A0P9BDW1 | Ribose import ATP-binding protein RbsA OS | Down | 0.6737 | 0.02709 |
| A0A3S4PXV5 | Transcriptional activator CopR OS | Down | 0.731 | 0.03974 |
| A0A423N111 | TetR family transcriptional regulator OS | Down | 0.00001 | 0 |
| A0A0B7DDH5 | Iron-sulfur cluster assembly scaffold protein IscU OS | Down | 0.00001 | 0 |
| A0A125QFU4 | Al-2E family transporter OS | Down | 0.00001 | 0 |
| A0A386Y9V3 | Al-2E family transporter OS | Down | 0.00001 | 0 |
Bioinformatics Functional Analysis of *A. johnsonii*, *P. fluorescens*, and Co-culture

**GO and COG Enrichment Analysis of *A. johnsonii*, *P. fluorescens*, and Co-culture**

A GO classification and COG enrichment of the 177 and 155 differentially expressed proteins were performed in A vs. AP and P vs. AP, respectively (Figures 4A,B). There were three main categories of cellular components, biological processes, and molecular functions in the GO classification. The cellular process (GO:0009987), the metabolic process (GO:0008152), and the single-organism process (GO:0044699) were the three main distributed terms in the biological processes. The 85 and 79 differentially expressed proteins were annotated as belonging to the cell in A vs. AP and P vs. AP, respectively. In addition, A vs. AP (80 out of 177) and P vs. AP (76 out of 155) of the differentially expressed proteins were localized in the cell part. This suggested that *A. johnsonii*, *P. fluorescens*, and their co-culture played an essential role in the transmembrane transport function and intracellular and extracellular substance migration, thereby promoting nutrient absorption and excretion of metabolic products. In the molecular functions category, the proteins were related to catalytic activity and binding.

**Figures 4C,D** displays the COG enrichment analysis. A total of 18 categories were classified, in which the top 6 COG terms were (i) energy production and conversion, (ii) amino acid transport and metabolism, (iii) translation, ribosomal structure,
and biogenesis, (iv) post-translational modification, (v) protein turnover, and (vi) chaperones. A total of 22 down-regulated proteins were involved in energy production and conversion in A vs. AP, while 23 up-regulated proteins were involved in translation, ribosomal structure, and biogenesis in A vs. AP. Interestingly, translation, ribosomal structure, and biogenesis were significantly up-regulated in P vs. AP, and amino acid transport and metabolism and energy production and conversion were significantly down-regulated in P vs. AP, suggesting that translation, ribosomal structure, and energy production and conversion were valuable targets for co-culturing, and thus deserve further investigation. It has been suggested that under co-culture conditions, energy production is capable of involving translation of the bacteria, resulting in the activation of the ribosomal structure (Gupta et al., 2013). These proteins might also participate in nucleotide catabolism, allowing bacteria to use deoxynucleotides as energy sources. The results of GO analysis and COG enrichment provide a significant view of the proteins differentially expressed in A. johnsonii, P. fluorescens, and their co-culture that can elevate protein functions.

Pathway Enrichment Analysis of A. johnsonii, P. fluorescens, and Co-culture
Pathway enrichment analysis of the KEGG database was conducted on the differentially expressed proteins to reveal the metabolic and signal transduction pathways of A. johnsonii, P. fluorescens, and their co-culture (Figures 4E,F). In our study, taken together, the up/down-regulated differentially expressed proteins of A. johnsonii, P. fluorescens, and their co-culture involved in amino acid metabolism, carbohydrate and energy metabolism, and nucleotide metabolism enabled predictions of the change in the culture of the A. johnsonii, P. fluorescens samples in the co-culture, indicating that protein changes and carbohydrate transformation contributed to bacteria co-culturing. A total of 84 and 77 differentially expressed proteins in A vs. AP and P vs. AP, respectively, were divided into 20 KEGG pathways, and a majority of the metabolic pathways included genetic information processing, environmental information processing, cellular processes, organismal systems, and human diseases. The KEGG pathways of amino acid metabolism, carbohydrate metabolism, energy metabolism, and translation were significantly enriched. A similar result for carbohydrate metabolism and energy metabolism of Vibrio parahaemolyticus revealed that carbohydrate metabolism is the key factor, indicating that carbohydrate can either be converted into glucan and fructose through a glycosyltransferase reaction or transported by sugar transport systems and subsequently metabolized through glycolysis (Zhong et al., 2019).

The main significant items relevant to the regulation of biofilm formation of A vs. AP and P vs. AP included the map 02026, map 05111, and map 02025 pathways (Supplementary Tables S1, S2). Analysis of the proteins of A. johnsonii, P. fluorescens, and their co-culture indicated that all of the pathways were present. In addition, the pathway of map 02024 was that which regulated QS, which is a crucial feature affecting the regulation of biofilm formation by AHLs, bacterial growth, protease activity, and the spoilage potential of bacteria (Jie et al., 2018).

Furthermore, as shown in Supplementary Tables S1, S2, ribosome was the most significantly enriched pathway, which indicated that protein expression was substantially promoted to achieve a large demand for bacterial growth (Li J. et al., 2020). Ribosome was the most significant pathway, with 43 differentially expressed proteins, of which 22 were up-regulated proteins and 21 were down-regulated proteins. In addition, abundant proteins were associated with the tricarboxylic acid (TCA) cycle and oxidative phosphorylation in response to co-culture conditions (Supplementary Figure S1). The TCA cycle
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pathway has 7 up-regulated proteins and 9 down-regulated proteins. The most abundant protein detected among the 6 up-regulated and 11 down-regulated proteins was associated with oxidative phosphorylation. These pathways were essential for bacterial growth and cell interactions, which have potential for enhancing bacterial spoilage and QS regulation (Remenant et al., 2015; Otwell et al., 2018).

Subcellular Localization Prediction of A. johnsonii, P. fluorescens, and Co-culture
The subcellular localization prediction of proteins has attracted considerable attention in protein functional annotation. There were two subcellular localization predictions, including for cytoplasm and plasma membranes. Figure 5 shows that subcellular localization in P vs. AP was located mainly in the cytoplasm and the plasma membrane, but mainly in the cytoplasm in A vs. AP. Previous studies have focused mainly on single bacteria producing a few metabolites that migrate from the inner membrane to cell-extracellular membranes during culture (Ellepola et al., 2019), resulting in cell communications. However, few studies have reported explanations for two bacterial subcellular localization predictions. As shown in Figure 5, expression of cytoplasmic proteins was also regulated in the co-culture.

Quorum Sensing and Spoilage-Related Proteins
The QS mechanism is the means by which cell population communication can regulate specific proteins to express the physiological characteristics of microorganisms (Li S. et al., 2019). As given in Table 4, the QS system was related to the protein of the AI-2E family transporter OS and the LuxR family transcriptional regulator OS. The LuxR family transcriptional regulator was found in A vs. AP and P vs. AP. The LuxR protein (AHL receptor) commonly consists of 200–260 amino acids binding with the key protein, resulting in the expression of the key protein. Five AI-2E family transporter up-regulated proteins and 11 AI-2E family transporter up-regulated proteins were found in A vs. AP and P vs. AP, respectively. This suggests that the AI-2E family transporter played a vital role in regulating QS. Previous studies have shown that P. fluorescens can

| Function | Protein name | Accession number | Regulate |
|----------|--------------|------------------|----------|
| QS system | AI-2E family transporter OS | A0A2S8XHJ7, A0A299P207, A0A2N1DUG2, A0A165YH66, A0A2S8XHJ7, A0A1T3AM62, A0A161G5M51, A0A2K9M819, A0A2S9REX4, A0A386Y9V3, A0A1B3D5W7, A0A327NA26, A0A327MWL2, A0A423L5V9, A0A2S9R442, A0A1T2YCZ7 | Up | Down |
| Spoliation | LuxR family transcriptional regulator OS | A0A0U3TA42 | Down |
| Thioredoxin reductase OS | A0A0W8H2Q7, A0A4P6V9D1, Q4K9V2, B3PL47, A0A2K9M4Z2, A0A379J3N3 | Down |
| Cysteine synthase OS | A0A380JBL3, G8Q5M5, A0A379J3Q0, J2Y774, A0A2A5RE67, A0A3S4RHU3, A0A4P7I1W5 | Up |
| Pyridoxal-phosphate dependent enzyme family protein OS | A0A379J407, A0A3S4PDU0 | Down |
| Sulfate adenylytransferase subunit 2 OS | A0A0F4TQL9 | Down |
| Multifunctional fusion protein OS | A0A010RIC6 | Down |
| Putative ABC transporter ATP-binding protein OS | A0A379J4E6 | Down |
| Glycine betaine transport ATP-binding protein Os | A0A0B7DI3 | Down |
| Microcin ABC transporter ATP-binding protein OS | A0A432M0U6 | Down |
| ABC transporter ATP-binding protein OS | A0A379J2D7 | Up |
| Glutathione import ATP-binding protein GisA OS | A0A0B7D6X0 | Down |
| Ribose import ATP-binding protein RbsA OS | A0A0P6BDW1 | Down |
| Spermidine/putrescine import ATP-binding protein PotA OS | A0A010RIC6 | Down |
| Spermidine/putrescine ABC transporter substrate-binding protein OS | A0A010RQY0 | Down |
| Spermidine/putrescine ABC transporter substrate-binding protein PotA OS | A0A448BW3 | Up |
| Transcriptional regulator, Crp/Fnr family OS | E2XZ43 | Down |
| Transcriptional activator CopR OS | A0A3S4PXV5 | Down |
| Urocanate hydratase OS | A0A0N7H007 | Up |
produce AI-2 proteins and AHLs (Sharma et al., 2006), which is consistent with the results described in Section “Protease Activity of A. johnsonii, P. fluorescens, and Co-culture.” Moreover, there were too many spoilage-related proteins, including thioredoxin reductase OS (6 down-regulated proteins, 5 up-regulated proteins), cysteine synthase OS (7 down-regulated proteins, 1 up-regulated protein), and pyridoxal phosphate-dependent enzyme family protein OS. Notably, the spoilage-related proteins in A. johnsonii, P. fluorescens, and their co-culture were similar to those of Shewanella baltica and P. fluorescens ITEM 17298 (Quintieri et al., 2019).

CONCLUSION

This study was carried out in order to explore cultures of A. johnsonii and P. fluorescens and compare them with their co-cultured state for QS and spoilage potential by means of their proteomic profiles. The results show that the products of AHL production (C4-HSL, C6-HSL, C8-HSL), biofilm production, protease activity, and spoilage potential were at a higher level in the co-culture than those of A. johnsonii and P. fluorescens single cultures alone. The proteomic results revealed that there were differences in the proteins involved in the metabolism of amino acids, carbohydrates, energy, and translation. The differentially expressed proteins that were spoilage-related included thioredoxin reductase OS, cysteine synthase OS, and pyridoxal phosphate-dependent enzyme family protein OS, as well as specific QS system proteins of the AI-2E family transporter OS and the LuxR family transcriptional regulator OS, which could be used as biomarkers in A. johnsonii, P. fluorescens, and their co-cultured state. These results may provide an understanding of how the QS and spoilage mechanisms of A. johnsonii, P. fluorescens, and their co-cultures can be regulated in future.

DATA AVAILABILITY STATEMENT

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomcentral.proteomexchange.org/cgi/GetDataset?ID=PXD018646) and the iProX (https://www.iprox.org/page/project.html?id=IPX0002129000).

AUTHOR CONTRIBUTIONS

X-YW analyzed the data, wrote the manuscript, and performed the experiments. JX made suggestions for revision and guided the experiments.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.00940/full#supplementary-material

FIGURE S1 | (A) KEGG pathway map of ribosome; (B) TCA cycle; and (C) oxidative phosphorylation. Proteins in the blue block belong to the experimental species. Red frames indicate up-regulated proteins, and green frames indicate down-regulated proteins.

FIGURE S2 | Chromatograms of C6-HSL produced by: (A) A. johnsonii; (B) P. fluorescens; and (C) their co-culture. Chromatograms of C8-HSL produced by: (D) A. johnsonii; (E) P. fluorescens; and (F) their co-culture.

TABLE S1 | KEGG pathway of the A group compared with the AP group.

TABLE S2 | KEGG pathway of the P group compared with the AP group.

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